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PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

EFEITO DA SUPLEMENTAÇÃO CRÔNICA COM O ÁCIDO GRAXO POLIINSATURADO ÔMEGA-3 E GORDURA VEGETAL HIDROGENADA EM UM MODELO DE COMPORTAMENTO DOENTIO: INFLUÊNCIA DA VIA DA QUINURENINA NAS ALTERAÇÕES NEUROCOMPORTAMENTAIS

TESE DE DOUTORADO

Marcelo Gomes de Gomes

Uruguaiana, RS, Brasil.
2019

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por

Marcelo Gomes de Gomes

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Elaborada por

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Como requisito parcial para obtenção do grau de **Doutor em Bioquímica**

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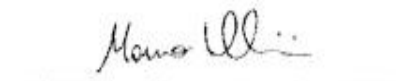

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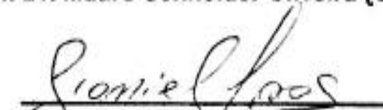

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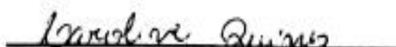

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Ao amigo, orientador e professor Cristiano Ricardo Jesse “Mestre”.
Por me encontrar quando eu estava perdido, por todo o tempo
preparatório, de amizade, fidelidade e aprendizado,
o qual tive a felicidade de viver nos últimos anos.
Me sinto eternamente honrado e agraciado por Deus,
ter entrelaçado nossos caminhos na vida terrena.

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“Cada ato de bondade requer uma haste rigorosa
de sacrifício e amor.”
(Luiz Alberto Gomes)

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Bioquímica
Universidade Federal do Pampa

EFEITO DA SUPLEMENTAÇÃO CRÔNICA COM O ÁCIDO GRAXO POLIINSATURADO ÔMEGA-3 E GORDURA VEGETAL HIDROGENADA EM UM MODELO DE COMPORTAMENTO DOENTIO: INFLUÊNCIA DA VIA DA QUINURENINA NAS ALTERAÇÕES NEUROCOMPORTAMENTAIS

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Local e data da defesa: Uruguaiana-RS, 18 de janeiro de 2019.

O comportamento doentio já foi bem descrito experimentalmente e observacionalmente em diversas espécies animais. Nesse contexto, o lipopolissacarídeo (LPS) é uma endotoxina oriunda de bactérias gram-negativas, o qual é utilizado como modelo preditivo para indução do comportamento doentio em roedores. Assim, este estudo teve por objetivo investigar o efeito da suplementação crônica com ácido graxo poliinsaturado ômega-3 (AGPI n-3) e gordura vegetal hidrogenada (GVH) no modelo de comportamento doentio induzido pelo LPS em camundongos idosos. Os animais idosos receberam a suplementação com AGPI n-3 e GVH por seis meses, após foi feita a administração do LPS para indução do comportamento doentio. Vinte e quatro horas após, foram realizados os testes comportamentais e posteriormente as determinações bioquímicas. De maneira geral, o LPS causou uma grande perda de peso corporal e graves alterações nos testes comportamentais. A suplementação crônica com AGPI n-3 foi capaz de impedir essas alterações geradas pelo LPS nos testes comportamentais em camundongos idosos e a suplementação com GVH não modulou essas alterações. Investigando o envolvimento do sistema serotoninérgico, encontramos que o LPS gerou um aumento nos níveis de serotonina (5-HT) e seu metabólito ácido 5-hidroxi-indolacético (5-HIAA) no hipocampo, estriado e córtex pré-frontal dos animais. A suplementação com AGPI n-3 protegeu contra o aumento nos níveis de 5-HT e 5-HIAA gerados pelo LPS, já a suplementação com GVH não modulou essas alterações e ainda potencializou o aumento nos níveis de 5-HT no hipocampo. A administração de LPS gerou um drástico aumento nos níveis das citocinas pró-inflamatórias (IL-1β, IL-6, TNF-α e IFN-γ), nas três estruturas cerebrais estudadas. Diante da suplementação com AGPI n-3 houve uma atenuação no aumento dos níveis das citocinas pró-inflamatórias. A suplementação com GVH não alterou o aumento nos níveis das citocinas causado pelo LPS e ainda potencializou o aumento nos níveis de TNF-α nas estruturas cerebrais estudas. Esse aumento nos níveis das citocinas pró-inflamatórias causado pelo LPS, acabou por ativar a via da

quinurenina pela ação da enzima indoleamina-2,3-dioxigenase (IDO). A suplementação com AGPI n-3 modulou a atividade da enzima IDO impedindo a alteração na atividade enzimática causada pelo LPS, já a suplementação com GVH não modulou a atividade da enzima nas três estruturas cerebrais. Diante da ativação exacerbada na via da quinurenina causada pelo LPS, investigamos os ramos neurotóxicos e neuroprotetores da via. Neste contexto, o LPS causou uma diminuição nos níveis de ácido quinurênico, o qual tem propriedade neuroprotetora. A suplementação com AGPI n-3 foi capaz de impedir a diminuição nos níveis de ácido quinurênico causada pela administração de LPS no hipocampo, estriado e córtex pré-frontal. Já a suplementação com GVH não influenciou nas alterações nos níveis de ácido quinurênico causadas pelo LPS e ainda contribuiu para a diminuição na razão ácido quinurênico/quinurenina nas estruturas cerebrais estudadas. Investigando o ramo neurotóxico da via, o LPS gerou um aumento nos níveis de 3-hidroxiquinurenina (3-HQ) e ácido quinolínico (AQ) no hipocampo, estriado e córtex pré-frontal dos animais idosos. A suplementação com AGPI n-3 foi capaz de impedir o aumento nos níveis de 3-HQ e AQ em todas as estruturas cerebrais. Já a suplementação com GVH agravou o aumento nos níveis de 3-HQ e AQ exacerbados pelo LPS, aumentando a neurotoxicidade causada pelo comportamento doentio. Assim, os dados do presente trabalho mostraram que a suplementação com AGPI n-3 foi capaz de proteger contra as alterações neurocomportamentais e neurometabólicas causadas pelo comportamento doentio. Também demonstramos que a suplementação com GVH agravou as alterações causadas pelo comportamento doentio. (1)A suplementação com GVH, aumentou a neuroinflamação via TNF-α que, por sua vez ativou ainda mais a via da quinurenina e ainda deslocou o equilíbrio da via em direção ao ramo neurotóxico. Finalmente, contribuímos para elucidação dos efeitos farmacológicos do AGPI n-3 e também demonstramos os efeitos deletérios da GVH no modelo de comportamento doentio. Esperamos, que os resultados do presente estudo possam servir de embasamento para estudos epidemiológicos e de saúde pública na população idosa que é gravemente acometida por doenças neurológicas.

Palavras-chave: Comportamento doentio, quinurenina, neuroinflamação, nutracêutico

ABSTRACT

Doctoral Thesis
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EFFECT OF CHRONIC SUPPLEMENTATION WITH OMEGA-3 POLYUNSATURED FATTY ACID AND HYDROGENATED VEGETABLE FAT IN A MODEL OF SICKNESS BEHAVIOR: INFLUENCE OF KYNURENINE PATHWAY ON NEUROBEHAVIORAL ALTERATIONS

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The sickness behavior has been well described experimentally and observationally in several animal species. In this context, lipopolysaccharide (LPS) is an endotoxin derived from gram-negative bacteria, which is used as a predictive model for the induction of sickness behavior in rodents. Thus, this study aimed to investigate the effect of chronic supplementation with omega-3 polyunsaturated fatty acid (n-3 PUFA) and hydrogenated vegetable fat (HVF) on the model of sickness behavior LPS-induced in aged mice. Aged animals received supplementation with n-3 PUFA and HVF for six months after administration of LPS for induction of sickness behavior. Twenty-four hours later, behavioral tests and biochemical determinations were performed. In general, LPS caused a severe body weight loss and alterations in behavioral tests. Chronic n-3 PUFA supplementation was able to prevent these LPS-generated alterations in behavioral tests in aged mice and HVF supplementation did not modulate these alterations. We investigated the involvement of the serotonergic system, LPS generated an increase in the levels of serotonin (5-HT) and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in the hippocampus, striatum and prefrontal cortex of the animals. Supplementation with n-3 PUFA protected against the increase in 5-HT and 5-HIAA levels generated by LPS, already HVF supplementation did not modulate these alterations and even potentiated the increase in 5-HT levels in the hippocampus. The administration of LPS generated a drastic increase in the levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ) in the three brain structures studied. In the presence of n-3 PUFA supplementation there was an attenuation in the increase of the levels of proinflammatory cytokines. HVF supplementation did not alter the increase in cytokine levels caused by LPS and also potentiated the increase of TNF-α levels in the brain structures studied. This increase in proinflammatory cytokines levels caused by LPS eventually activated the kynurenine pathway by the action of the

indoleamine-2,3-dioxygenase (IDO) enzyme. Supplementation with n-3 PUFA modulated the activity of enzyme IDO, preventing the alteration in the enzymatic activity caused by LPS, already HVF supplementation did not modulate enzyme activity in the three brain structures. In view of the exacerbated activation in the kynurenine pathway caused by LPS, we investigated the branches neurotoxic and neuroprotective. In this context, LPS caused a decrease in the levels of kynurenic acid, which has neuroprotective properties. Supplementation with n-3 PUFA was able to prevent the decrease in levels of kynurenic acid caused by administration of LPS in hippocampus, striatum and prefrontal cortex. However, HVF supplementation did not influence alterations in levels of kynurenic acid caused by LPS and also contributed to the decrease in kynurenic acid/kynurenine ratio in brain structures studied. Investigating the pathway's neurotoxic branch, LPS generated an increase in levels of 3-hydroxykynurenine (3-HQ) and quinolinic acid (AQ) in hippocampus, striatum and prefrontal cortex of the aged animals. Supplementation with n-3 PUFA was able to prevent the increase in 3-HQ and AQ levels in all brain structures. On the other hand, supplementation with HVF aggravated the increase in 3-HQ and AQ levels exacerbated by LPS, increasing the neurotoxicity caused by sickness behavior. Thus, data from the present study showed that n-3 PUFA supplementation was able to protect against neurobehavioral and neurometabolic alterations caused by sickness behavior. We have also shown that HVF supplementation aggravated the alterations caused by sickness behavior. HVF supplementation, increased neuroinflammation via TNF-α which activated kynurenine pathway and further shifted the pathway balance towards the neurotoxic branch. Finally, we contributed to elucidate the pharmacological effects of n-3 PUFA and also demonstrated the deleterious effects of HVF in sickness behavior model. We hope that results of the present study may serve as a basis for epidemiological and public health studies in aged population that is severely affected by neurological diseases.

Keywords: Sickness behavior, kynurenine, neuroinflammation, nutraceutical

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LISTA DE ABREVIATURAS

3-HQ, 3-Hidroxiquinurenina;

5-HIAA, 5-hidroxi-indolacético;

5-HT, Serotonina;

AGPI n-3, Ácido graxo poliinsaturado ômega-3;

AGT, Ácidos graxos trans;

ALA, Ácido α-linolênico;

AQ, Ácido quinolínico;

BCG, bacilo *Calmette-Guérin;*

CHM, Complexo de histocompatibilidade de membrana;

DHA, Ácido docosahexaenóico;

EPA, Ácido eicosapentaenóico;

EROS, Espécies reativas de oxigênio;

GVH, Gordura vegetal hidrogenada;

HPA, Hipotálamo-hipófise-adrenal;

IDO, Indoleamina-2,3-dioxigenase;

IFN-γ, Interferon gama;

IL-1β, Interleucina 1-beta;

IL-6, Interleucina 6;

LPS, Lipopolisscarídeo;

NMDA, N-metil-D-aspartato;

Poly I:C, Ácido policitidílico;

QAT, Quinurenina aminotransferase;

QMO, Quinurenina 3-monooxigenase;

RTT, Receptores do tipo Toll;

SNC, Sistema nervoso central;

TDO, Triptofano 2,3-dioxigenase;

TNF-α, Fator de necrose tumoral alfa;

TRP, Triptofano;

α7nACh, Acetilcolina-7-nicotínica.

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APRESENTAÇÃO

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos publicados, os quais encontram-se no item ARTIGOS CIENTÍFICOS. As seções Introdução, Materiais e Métodos, Discussão, Conclusão e Referências Bibliográficas encontram-se nos ARTIGOS CIENTÍFICOS e representam a íntegra deste estudo. Os itens DISCUSSÃO E CONCLUSÕES, encontram-se no final desta tese e apresentam interpretações e comentários gerais sobre os artigos científicos neste trabalho. As REFERÊNCIAS BIBLIOGRÁFICAS referem-se somente às citações que aparecem itens INTRODUÇÃO e DISCUSSÃO desta tese.

1. INTRODUÇÃO

1.1. Ácidos Graxos

Os ácidos graxos são compostos com a característica química de serem insolúveis em água e solúveis em solventes orgânicos, pertencentes ao abrangente grupo dos lipídeos. Sendo, substâncias encontradas em uma ampla variedade de alimentos e possuem funções estruturais, protetoras e de fornecimento e armazenamento de energia (COSTA E SILVA, 2002). Sua constituição química, consiste de uma cadeia hidrocarbonada, contendo um grupo carboxila em um extremo da cadeia carbonada e um grupo metil na extremidade oposta (GOGUS E SMITH, 2010). Em relação a nomenclatura de ácidos graxos, são classificados de acordo com o número de carbonos, quantidade e posição das duplas ligações em relação ao grupamento metil. Em se tratando do tamanho de cadeia carbonada, os ácidos graxos podem variar de 4 a 30 carbonos (LEHNINGER, NELSON e COX, 2002). Para extensão da cadeia, os ácidos graxos classificam-se em: Ácidos graxos de cadeia curta (menos de 6 carbonos), de cadeia média (6 a 12 carbonos); de cadeia longa (mais de 12 carbonos) e de cadeia muito longa (mais de 22 carbonos). São classificados também de acordo com o número de insaturações presentes em sua cadeia carbonada (LEHNINGER, NELSON e COX, 2002). Assim, os ácidos graxos saturados (Fig. 1), são aqueles que não possuem duplas ligações e os insaturados (Fig. 2) são os que contêm em sua cadeia uma ou mais duplas ligações.

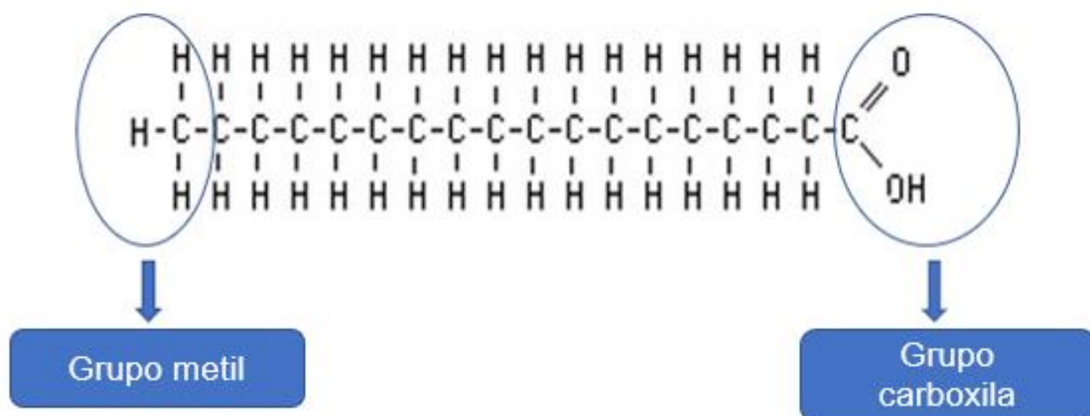

Figura 1. Estrutura química de um ácido graxo saturado (Elaborada pelo autor).

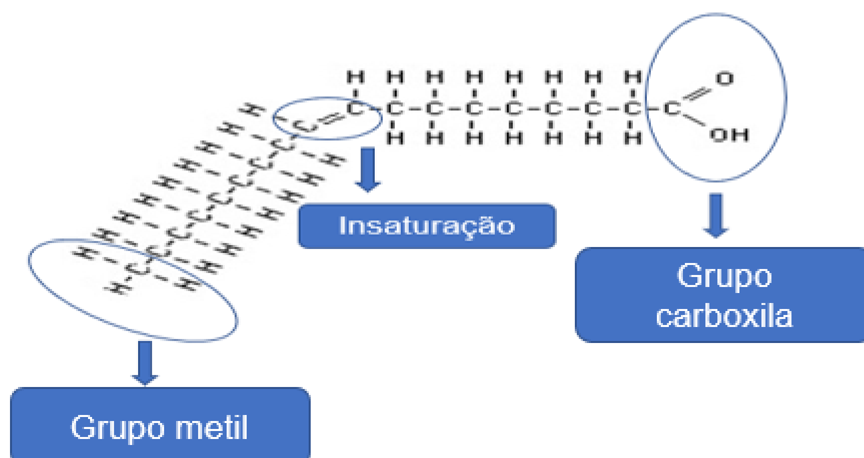

Figura 2. Estrutura química de um ácido graxo insaturado (Elaborada pelo autor).

1.1.1. Ácido Graxo Poliinsaturado Ômega-3 (AGPI n-3)

Os mamíferos em geral, não são capazes de sintetizar os ácidos graxos poliinsaturados (AGPI), assim esses ácidos graxos são considerados essenciais, pois os mesmos têm que ser oriundos de uma fonte dietética. Assim, as células animais não possuem as enzimas dessaturases capazes de especificamente colocar as duplas ligações nas posições n-3 e n-6, de forma que seu suprimento depende unicamente da dieta alimentar (OKEN e BELFORT, 2010).

O ácido α-linolênico (C18:3, ALA) (Fig. 3A), principal representante da série n-3, é encontrado em peixes marinhos de águas geladas e profundas (sardinha, salmão, cavala, truta, arenque), óleos e produtos derivados de pescados, nozes e óleos vegetais (chia, canola e linhaça) (LARSSON et al., 2004). Quando o ALA é consumido e posteriormente metabolizado, ele pode sofrer a ação de enzimas conhecidas como elongases, as quais desdobram o ALA dando a origem a outros dois AGPI n-3, o ácido eicosapentaenóico (C20:5, EPA) e o ácido docosahexaenóico (C22:6, DHA).

O DHA (Fig. 3B) é abundantemente presente no cérebro de mamíferos, compreendendo 40% dos ácidos graxos cerebrais totais, em contraste o EPA (Fig. 3C) representa apenas menos de 1% da quantidade total de ácidos graxos no cérebro (MCNAMARA E CARLSON, 2006).

Sabe-se que os AGPI n-3 contribuem para a modulação, estrutura e funções biológicas das membranas celulares cerebrais, incluindo elasticidade, organização

da membrana e permeabilidade aos íons (GORJÃO et al., 2009). Podendo, portanto facilitar a captação cerebral de glicose, neurotransmissão e função neuronal (LUCHTMAN E SONG, 2013).

Nesse contexto, o EPA especificamente já foi sugerido ter importantes propriedades neuroprotetoras, atividade antioxidante e regulação de processos anti-inflamatórios (CUNNANE et al., 2009;). O DHA é o principal componente dos fosfolipídios das membranas neuronais, abrangendo cerca de 17% do total dos ácidos graxos nesse tecido (SALEM et al., 2001), sendo um dos principais responsáveis por aumentar a fluidez da membrana e a plasticidade sináptica, contribuindo para as funções cerebrais (MITCHELL et al., 2003).

A presença do DHA nessas estruturas favorece a flexibilidade e a mobilidade das proteínas na bicamada de fosfolipídios, características essenciais para as respectivas funções (INNIS, 2007). Diante do exposto, fica ressaltada a importância dos ácidos graxos essenciais para que o sistema nervoso central (SNC), mantenha suas funções fisiológicas normais.

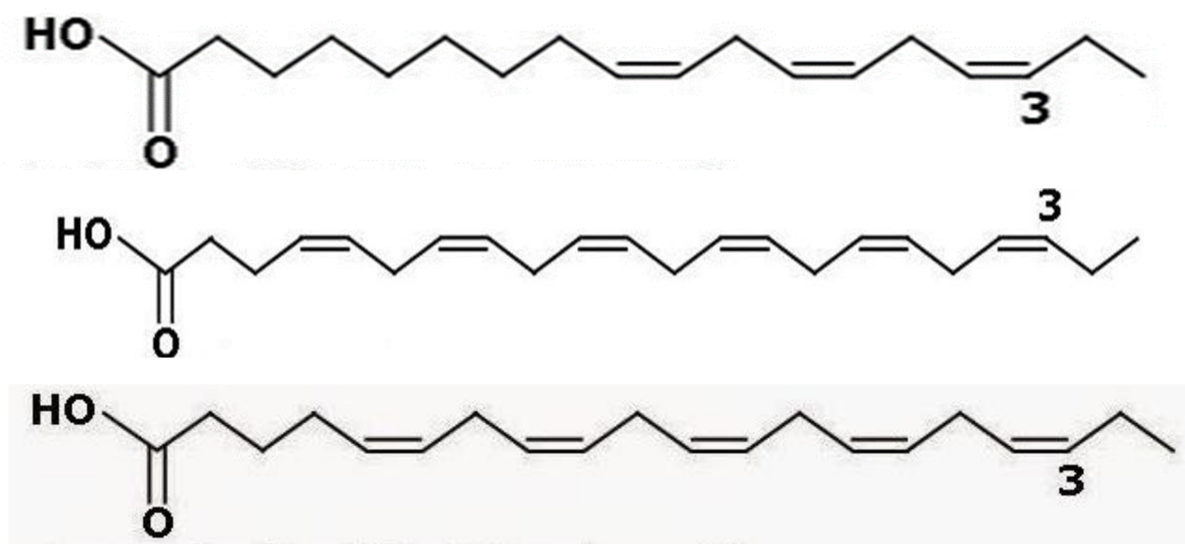

Figura 3. (**A**) Estrutura química do ácido α-linolênico (ALA). (**B**)Estrutura química do ácido docosahexaenóico (DHA). (**C**) Estrutura química do ácido eicosapentaenóico (EPA) (Elaborada pelo autor).

1.1.2. Ácidos Graxos Trans

Os ácidos graxos trans (AGT) são formados pela hidrogenação de óleos vegetais e encontrados principalmente em produtos industriais. Por definição, são

um tipo de ácidos graxos insaturados que contêm pelo menos uma dupla ligação não conjugada na configuração molecular trans (TEEGALA et al., 2009). Os AGT dietéticos basicamente são provindos de duas fontes diferentes, natural e industrial.

Os AGT naturais são produzidos a partir da transformação microbiana de ácidos graxos insaturados em animais ruminantes e estão presentes principalmente no leite, produtos lácteos e na própria carne dos animais ruminantes (MOZAFFARIAN, 2010). Os AGT industriais são formados pela hidrogenação parcial de óleos vegetais que converte esses óleos líquidos em gorduras semissólidas, visando aumentar sua estabilidade e versatilidade (BHARDWAJ et al., 2011), sendo o ácido elaídico (C18: 1 t9; Fig. 4) o principal isômero trans formado na produção de AGT industriais.

Atualmente, vários esforços têm sido feitos para reduzir e ou eliminar os ácidos AGT dos alimentos em vários países (MOZAFFARIAN, 2010; DOWNS et al., 2013). Esses esforços vêm sendo feitos, devido à crescente evidência de que os AGT afetam adversamente a saúde cardiovascular (MOZAFFARIAN et al., 2009) e aumentam os níveis séricos de colesterol (MENSINK e KATAN, 1990). Evidências experimentais também demonstraram que ingestões de AGT aumentam o risco de inflamação (MOZAFFARIAN et al., 2009), disfunção cognitiva (KUHN et al., 2013; PASE et al., 2017) e sintomas recorrentes de depressão em mulheres (AKBARALY et al., 2013). Além disso, os AGT podem ser facilmente incorporados nos fosfolipídios da membrana neural, alterando a fluidez da membrana, propriedades bioquímicas e assim afetando a função celular (LARQUE et al., 2003).

Figura 4. Estrutura química do ácido elaídico (Elaborada pelo autor).

1.2. Ácidos Graxos e Sistema Nervoso Central

Desde a descoberta dos AGPI n-3 em 1929 por George Burr e Mildred Burr (SPECTOR E KIM, 2015), vários estudos foram realizados avaliando o papel dos AGPI n-3 no risco cardiovascular, depressão, ansiedade, declínio cognitivo e doenças neurodegenerativas (BAZINET E LAYÉ, 2014; COULOMBE et al., 2017). A relevância dos lipídeos na função cerebral é comprovada, pelo fato de o SNC ter a

maior concentração de lipídios no organismo após o tecido adiposo (50 a 60% do peso seco do cérebro) (SASTRY, 1985). No cérebro humano, boa parte da composição lipídica é composta por AGPI. Assim, os AGPI são indispensáveis para o desenvolvimento normal e funcionamento do SNC (BAZINET E LAYÉ, 2014).

Uma hipótese que explica essa abundância de AGPI no tecido cerebral é que o *Homo sapiens* no período paleolítico, se estabeleceu em torno dos lagos e mares, onde o acesso a alimentos ricos em AGPI n-3 era abundante (BRADBURY, 2011). Nesse contexto, se considera que os seres humanos evoluíram com uma dieta com proporções equivalentes de AGPI n-3 e n-6 (LARRIEU E LAYÉ, 2018). Porém, durante a revolução industrial com as altas inovações tecnológicas, a indústria alimentícia acabou por afetar a proporção dos AGPI dietéticos, acarretando na diminuição na oferta do AGPI n-3 dietético.

Essa redução do suprimento dietético de AGPI n-3 para o cérebro está associada a muitas doenças cerebrais, incluindo depressão e transtornos de ansiedade (MÜLLER et al., 2015). O estudo epidemiológico realizado por HIBBELN (1998), associou a baixa ingesta de AGPI n-3, com a prevalência de depressão na população em geral. Estudos clínicos revelaram ainda que os pacientes diagnosticados com depressão ou ansiedade apresentam níveis baixos de AGPI n-3, e uma alta proporção de AGPI n-6 para AGPI n-3, no sangue e no cérebro respectivamente (MCNAMARA E LIU, 2011; PARLETTA et al., 2016). Apoiando observações clínicas, estudos pré-clínicos realizados em roedores mostraram que o consumo deficiente de AGPI n-3 dietético induz sintomas tipo depressivos e de ansiedade, bem como comportamento social anormal. (LAFOURCADE et al., 2011; LARRIEU et al., 2012; BONDI et al., 2014).

Assim, é importante ressaltar que o uso de modelos animais envolvendo alterações dietéticas é fundamental para estudar os mecanismos neurobiológicos e comportamentais os quais envolvem ácidos graxos e funções cerebrais.

1.3. Contextualização do comportamento doentio

Quando ocorre a ativação do sistema imunológico, independente da origem da mesma, seja ocasional ou intencional acaba acarretando mudanças profundas no comportamento. Por exemplo, a administração periférica de lipopolisscarídeo (LPS), o qual é um componente da parede celular de bactérias gram-negativas, diminui a exploração social, atividade locomotora, ingestão de alimentos, comportamento

motivado por alimentação e aumenta as horas de sono em roedores (DANTZER et al., 1999; LARSON e DUNN, 2001). Quando se faz a administração periférica de LPS o mesmo acaba por aumentar os níveis de citocinas pró-inflamatórias, as quais geram uma cascata de sinalização que desencadeia o comportamento doentio. Como parte da via endócrina, as citocinas pró-inflamatórias circulantes atingem o cérebro, a nível do plexo coroide e órgãos circunventriculares, assim induzem a expressão de citocinas no interior do cérebro (DANTZER et al., 2000). A ativação do sistema imune tantos a níveis centrais como periféricos ocorre basicamente por três vias.

Em primeiro lugar, a ativação imunológica acaba induzindo a imunorreatividade do tipo C-FOS, um indicador de ativação neuronal dentro do cérebro o qual pode fornecer percepções sobre as redes neurais subjacentes aos sintomas da doença (GAYKEMA e GOEHLER, 2011). Em segundo lugar, a ativação imune leva a um aumento dos níveis circulantes de corticosterona indicando uma estimulação do eixo hipotálamo-hipófise-adrenal (HPA) (LENCZOWSKI et al., 1997).

Ainda ocorre uma terceira ativação, o catabólito do triptofano (TRP), a quinurenina o qual é gerada pela indoleamina-2,3-dioxigenase (IDO) após ativação pelas citocinas, tem emergido como principal mediador na indução de comportamento tipo anedônico e de ansiedade (HAROON et al, 2012).

1.4. Mecanismo de indução do comportamento doentio pelo LPS

A administração periférica de um indutor de citocinas como o LPS, ou de citocinas recombinantes como por exemplo a interleucina 1-beta (IL-1β) ou fator de necrose tumoral alfa (TNF-α), acabam mimetizando todos os sintomas não específicos de doença, incluindo febre, ativação do eixo HPA, redução do consumo de alimentos e outras atividades comportamentais e a redução das condições físicas e sociais do meio (DANTZER et al., 2008).

Basicamente o LPS tem seu mecanismo de ação mediado por citocinas pró-inflamatórias, quando usado um antagonista dessas citocinas o LPS tem seu efeito nulo, então acredita-se que as citocinas são mediadoras fundamentais na resposta clínica na instalação da patologia. Consequentemente, as alterações neurocomportamentais são todas mediadas via SNC. As alterações fisiológicas e comportamentais características da doença são mediadas no SNC. A febre, por exemplo, representa um aumento regulado da temperatura corporal resultante da

produção de calor (termogênese) e diminuição da perda térmica (termólise) em resposta a um ponto de ajuste elevado para a regulação da temperatura corporal (DANTZER et al., 2008).

Chega um ponto que temperatura corporal é controlada por neurônios sensíveis no hipotálamo, citocinas pirogênicas, tais como IL-1β e interleucina 6 (IL-6), atuam no SNC para induzir a febre (ROMANOVSKY et al, 2005). Da mesma forma, a IL-1 β atua no núcleo paraventricular do hipotálamo onde estão localizados os neurônios que contêm hormônio liberador de corticotropina (ERICSSON et al.,1994). Assim, ocorre à liberação de corticotropina pela pituitária, o que por sua vez aumenta a liberação e secreção de glicocorticóides pelo córtex adrenal (DANTZER et al., 2008). Os mecanismos pelo qual essa citocinas agem no SNC não estão bem elucidados, acredita-se que o sistema imune tenha uma ligação com SNC através de receptores saturáveis específicos ou ainda através da ação de prostaglandinas.

1.5. Mecanismo de ação das citocinas na transmissão neural

Seguindo a hipótese que as citocinas atuam diretamente no SNC, a mesma se sustenta porque elas causam um processo inflamatório. Assim, requer um processamento sensorial, ou seja, vai ocorrer a liberação de uma sinalização a qual atinge o SNC. Quando se administra o LPS ou citocinas na cavidade abdominal, elas induzem inflamação do peritônio (DANTZER, 2008). Uma das principais vias de comunicação visceral com o SNC é nos ramos aferentes do nervo vago (DANTZER, 2008). Estes ramos contêm, na sua bainha perineural macrófagos e células dendríticas que expressam receptores do tipo Toll (RTT) de membrana e produzem IL-1β em resposta a uma injeção intraperitoneal de LPS (GOEHLER et al., 1999). Os neurônios sensoriais do nervo vago expressam receptores de IL-1, e a IL-1β circulante estimula a atividade sensorial vagal (EK et al., 1998).

A importância da via neural na transmissão da mensagem imunológica periférica, ao cérebro não é a mesma para todos os componentes do comportamento doentio (DANTZER, 2008). Por exemplo, ratos vagotomizados não desenvolveram alterações comportamentais, características da doença, mas eles ainda são capazes de ter os sintomas de uma febre (KONSMAN et al., 2000). Assim, se demonstra que as aferências vagais são menos importantes para a febre induzida por citocinas, do que a ativação do eixo HPA também induzido por citocinas para

que o ocorra o comportamento doentio (DANTZER, 2008). Assim a hipótese mais aceita atualmente é de que outras vias de comunicação funcionam em paralelo com a via neural.

1.6. Ativação da via da quinurenina mediada pela enzima IDO

O TRP é um aminoácido o qual é usado na síntese da serotonina (5-HT), além de ser metabolizado e degradado pela enzima IDO, em condições normais o seu produto é a quinurenina. A depleção aguda de TRP, junto com quantidades em excesso de grandes aminoácidos neutros, provindos de uma alimentação inadequada, os quais competem com o TRP para a entrada no SNC, resulta no desenvolvimento de humor deprimido em indivíduos com risco de depressão (DANTZER, 2008).

A principal responsável por esta diminuição do TRP plasmático em humanos com risco de depressão, é a enzima IDO, que degrada o TRP em quinurenina e ácido quinolínico (AQ) (Fig. 5). A IDO está presente em macrófagos, monócitos, células endoteliais e células gliais cerebrais em seres humanos e animais. É potencialmente ativada por citocinas pró-inflamatórias, como TNF-α e interferon gama (IFN-γ), tanto perifericamente quanto no SNC (LESTAGE et al., 2002). Sua ativação resulta em uma diminuição na biodisponibilidade do TRP para a síntese de 5-HT e na formação de compostos neuroativos, como a quinurenina, que atua como um antagonista dos receptores do glutamato, e o AQ, que atua como um agonista dos receptores do glutamato (MOREAU et al., 2008).

Experimentos realizados em camundongos submetidos à inflamação aguda em resposta ao LPS, ou à inflamação crônica com bacilo *Calmette-Guérin*, uma forma atenuada de *Mycobacterium bovis*, revelam o aparecimento gradual de comportamento tipo depressivo após o declínio da resposta à doença (FRENOIS et al., 2007). O surgimento de comportamento tipo depressivo está diretamente associado com a ativação de IDO (ANDRE et al., 2008). A interferência das citocinas pró-inflamatórias na neurotransmissão serotoninérgica não é considerada anedonia, fadiga ou atraso da psicomotricidade (CAPURON et al., 2004). Esses sintomas refletem uma diminuição da neurotransmissão dopaminérgica. Esta hipótese, é suportada por estudos de neuroimagem os quais demonstram alterações na atividade dos gânglios basais (JUENGLING et al., 2000).

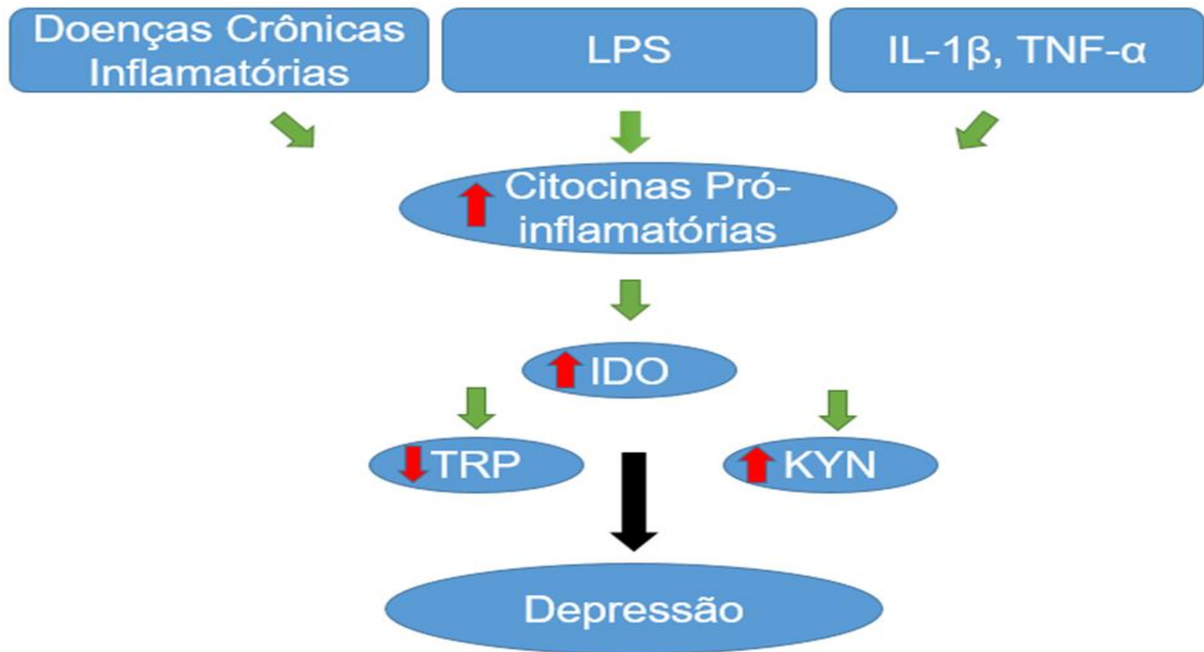

Figura 5. Ativação da enzima IDO pelo LPS, IL-1β, TNF-α e doenças crônicas inflamatórias. Com a ativação da IDO, se tem uma diminuição dos níveis de triptofano e aumento no seu metabólito a quinurenina, estando diretamente relacionada com o comportamento tipo depressivo no modelo de comportamento doentio (Adaptado de DANTZER, 2008).

1.7. Metabólitos neuroativos da via da quinurenina

Basicamente, os metabólitos da via da quinurenina apresentam propriedades neuroativas, que causam efeitos contrastantes nos neurônios, por isso são conhecidos como “braços” da via da quinurenina. Um dos braços da via da quinurenina, vêm da metabolização da quinurenina quando catalisada pela enzima quinurenina 3-monooxigenase (QMO), produz os metabólitos 3-Hidroxiquinurenina (3-HQ) e o AQ, ambos neurotóxicos (MADDISON E GIORGINI, 2015; Fig. 6). Já foi exposto, que o AQ ativa seletivamente os receptores de N-metil-D-aspartato (NMDA), assim foi identificado como uma neurotoxina em potencial, quando via intracerebroventricular em camundongos causando fortes convulsões (LAPIN, 1978). Em estudo subsequente, foi descoberto que uma injeção de AQ no estriado em cérebro de ratos era excitotóxica, causando lesões emparelhadas de tamanho dependente da dose, próximo ao local da injeção (SCHWARCZ et al., 1983). Um aumento nos níveis do AQ, passando dos níveis fisiológicos já foi demonstrado como suficiente para causar uma rápida neurodegeneração em uma cultura de

células corticoestriatais em ratos (WHETSELL E SCHWARCZ, 1989). Os mecanismos pelo qual o AQ exerce seu potente efeito neurotóxico são: Via estimulação da liberação neuronal do glutamato e inibição da recaptação astroglial deste neurotransmissor (TAVARES et al., 2002), concentrações elevadas de glutamato extracelular e ativação persistente de neurônios excitatórios causam excitotoxicidade devido ao influxo aumentado de Ca^{2+}, levando à disfunção mitocondrial, ativação de proteases e caspases, bem como ativação de óxido nítrico sintase (PEREZ et al., 2012); Peroxidação lipídica de maneira dependente nos receptores NMDA (SANTAMARIA E RIOS, 1993); Por conseguinte, o AQ forma um complexo com ferro causando a produção de espécies reativas de oxigênio (EROS) e após uma auto-oxidação (PLATENIK et al., 2001).

Ainda, nesse braço da via da quinurenina temos a síntese do 3-HQ, metabólito que gera a formação de radicais livres por interação com as moléculas (GILES et al., 2003). Estudos demonstraram, que o tratamento com 3-HQ em culturas celulares de neurônios do estriado e córtex, resultou na diminuição da viabilidade celular, esse efeito foi impedido pela ação da enzima antioxidante catalase, mas a enzima superóxido dismutase não teve essa mesma capacidade (OKUDA et al., 1998; SMITH et al., 2009).

O outro braço da via da quinurenina, é o metabólito ácido quinurênico (Fig. 8) o qual possui propriedades ativas com capacidades neuroprotetoras, sendo gerado pela ação da enzima quinurenina aminotransferase (QAT). O ácido quinurênico possui propriedades antioxidantes, devido à sua capacidade de eliminar radicais livres, como hidroxilas e ânions superóxido (LUGO-HUITRON et al., 2011). Em níveis fisiológicos, ele também atua como um antagonista não competitivo dos receptores da acetilcolina-7-nicotínica (α7nACh), reduzindo subsequentemente a sinalização de acetilcolina, dopamina e glutamato (HILMAS et al., 2001). Sabe-se que, em altas concentrações micromolares, ácido quinurênico é um antagonista não seletivo dos receptores NMDA (PERKINS E STONE, 1982). Através, desta modulação da sinalização do glutamato e de sua atividade e antioxidante, provavelmente seja o mecanismo pelo qual o ácido quinurênico é capaz de neutralizar a neurotoxicidade causada por AQ e 3-HQ (MADDISON E GIORGINI, 2015). Assim, durante condições fisiológicas normais é de fundamental importância a manutenção do equilíbrio dos braços da via da quinurenina, para assegurar que a razão destes metabólitos seja mantida a um nível que evite a toxicidade celular.

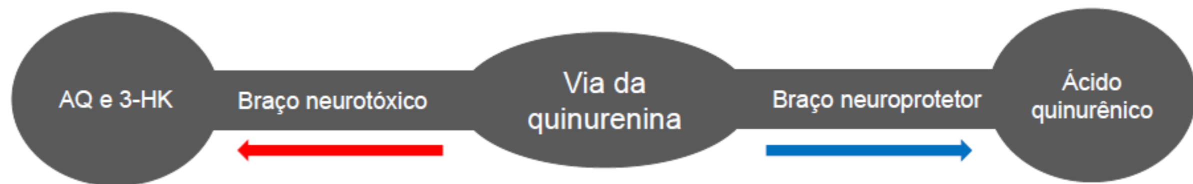

Figura 6. Esquematização dos metabólitos neuroativos da via da quinurenina (Elaborada pelo autor).

1.8. Via da quinurenina e depressão

Sabe-se que a ativação da via da quinurenina está relacionada com a fisiopatologia da depressão. Um aumento nas concentrações de quinurenina, AQ tem sido identificado no líquido cefalorraquidiano (RAISON et al., 2010), enquanto diminuições nas concentrações plasmáticas de ácido quinurênico também foram identificadas em pacientes com depressão (MYINT et al., 2007). Essas alterações, indicam que a ativação da via da quinurenina pode estar mediando subtipos de depressão (REUS et al., 2015). A primeira relação do envolvimento do processo inflamatório na patogênese da depressão foi proposta por SMITH (1991), que desenvolveu a teoria macrofágica da depressão, na qual ele enfatizou que era a secreção excessiva de citocinas pelos macrófagos que causam depressão. Posteriormente, vários estudos pré-clínicos e clínicos comprovaram essa relação do aumento dos níveis plasmáticos de citocinas pró-inflamatórias e depressão (RAISON et al., 2005; JESSE et al., 2010; SOUZA et al., 2017).

Pode ocorrer também a depressão secundária a qual ocorre em detrimento de uma infecção, inflamação crônica ou estresse oxidativo, a qual geralmente está relacionada à ativação da via da quinurenina pela enzima IDO (FUKUDA, 2014). Nesse contexto, existe a depressão reativa a qual está associada a exposição de fatores estressores relacionados com o estilo de vida, ativação e desregulação do eixo HPA via a indução pela enzima triptofano 2,3-dioxigenase (TDO), acarretando também a ativação da via da quinurenina.

Assim, esses vínculos entre os tipos de depressão, fatores estressores, ativação da via da quinurenina não foram muito explorados em estudos clínicos ainda. Nesse contexto, se destaca a importância de estudos pré-clínicos os quais

possam ajudar a elucidar e compreender os mecanismos da ativação da via quinurenina os quais estão envolvidos na fisiopatologia da depressão.

1.9. Envolvimento do processo fisiológico de envelhecimento e neuroinflamação

Sabe-se que em um cérebro adulto e saudável, existe um equilíbrio entre mediadores pró-inflamatórios e anti-inflamatórios, assim as células da micróglia permanecem inativas. Porém, diante de uma doença neurodegenerativa por exemplo as células da micróglia sofrem um processo de "ativação" (PERRY et al., 2013). Uma vez ocorrida a ativação da micróglia, gera um aumento na expressão gênica de marcadores fisiológicos que sugerem a ativação de receptores do complexo de histocompatibilidade de membrana classe II (CHM) e alguns receptores complementares (GODBOUT et al., 2005). Entretanto, é necessário a ativação das células microgliais para que se tenham níveis expressivos de citocinas inflamatórias, assim essas células são hiperresponsivas a estímulos secundários produzindo uma resposta exacerbada quando necessário. Essa resposta exacerbada da micróglia, diante de estímulos secundários do sistema imunológico é importante por várias razões.

Primeiramente, as citocinas inflamatórias no SNC são importantes mediadoras do comportamento doentio (KONSMAN et al., 2002). As citocinas inflamatórias, IL-1β, IL-6 e TNF-α, induzem anorexia, déficits na função cognitiva e motora podendo levar a depressão em pacientes cronicamente infectados (POLLMACHER et al., 2002). Em segundo lugar, as citocinas inflamatórias estão envolvidas em doenças neurodegenerativas, como a esclerose múltipla e a doença de Alzheimer (WILSON et al., 2002). Assim, pode-se inferir que indivíduos com ativação das células microgliais quando comparados a indivíduos os quais a micróglia está inativada, apresentam um risco aumentado de déficits neurocomportamentais graves e desencadeamento de uma neuropatologia.

Dessa maneira, um evento fisiológico que pode estimular as células da micróglia a uma resposta exacerbada é o envelhecimento normal. Essa afirmação baseia-se no fato de que a expressão do CHM classe II, um marcador da ativação da micróglia, está aumentado nos cérebros de humanos, primatas e roedores idosos, porém todos saudáveis (SHEFFIELD E BERMAN, 1998; GODBOUT et al., 2005; DANTZER et al., 2011). Assim, os indivíduos idosos por possuírem uma

ativação maior da micróglia devido ao processo fisiológico de senescência, diante de uma infecção sistêmica terão uma resposta inflamatória exagerada no cérebro (Fig. 7). Também, corrobora com a maior susceptibilidade da população idosa a infecções sistêmicas, com altos níveis de mortalidade para indivíduos com 65 anos ou mais (PINNER, 1996).

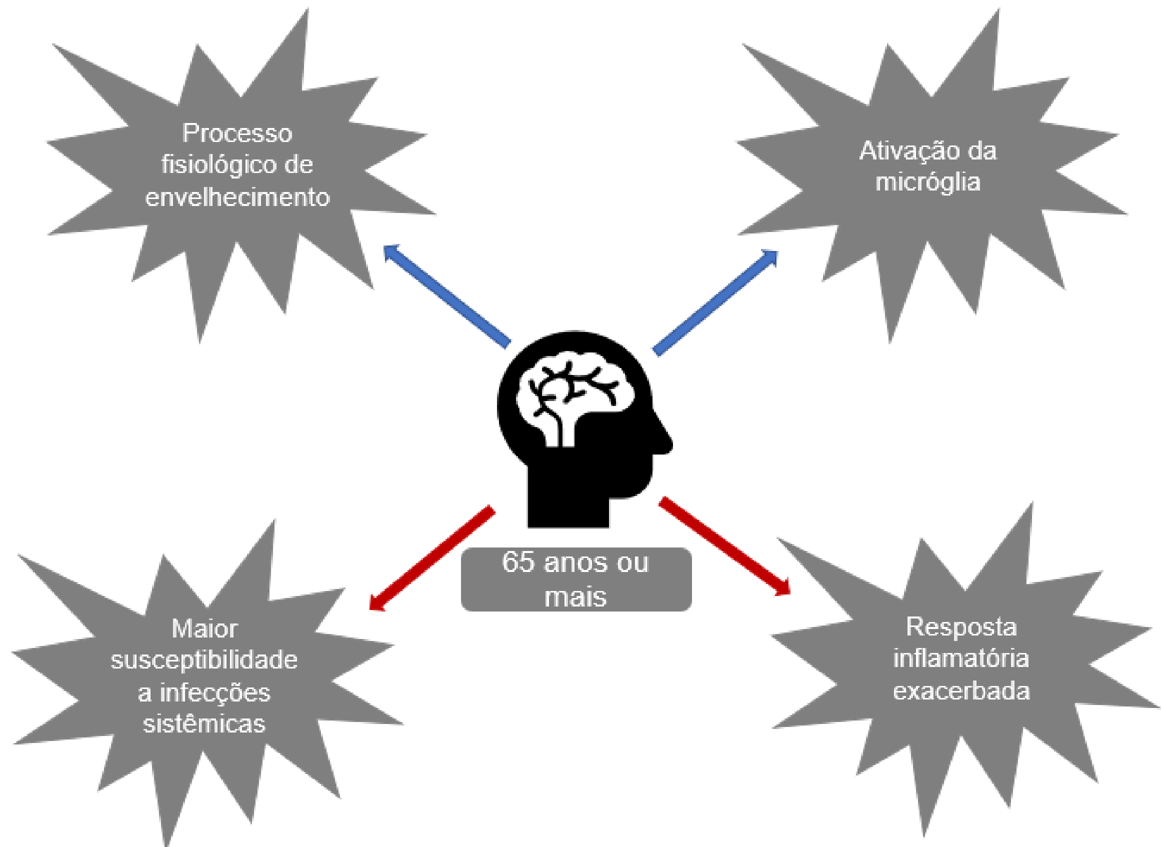

Figura 7. Processo fisiológico de envelhecimento e o aumento na ativação da micróglia, causando uma resposta inflamatória exacerbada (Elaborada pelo autor).

2. OBJETIVOS

2.1. Objetivo Geral

O objetivo do presente estudo foi avaliar o efeito da suplementação crônica com AGPI n-3 ou gordura vegetal hidrogenada (GVH) em um modelo de comportamento doentio induzido por LPS em camundongos idosos.

2.2. Objetivos Específicos

2.2.1. Objetivos específicos para suplementação crônica com AGPI n-3

- Analisar alterações neurocomportamentais;
- Avaliar as alterações neuroquímicas de diferentes estruturas cerebrais;
- Investigar o processo inflamatório em diferentes estruturas cerebrais;
- Avaliar o envolvimento do sistema serotoninérgico;
- Verificar as alterações decorrentes da ativação da via da quinurenina;
- Quantificar os AGPI n-3 em diferentes estruturas cerebrais.

2.2.2. Objetivos específicos para suplementação crônica com GVH

- Analisar alterações neurocomportamentais;
- Analisar alteração hormonal (corticosterona);
- Avaliar as alterações neuroquímicas de diferentes estruturas cerebrais;
- Investigar o processo inflamatório em diferentes estruturas cerebrais;
- Avaliar o envolvimento do sistema serotoninérgico;
- Verificar as alterações decorrentes da ativação da via da quinurenina.

3. ARTIGO CIENTÍFICOS

Os resultados inseridos nesta tese apresentam-se sob a forma de artigos científicos 1 e 2, os quais se encontram aqui estruturados. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se nos artigos, os quais estão dispostos na mesma maneira que foram publicados (1 e 2).

3.1. Artigo 1

O óleo de peixe melhora comportamento doentio induzido por lipopolissacarídeo em camundongos idosos através da modulação da via da quinurenina

Marcelo Gomes de Gomes, Leandro Cattelan Souza, André Rossito Goes, Lucian Del Fabbro, Carlos Borges Filho, Franciele Donato, Marina Prigol, Cristiane Luchese, Silvane Souza Roman, Robson Luiz Puntel, Silvana Peterini Boeira, Cristiano Ricardo Jesse

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Fish oil ameliorates sickness behavior induced by lipopolysaccharide in aged mice through the modulation of kynurenine pathway

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Abstract

Sickness behavior is an expression of a central motivational state triggered by activation of the immune system, being considered a strategy of the organism to fight infection. Sickness behavior is induced by peripheral administration of lipopolysaccharide (LPS). LPS can increase the levels of proinflammatory cytokines, which induce the activation of the kynurenine pathway (KP) and behavioral alterations. Previous studies have shown that omega-3 (n-3) polyunsaturated fatty acid (PUFA) has anti-inflammatory properties. Because of this, the purpose of the present study was to evaluate the protective effect of fish oil (FO) supplementation against LPS-induced sickness behavior in aged mice with respect to anhedonia, locomotor activity and body weight. Moreover, we evaluated the ability of FO treatment on the regulation of neuroinflammation (levels of interleukin-1β, interleukin-6, tumor factor necrosis-α and interferon-γ), KP biomarkers (levels of tryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine and quinolinic acid and activities of indoleamine-2,3-dioxygenase, kynurenine monooxygenase and kynurenine aminotransferase) and serotonergic system (levels of serotonin and 5-hydroxyindoleactic acid) in the hippocampus, striatum and prefrontal cortex of LPS-treated mice. We found that FO prevented the LPS-mediated body weight loss, anhedonic behavior, reduction of locomotor activity, up-regulation of the proinflammatory cytokines and serotoninergic alterations. We also found that FO was effective in modulating the KP biomarkers, inhibiting or attenuating KP dysregulation induced by LPS. Together, our results indicated that FO may have beneficial effects on LPS induced sickness-behavior in aged mice either by modulating central inflammation, KP and serotonergic signaling (indirectly effect) or by fatty acids incorporation into neuronal membranes (direct effect).
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Keywords: Neuroinflammation; Neurotoxic; Nutraceutical; Cytokines; Omega-3

1. Introduction

Sickness behavior is an expression of a motivational state triggered by activation of the peripheral innate immune system whereby an organism reorganizes its priorities to fight infection [1–3]. Sickness behavior is easily induced in laboratory animals by the administration of endotoxins, which are the main active components of the outer membrane of gram-negative bacteria [4]. Activation of the immune system by microbial invasion results in the production of proinflammatory cytokines leading to sickness behavior, an adaptive behavioral response whose prototypical symptoms comprise a reduction of locomotor activity, anorexia and anhedonia [2,5,6].

Experimentally, the increase of proinflammatory cytokines expression is commonly achieved through peripheral administration of lipopolysaccharide (LPS), an isolated portion of gram-negative bacteria [7]. The peripheral and central effects of LPS on immune activation can be assessed by several biological parameters. First, immune activation induces c-Fos-like immunoreactivity, an indicator of neuronal activation within the brain, which precipitates sickness symptoms [8,9]. Second, the inflammation-induced overstimulation

Abbreviations: 3-HK, 3-hydroxykynurenine; 5-HIAA, 5-hydroxyindoleactic acid; 5-HT, serotonin; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; IDO, indoleamine-2,3-dioxygenase; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KP, kynurenine pathway; KYN, kynurenine; KYNA, kynurenic acid; LPS, lipopolysaccharide; n-3, omega-3; PUFA, polyunsaturated fatty acid; QA, quinolinic acid; TNF-α, tumor necrosis factor-α; TRP, tryptophan

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of the tryptophan (TRP)-degrading route, kynurenine pathway (KP), has emerged as a key mediator for the induction of anhedonic behavior [10–12]. Effects of proinflammatory cytokines have been linked to sickness symptoms *via* induction of indoleamine-2,3-dioxygenase (IDO) activation [13], the first rate-limiting enzyme of KP. Since exaggerated immune function in sickness behavior has been associated with several neuropsychiatric disorders that are linked to altered glutamate and serotonin (5-HT) neurotransmission [14,15], the ability to use nutritional supplementation to modify neural systems in a low-cost and physiologically safe manner is an attractive proposition for the development of new treatments.

Previous clinical and epidemiological studies suggest that n-3 polyunsaturated fatty acids (PUFAs), composed of eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3), are effective in the treatment of sickness disturbances [16,17]. It has been proposed that PUFA deficiency may affect neurotransmission, especially within the glutamatergic and serotonergic systems, as a consequence of altered membrane fluidity and related receptor functions [18]. Furthermore, PUFAs are important for the regulation of synaptic plasticity [19] and anti-inflammatory effects, particularly by inhibiting the tumor necrosis factor-α (TNF), interleukin-6 (IL-6) and interleukin-1β (IL-1β) [20,21]. Thus, it has been suggested that n-3 PUFAs represent a potential novel therapeutic agent for severely injured patients who commonly present inflammation and severe weight loss. The majority of studies in this area have focused on the preventive effects of fish oil (FO) through dietary supplementation [21,22].

Here, we tested the hypothesis that supplementation with FO is able to prevent LPS-induced sickness behavior in aged mice. We evaluated the FO administration on LPS-induced sickness state in aged mice with respect to anhedonia, locomotor activity and body weight. Moreover, we evaluated the ability of FO treatment on the regulation of neuroinflammation (levels of IL-1β, IL-6, TNF-α and interferon-γ), KP biomarkers [levels of TRP, kynurenine (KYN), kynurenic acid, 3-hydroxykynurenine and quinolinic acid and activities of indoleamine-2,3-dioxygenase, kynurenine monooxygenase and kynurenine aminotransferase] and serotonergic system (levels of serotonin and 5-hydroxyindoleactic acid) in the hippocampus, striatum and prefrontal cortex of LPS-treated mice.

2. Methods

2.1. Animals

Experiments were performed using male C57BJ/6 mice (30–40 g, 2 years old). Animals were maintained at 22°C–25°C with free access to water and food, under a 12-h:12-h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out during light phase on the day. All efforts were made to minimize animal suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for Animal Use (CEUA protocol #010/2016) of Federal University of Pampa, Brazil.

2.2. Mouse treatments

Food was placed in dry-diet feeder jars to avoid significant food loss that often accompanies overhead pellet feeding, and the jars were weighed daily to determine the amount of food consumed. General animal health was also monitored during the test periods. Diet consisted of defined mouse diet AIN-93M (Puro Trato, RS). The animals were divided into four groups: control group (corn oil, equivoluminal to fish oil+PBS), LPS group (LPS+corn oil), FO group (FO+PBS) and FO/LPS group (FO+LPS). Animals treated with FO (Tratto Saúde, Brazil; EPA 43%, DHA 20%, other n-3 2%) were daily intragastrically administrated (per os) with 50 μl for 180 days prior LPS injection (Fig. 1). The administered dose of FO was equivalent to a human daily dose of 486 mg/kg/day when adjusted according to the FDA converting factor [23]. The supplementation protocol was performed as earlier described [23], and we normalized the doses used in humans to be used in the mice. This diet was isocaloric, isonitrogenous, 60.1% carbohydrate, 18.2% protein, 10.2% fat and approximately 90% dry matter. LPS treatment involved an intraperitoneal (i.p.) injection of freshly made solutions of 0.15 mg/kg LPS (L-020M4062, serotype 0127:B8; Sigma, St. Louis, MO, USA) prepared in sterile pyrogen-free PBS, and control animals received the equivalent volume of PBS. This dose of LPS was chosen to induce sickness behaviors and cytokine activation at the time point of measurement while still providing room for the potential of elevated responses. This range of LPS doses was selected based upon previous studies demonstrating that 0.33 mg/kg LPS produced prolonged sickness behavior in aged compared to young mice [24] and 0.02 mg/kg being the lowest dose capable of inducing statistically significant changes in sickness behaviors when compared to saline-treated mice.

2.3. Measurement of sickness behavior: body weight loss and locomotor activity

Sickness behavior was assessed by changes in body weight loss and locomotor activity (LMA) at 24 h after LPS injection. Decreased LMA in a novel environment is a sensitive measure of sickness behavior [25]. For this test, mice were individually placed into a clean, novel cage [30×19×12 cm (L×W×H cm)] devoid of bedding or litter, and LMA was video-recorded for a 5-min period. Videos were analyzed by dividing the cage into four virtual quadrants and counting the number of quadrant entrances over the 5-min period; counting was done by a trained observer who was blind to experimental treatments. Changes in locomotor activity and depression-like behavior were assessed 24 h after drug administration. We selected behavioral end points that we had already demonstrated to be very sensitive to systemic inflammation-induced sickness [26].

2.4. Sucrose preference test (SPT)

At 24 h after treatment, anhedonia was measured by preference for a sucrose solution over water using a two-bottle free-choice method [27]. Each animal was presented simultaneously two bottles, one containing 1% sucrose solution (w/v) and the other containing tap water. Blunted sucrose intake, in this test, is proposed to reflect impaired sensitivity to reward and model anhedonia, a core symptom of major depression [28]. Tap water and 1% sucrose solution were placed in premeasured bottles in the cages, and fluid intake was monitored for 24 h [2]. Before the start of the protocol, the mice were exposed only to a 1% sucrose solution for 24 h aiming for the adaptation to sucrose solution. Mice were deprived of food and water for 20 h before the start of the test. Sucrose preference was evaluated *via* the sucrose uptake rate, namely, the ratio of volume of sucrose consumption to the volume of sucrose consumption plus tap water consumption [sucrose preference=sucrose consumption/(sucrose consumption+water consumption)×100%].

2.5. Tissue preparation for neurochemical determinations

After behavioral tests, mice were euthanized with barbiturate overdose (pentobarbital sodium 150 mg/kg; i.p. route) and transcardiacally perfused with 10 ml ice-cold saline *via* the aorta. The brain dissection was performed according to the method of Spijker [29], a method to dissect multiple brain regions from a single brain based on existing atlases [30]. Hippocampus, striatum and prefrontal cortex

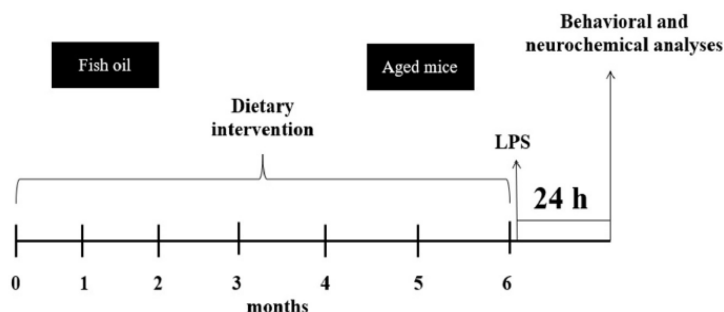

Fig. 1. Overview of the study design demonstrating the experimental procedures. The animals were supplemented with FO for 6 months. The sickness behavior was induced by intraperitoneal injection of LPS. After 24 h, the behavioral and neurochemical analyzes were performed.

were bilaterally removed and rapidly homogenized in 50 mM Tris–Cl, pH 7.4. The homogenate was centrifuged at 2400×g for 15 min at 4°C, and a low-speed supernatant fraction (S1) was used for assays.

2.6. Neurochemical assays

2.6.1. Cytokine levels

Levels of IL-1β, IL-6, TNF-α and IFN-γ in the hippocampus, striatum and prefrontal cortex were measured using sample aliquots of 100 μl and mouse cytokine ELISA DuoSet Kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions (protein range of 31.25–2000 pg). The level of cytokine was estimated by interpolation from a standard curve by colorimetric measurements at 450 nm (correction wavelength 540 nm) on an ELISA plate reader (Berthold Technologies-Apollo 8-LB 912, KG, Germany). Results are shown as pg/mg of protein.

2.6.2. TRP and KYN levels

The levels of TRP and its metabolite KYN in the hippocampus, striatum and prefrontal cortex were performed in a Shimadzu LC-10A liquid chromatograph according to Silva et al. [31]. The chromatographic separation was achieved using a 250×4.6-mm (inner diameter) C_{18} reverse-phase column (particle size, 4 μm; Aquapore RP-300 C-18). For TRP measurement, the column was eluted isocratically at flow rate of 1.0 ml/min with 0.015 M sodium acetate (pH 4.5) containing 15% methanol. For KYN determination, the column was eluted with acetonitrile at a 1:47 dilution in 0.1 M acetic acid–0.1 M ammonium acetate (pH 4.65). The absorbance of the column effluent was monitored at 280 and 365 nm for TRP and KYN, respectively. The peaks of TRP or KYN were identified by comparison with the retention times of standard compounds (Sigma), and quantification was based on the ratios of the peak areas of compound to the internal standard. The tissue levels were expressed in pg/mg tissue. KYN/TRP ratio was calculated by ratio between KYN and TRP concentrations.

2.6.3. Analysis of KYNA, 3-HK and QA levels

KYNA, 3-HK and QA levels were measured in the hippocampus, striatum and prefrontal cortex samples using high-pressure liquid chromatography (HPLC). The mobile phase contained 50 nM glacial acetic acid, 100 mM zinc acetate and 3% acetonitrile dissolved in double-distilled NANO pure water HPLC-grade H_2O. The pH was adjusted to 4.9 using 5 M NaOH. S1 of PFC and HP was sonicated in 1 ml of mobile phase containing 7% perchloric acid spiked with 50 ng/20 μl of N-methyl-5-HT as an internal standard. The resultant solution was centrifuged at 20,000 rpm for 20 min, and the supernatants were placed into new Eppendorf tubes using a syringe fitted with a 0.45-μm filter (Phenomenex). Approximately 20 μl of the filtered supernatant was injected using a Waters auto sampler, and a Reverse Phase analytical column (Kinetex Core Shell Technology column with specific area of 4.6 mm and particle size of 2.6 μl, Phenomenex) was used for the separation of metabolites. A PDA-UV detector (Shimadzu SPD-M10A VP), calibrated to integrate at 230 and 250 nm, as well as a fluorescent detector (Shimadzu RF- 20A XS prominence fluorescence detector), set to excitation wavelength 254 nm and emission wavelength 404 nm, was used to detect the metabolites. Chromatographs were generated by CLASS-VP software (Shimadzu). The results are expressed as ng/g tissue.

2.6.4. 5-HT and 5-HIAA levels and 5-HIAA/5-HT ratio

The levels of 5-HT and its metabolite 5-HIAA in the hippocampus, striatum and prefrontal cortex were analyzed by HPLC with electrochemical detection, as previously described [32]. The mobile phase, used at a flow rate of 0.8 ml/min, consisted of 0.02 M phosphate/citrate buffer and 90/10 methanol (v/v), 0.12 mM Na2 EDTA and 0.0556% heptane sulfonic acid as ion pair. The pH was adjusted to 2.64 with H_3PO_4 at 22°C. A 5-μm (220×4.6) Spheri-5 RP-18 column from Brownlee Laboratory was used. Electrochemical detection was performed with a Shimadzu L-ECD-6A electrochemical detector with a potential of 0.75 V. The peak area of the internal standard (DHBA) was used to quantify the sample peaks. The tissue levels were expressed in ng/g tissue. 5-HIAA/5-HT ratio was calculated by ratio between 5-HIAA and 5-HT concentrations.

2.6.5. Determination of IDO, KMO and KAT activities

IDO activity in the hippocampus, striatum and prefrontal cortex was determined as previously described [33], with minor modifications: the amount of the enzyme was expressed in terms of its heme content based on the absorbance at 406 nm, and in our method, the protein concentration was measured by the method of Bradford [34] using bovine serum albumin as the standard; the temperature was 25°C, and in our study, it was 37°C. The S1 (0.2 ml) was added to 0.8 ml of the reaction mixture containing 400 μM L-TRP, 20 mM ascorbate, 10 μM methylene blue and 100 μg catalase in 50 mM potassium phosphate buffer, pH 6.5. The reaction was carried out at 37°C under agitation for 60 min. Then, it was blocked by adding 0.2 ml of 30% trichloroacetic acid and further incubated at 50°C for 30 min to convert the N-formylkynurenine to L-KYN. Samples were centrifuged at 13,000×g for 10 min at 4°C. The supernatants were filtered through microspin ultrafiltrates with a cutoff of 10,000 M_r before being taken for measurement of IDO. The amount of L-KYN formed from TRP was determined by reversed-phase HPLC. One hundred microliters of the reaction product was injected onto a Merck LiChrospher column (150 mm long, 4.6 mm diameter, packed with 5 lm silica beads holding 18C long-carbon chains). A cartridge guard column containing the same material as the analytical column was used. The mobile phase consisted of 0.1 M ammonium acetate buffer (pH 4.65) with 5% acetonitrile. Flow rate was 1 ml/min. KYN was detected using a

spectrometer measuring absorbency at a wavelength of 365 nm and was quantified using known amounts of L-kynurenine. The retention time of KYN was around 5.35 min. All determinations were performed in duplicate. One unit of the activity was defined as 1 nmol KYN/h/mg protein at 37°C.

For the determination of KMO activity in the hippocampus, striatum and prefrontal cortex tissues were homogenized 1:5 (wt/vol) in ultrapure water and further diluted 1:5 (vol/vol) in 100 mM Tris–HCl buffer (pH 8.1) containing 10 mM KCl and 1 mM EDTA. Eighty microliters of the tissue preparation was incubated for 40 min at 37°C in a solution containing 1 mM NADPH, 3 mM glucose-6-phosphate, 1 U/ml glucose-6 phosphate dehydrogenase, 100 μM KYN, 10 mM KCl and 1 mM EDTA in a total volume of 200 μl. The reaction was stopped by the addition of 50 μl of 6% perchloric acid. Blanks were obtained by adding the specific enzyme inhibitor Ro 61-8048 (100 μM) in the incubation solution. After centrifugation (16,000×g, 15 min), 20 μl of the supernatant was applied to HPLC to measure 3-HK.

The KAT activity was made using the method previously described by Guidetti et al. [35]. Briefly, the hippocampus, striatum and prefrontal cortex were harvested and homogenized in distilled water. After centrifugation (12,000×g, 10 min), KAT activity was measured in a total volume of 200 μl containing 80 μl of supernatant fluid, 150 mm Tris-acetate buffer, pH 7.4, 2 lm KYN, 1 mm pyruvate and 80 lm pyridoxal-5-phosphate. Samples were incubated for 24 h at 37°C, and the reaction was terminated by adding 50% (w/v) trichloroacetic acid. After successive washes with 0.1 m HCl and distilled water, KYNA was eluted from the column with 2×1 ml of distilled water and quantified by HPLC.

2.7. Protein determination

Protein concentration was measured by the method of Bradford [34] using bovine serum albumin as the standard.

2.8. Fatty acid compositions of brain areas

Hipocampus, striatuam and prefrontal cortex tissues (100 mg) were mixed with 5 ml of chloroform:methanol:distilled water at a ratio of 3:3:1 (vol:vol:vol). Tissue phospholipids were separated using thin-layer chromatography (silica gel G, Analtech, Newark, DE, USA) and reextracted in hexane:ether:acetic acid, 40:10:1 (vol:vol:vol). Fatty acid methyl esters were analyzed using gas chromatography (Shimadzu 2010AF; Shimadzu Scientific Instrument, Tokyo, Japan) with a 100-m SP2555 capillary column (Supelco, Bellefonte, PA, USA). Fatty acids were identified by comparison with standards (Elysian, MN, USA) [36].

2.9. Statistical analysis

The data distribution was verified by applying the Kolmogorov–Smirnov test. Results are presented as means±standard error of the mean (S.E.M.). Comparisons between the experimental and the control groups were performed by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test or two-way ANOVA followed by Bonferroni *post hoc* test when appropriate. A value of $P<.05$ was considered to be statistically significant. All tests were carried out using the GraphPad software 5.0 (San Diego, CA, USA).

3. Results

3.1. The behavioral alterations in SPT

Statistical analysis revealed no significant differences in sucrose preference test basal (FO×LPS, $F_{1,24}=0.07$, $P<.78$; Fig. 2A). Two-way ANOVA showed a significant effect for FO×LPS interaction ($F_{1,24}=8.61$, $P<.01$; Fig. 2B) in SPT. Results of *post hoc* indicated that LPS administration displayed a significant decrease in the sucrose intake

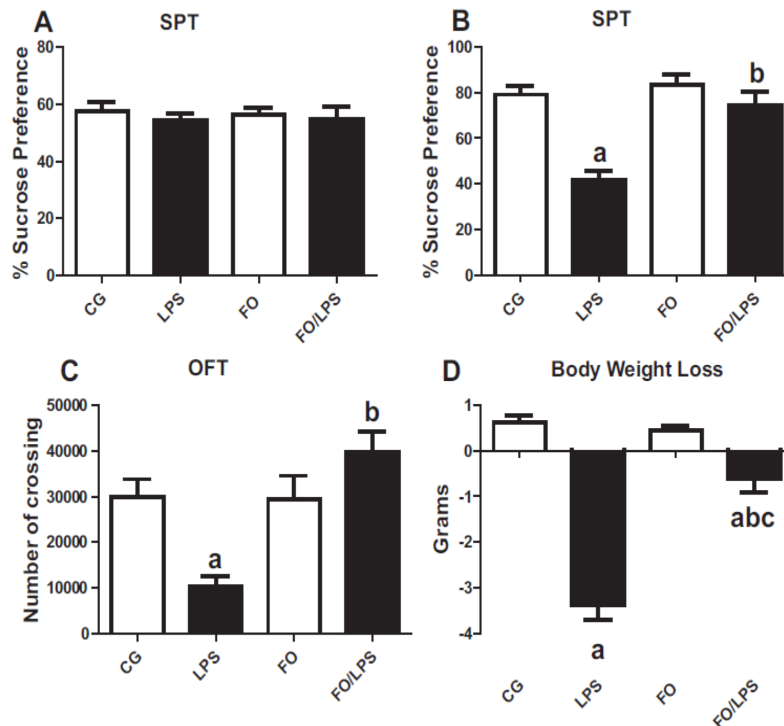

Fig. 2. Effect of FO administration in the SPT, OFT and body weight in mice. Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni-multiple comparison test). (a) $P<.05$ when compared to the control group (CG). (b) $P<.05$ when comparing the FO/LPS with the LPS. (c) $P<.05$ when comparing the FO and FO/LPS.

Table 1
Effect of administration of FO on interleukin levels in brain structures of mice in a sickness behavior model

Groups	IL-1β (pg/ mg)			IL-6 (pg/ mg)		
	Hippocampus	Striatum	Prefrontal cortex	Hippocampus	Striatum	Prefrontal cortex
CG	78.75±5.63	79.50±6.22	85.50±8.70	65.50±7.32	24.00±4.34	43.75±2.98
LPS	735.3±98.73[a]	687.0±105.0[a]	683.3±50.25[a]	220.8±24.88[a]	219.5±29.07[a]	267.3±28.06[a]
FO	43.00±7.55	36.25±4,19	60.25±10.96	31.50±7.05	24.75±3.63	30.00±5.21
FO/LPS	280.8±60.14[ab] c	224.5±36.66[b]	268.8±62.44[b]	157.5±11.29[a]	183.3±18.00[a]	214.8±39.57[a]

Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test).
[a] $P<.05$ when compared to the control group (CG).
[b] $P<.05$ when comparing the FO/LPS with the LPS.
[c] $P<.05$ when comparing the FO and FO/LPS.

compared to control animals. Treatment with FO prevented the decrease in the sucrose intake compared to LPS group, supporting the antianhedonic-like effect of FO (Fig. 2B).

3.2. Open field test

Two-way ANOVA of number of crossing in OFT demonstrated a significant effect of FO×LPS interaction ($F_{1,24}$=11.19, $P<.001$; Fig. 2C). Results showed that LPS injection displayed a significant decrease in the number of crossing in OFT when compared to control group. *Post hoc* comparisons revealed that the FO treatment significantly blocked the decrease in the number of crossing in OFT induced by LPS in mice.

3.3. Body weight loss

Two-way ANOVA of body weight loss demonstrated a significant effect of FO×LPS interaction ($F_{1,24}$=42.23, $P<.001$; Fig. 2D). *Post hoc* comparisons revealed that the FO treatment significantly blocked the body weight loss, a feature of the sickness model, induced by LPS when compared to control group.

3.4. Neuroinflammatory markers

Statistical analysis of IL-1β levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=13.55, $P<.001$; $F_{1,24}$=25.68, $P<.001$; and $F_{1,24}$=32.22, $P<.001$, respectively) (Table 1). *Post hoc* comparisons revealed that FO treatment significantly abrogated the increase of IL-1β levels in the hippocampus, striatum and prefrontal cortex induced by LPS.

Two-way ANOVA of IL-6 levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=8.68, $P<.001$; $F_{1,24}$=48.35, $P<.001$; and $F_{1,24}$=22.58, $P<.001$, respectively) (Table 1). *Post hoc* comparisons revealed that FO treatment attenuated the increase of IL-6 levels caused by LPS in the brain areas (hippocampus, striatum and prefrontal cortex).

Statistical analysis of TNF-α levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=12.87, $P<.001$; $F_{1,24}$=9.41, $P<.001$; and $F_{1,24}$=20.24, $P<.001$, respectively) (Table 2). *Post hoc* comparisons revealed that LPS significantly increased TNF-α levels in the hippocampus, striatum and prefrontal cortex of mice compared to control group. FO treatment partially protected against the increase of TNF-α levels induced by LPS in the brain areas in mice (Table 2).

Two-way ANOVA of IFN-γ levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=10.01, $P<.001$; $F_{1,24}$=8.66, $P<.001$; and $F_{1,24}$=11.37, $P<.001$, respectively). *Post hoc* comparisons revealed that LPS significantly increased IFN-γ levels in the hippocampus, striatum and prefrontal cortex of mice compared to control group. FO treatment protected against the increase of IFN-γ levels induced by LPS in the brain areas in mice but not at control levels (Table 2).

3.5. Serotonergic 5-HT and 5-HIAA levels

Two-way ANOVA of 5-HT levels in hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=12.77, $P<.001$, Fig. 3A; $F_{1,24}$=19.56, $P<.001$, Fig. 3B; and $F_{1,24}$=21.66, $P<.001$, Fig. 3C, respectively). *Post hoc* comparisons revealed that LPS significantly increased 5-HT levels in hippocampus, striatum and prefrontal cortex of mice compared to sham group. FO treatment significantly attenuated the decrease of 5-HT levels in brain areas induced by LPS.

Two-way ANOVA of 5-HIAA levels in hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=9.77, $P<.001$, Fig. 3D; $F_{1,24}$=13.24, $P<.001$, Fig. 3E; and $F_{1,24}$= 17.29, $P<.001$, Fig. 3F, respectively). *Post hoc* comparisons revealed that LPS significantly increased 5-HIAA levels in hippocampus, striatum and prefrontal cortex of mice compared to control group. FO treatment normalized the increase of 5-HIAA levels in these brain areas induced by LPS.

Statistical analysis of 5-HIAA/5-HT ratio in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=15.65, $P<.001$, Fig. 3G; $F_{1,24}$=10.25, $P<.001$, Fig. 3H; and $F_{1,24}$=13.55, $P<.001$, Fig. 3I, respectively). *Post hoc* comparisons

Table 2
Effect of administration of FO on interleukin levels in brain structures of mice in a sickness behavior model

Groups	TNF-α (pg/ mg)			IFN-γ (pg/ mg)		
	Hippocampus	Striatum	Prefrontal cortex	Hippocampus	Striatum	Prefrontal cortex
CG	58.50±11.38	34.75±3.81	28.50±5.33	34.00±2.73	29.25±2.78	58.75±6.95
LPS	515.8±68.38[a]	439.5±40.81[a]	436.8±37.67[a]	330.5±38.57[a]	337.3±44.82[a]	307.3±55.80[a]
FO	30.25±2.05	23.50±4.33	22.50±3.12	26.50±4.57	43.00±8.41	54.00±9.46
FO/LPS	198.3±27.13[b]	197.3±17.09[ab] c	193.0±17.48[ab]	246.5±50.05[a]	186.8±22.53[ab]	213.5±32.27[a]

Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test).
[a] $P<.05$ when compared to the control group (CG).
[b] $P<.05$ when comparing the FO/LPS with the LPS.
[c] $P<.05$ when comparing the FO and FO/LPS.

revealed that LPS significantly increased 5-HIAA/5-HT ratio in the hippocampus, striatum and prefrontal cortex of mice compared to control group. FO treatment protected against the increase of 5-HIAA/5-HT ratio induced by LPS in the brain areas in mice.

3.6. TRP and KYN levels

Two-way ANOVA of TRP levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=6.72, P<.01, Fig. 4A; $F_{1,24}$=21.32, P<.001, Fig. 4B; and $F_{1,24}$=7.69, P<.01, Fig. 4C, respectively). *Post hoc* comparisons showed that the increase of TRP levels (hippocampus, striatum and prefrontal cortex) induced by LPS was prevented by FO treatment in mice.

Statistical analysis of KYN levels in hippocampus, striatum and prefrontal cortex yielded a significant FO×LPS interaction ($F_{1,24}$=15.85, P<.001, Fig. 4D; $F_{1,24}$=6.23, P<.01, Fig. 4E; and $F_{1,24}$=8.12, P<.01, Fig. 4F, respectively). *Post hoc* comparisons demonstrated that the increase of KYN levels in brain areas induced by LPS was significantly attenuated by FO treatment.

Two-way ANOVA of KYN/TRP ratio in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=6.22, P<.01, Fig. 4G; $F_{1,24}$=25.22, P<.001, Fig. 4H; and $F_{1,24}$=18.11, P<.001, Fig. 4I, respectively). *Post hoc* comparisons revealed that the LPS-induced increase of KYN/TRP ratio was attenuated by FO treatment.

3.7. Neuroprotective branch of KP

Two-way ANOVA of KYNA levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=7.22, P<.01, Fig. 5A; $F_{1,24}$=15.68, P<.001, Fig. 5B; and $F_{1,24}$=28.55, P<.001, Fig. 5C, respectively). *Post hoc* comparisons revealed that LPS significantly decreased the KYNA levels in the hippocampus, striatum and prefrontal cortex of mice compared to control group. FO supplementation increased KYNA levels in brain areas of mice when compared to control group.

Two-way ANOVA of KYNA/KYN ratio in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}$=26.88, P<.001, Fig. 5D; $F_{1,24}$=41.02, P<.001, Fig. 5E; and $F_{1,24}$=31.21, P<.01, Fig. 5F, respectively). *Post hoc* comparisons yield that the decrease induced by LPS administration of KYNA/KYN ratio in brain areas is reversed by FO treatment.

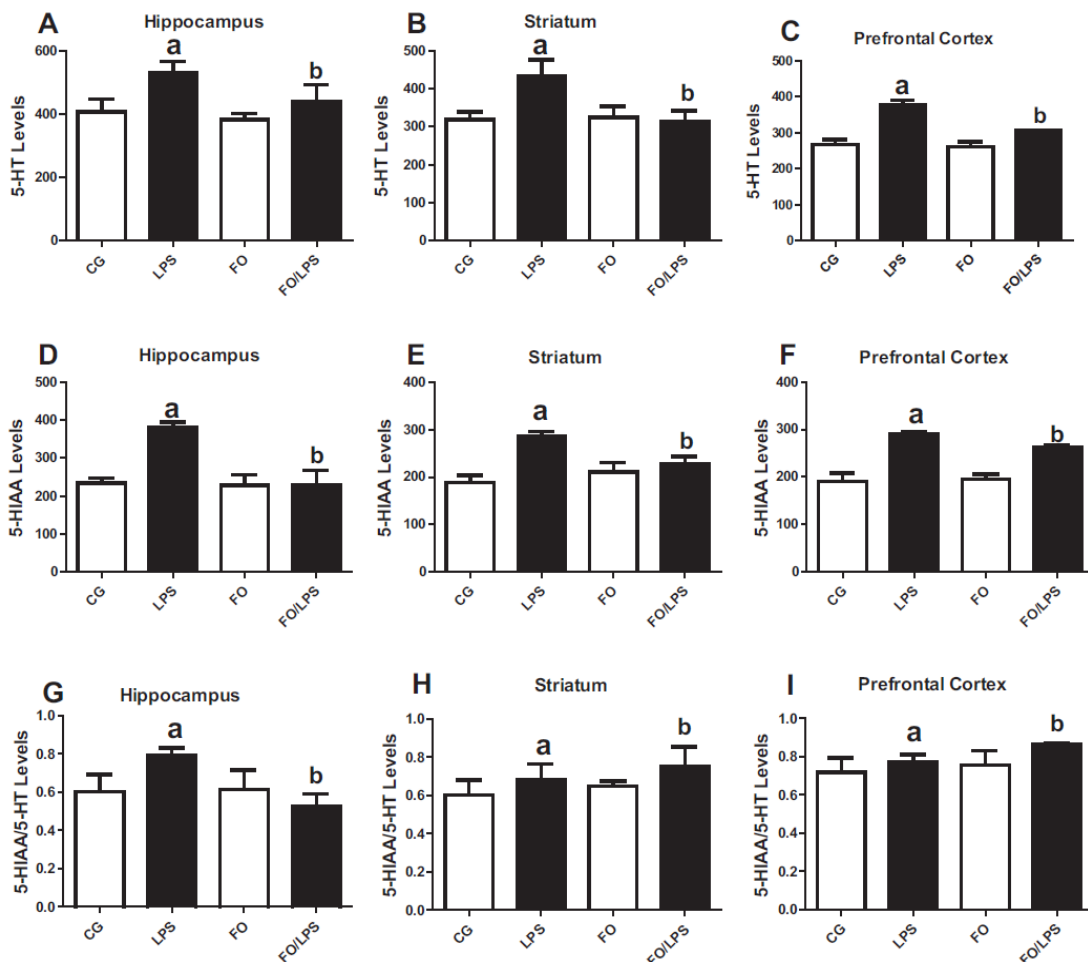

Fig. 3. The effect of FO administration in the serotonergic 5-HT levels and 5-HIAA metabolite in brain areas of mice. Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test). (a) P<.05 when compared to the control group (CG). (b) P<.05 when comparing the FO/LPS with the LPS. (c) P<.05 when comparing the FO and FO/LPS.

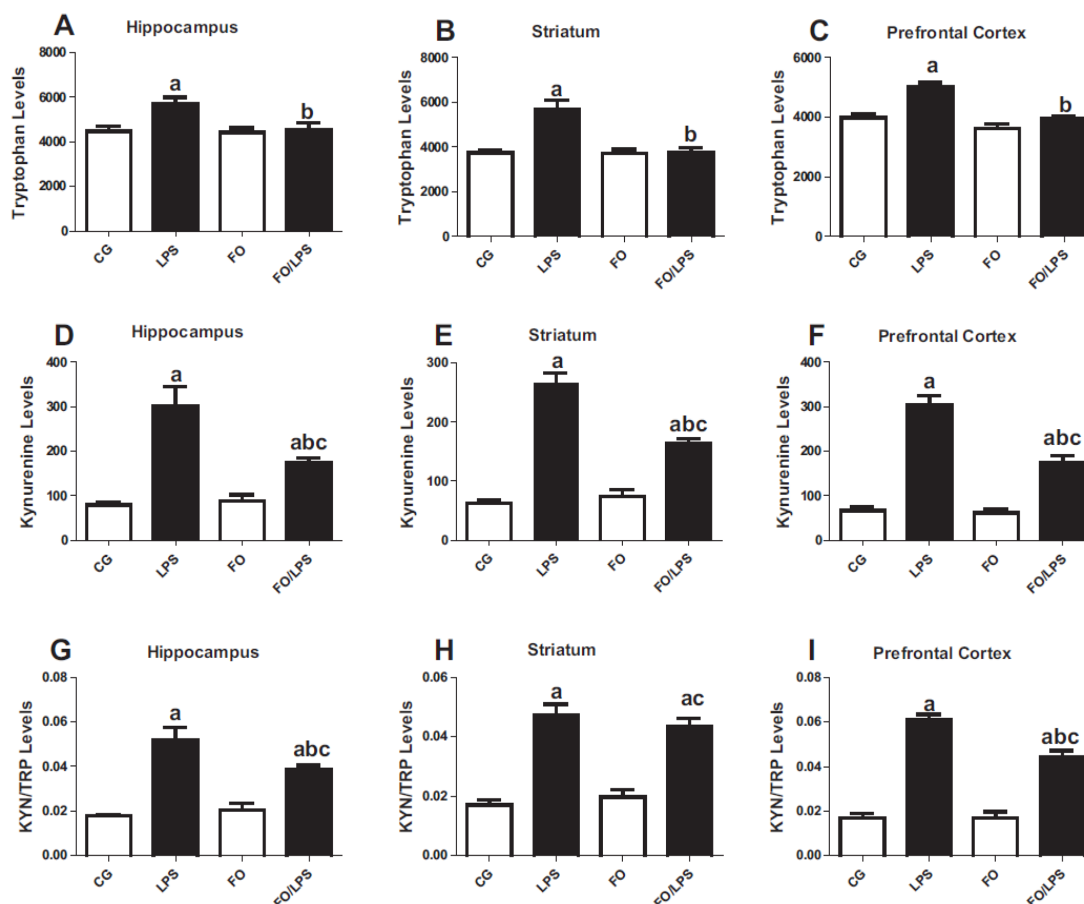

Fig. 4. Effect of FO administration in the TRP and KYN levels in brain areas of mice. Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test). (a) $P<.05$ when compared to the control group (CG). (b) $P<.05$ when comparing the FO/LPS with the LPS. (c) $P<.05$ when comparing the FO and FO/LPS.

3.8. Neurotoxic branch of KP

Two-way ANOVA of 3-HK levels in the hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}=56.02$, $P<.001$, Fig. 6A; $F_{1,24}=42.30$, $P<.001$, Fig. 6B; and $F_{1,24}=23.87$, $P<.01$, Fig. 6C, respectively). *Post hoc* comparisons showed that the LPS-induced increase of 3-HK levels in the hippocampus, striatum and prefrontal cortex was significantly attenuated by FO treatment.

Statistical analysis of QA levels in hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}=48.91$, $P<.001$, Fig. 6D; $F_{1,24}=28.52$, $P<.001$, Fig. 6E; and $F_{1,24}=31.41$, $P<.01$, Fig. 6F, respectively). *Post hoc* comparisons yield that the increase of QA levels induced by LPS was significantly prevented by FO treatment, but not to control levels.

3.9. IDO, KMO and KAT activities

Two-way ANOVA of IDO activity in hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}=24.10$, $P<.001$, Fig. 7A; $F_{1,24}=29.71$, $P<.001$, Fig. 7B; and $F_{1,24}=30.21$, $P<.01$, Fig. 7C, respectively). *Post hoc* comparisons revealed that LPS injection significantly increased IDO activity when compared to control group. FO treatment notably blocked the induction of IDO induced by LPS in the brain areas.

Statistical analysis of KMO activity in hippocampus, striatum and prefrontal cortex demonstrated a significant FO×LPS interaction ($F_{1,24}=24.10$, $P<.001$, Fig. 7D; $F_{1,24}=29.71$, $P<.001$, Fig. 7E; and $F_{1,24}=30.21$, $P<.01$, Fig. 7F, respectively). *Post hoc* comparisons showed that the increase of KMO activity in hippocampus, striatum and prefrontal cortex induced by LPS was significantly blocked by FO treatment in mice.

Two-way ANOVA of KAT activity in hippocampus, striatum and prefrontal cortex showed a significant FO×LPS interaction ($F_{1,24}=8.54$, $P<.01$, Fig. 7G; $F_{1,24}=12.51$, $P<.001$, Fig. 7H; and $F_{1,24}=19.54$, $P<.001$, Fig. 7I, respectively). *Post hoc* comparisons revealed that the LPS induced a decrease of KAT activity in hippocampus, striatum and prefrontal cortex when compared to control group. In addition, the FO treatment significantly attenuated the decrease of KAT activity in brain areas of mice (Fig. 7G, H and I).

3.10. Fatty acid compositions of hippocampus, striatum and prefrontal cortex

Table 3 shows that the diets with FO demonstrated a significant effect on the fatty acid composition of brain areas (hippocampus, striatum and prefrontal cortex) phospholipid. The levels of n-3 PUFA, EPA and DHA in the brain areas were elevated in the FO group when compared to control group. Monounsaturated fatty acid 16:1n7 levels in the brain areas were also significantly increased in the FO group comparing to control group. There were no significant differences in other fatty acids in brain areas among the groups.

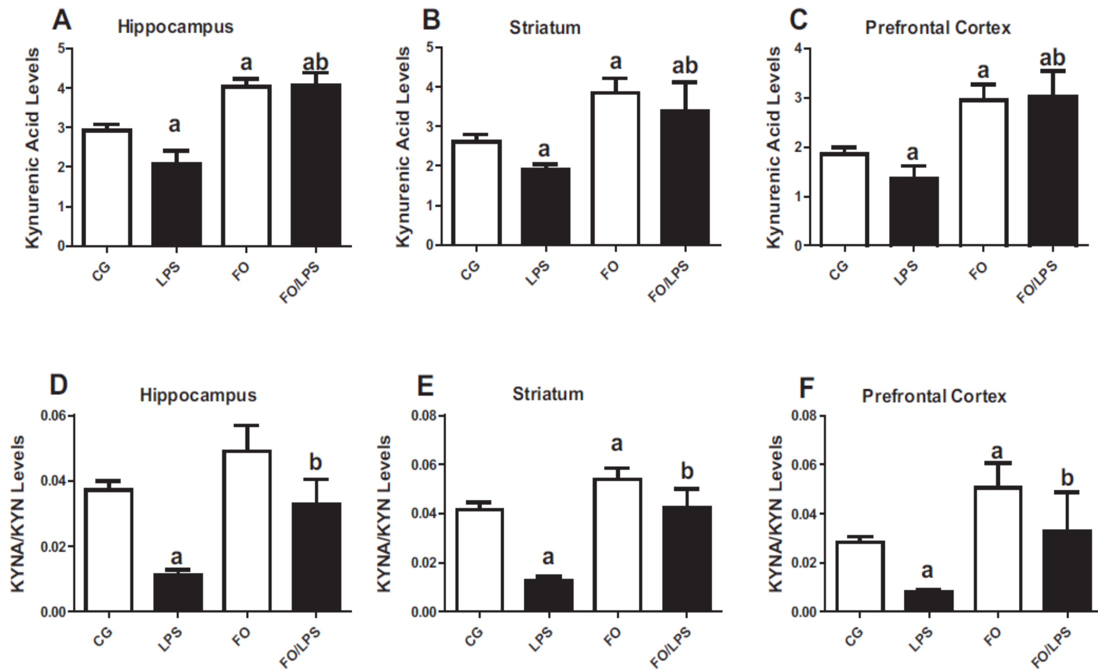

Fig. 5. The effect of FO administration in the KYNA and KYNA/KYN ratio in brain areas of mice. Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test). (a) $P<.05$ when compared to the control group (CG). (b) $P<.05$ when comparing the FO/LPS with the LPS. (c) $P<.05$ when comparing the FO and FO/LPS.

4. Discussion

The results of the present study showed that the blockade of proinflammatory cytokines by FO treatment abrogated LPS-induced sickness behavior in mice *via* modulation of KP in the brain of aged mice. PUFAs, such as EPA and DHA mainly found in FO, have been shown to decrease the production of inflammatory cytokines and exert beneficial effects on inflammation-related diseases in both preclinical and clinical studies [20,21]. Thus, our findings corroborate that LPS model is a well-designed methodology for identifying the

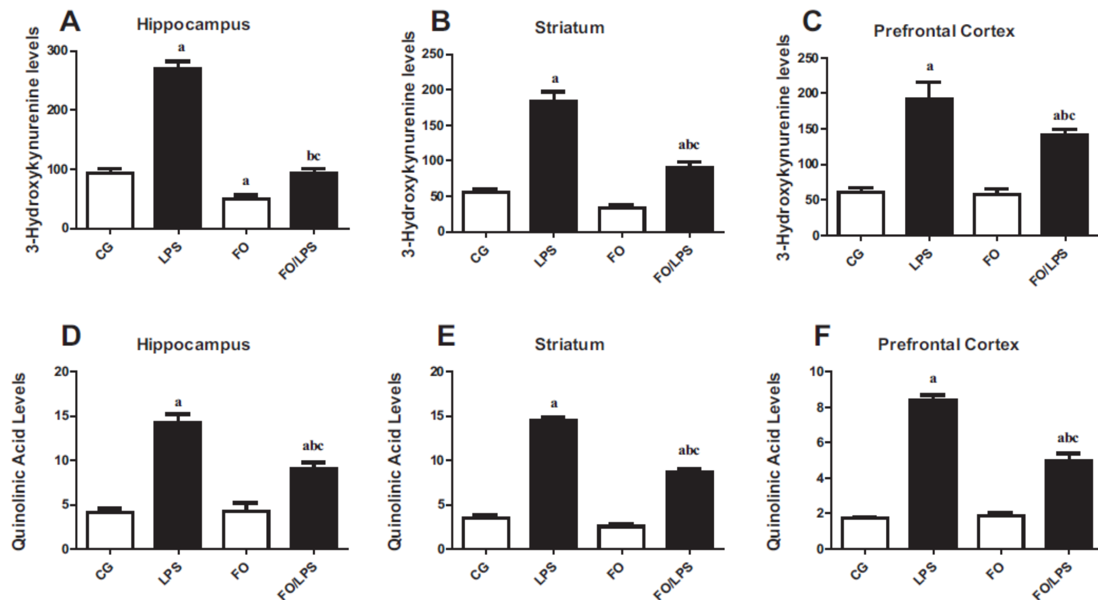

Fig. 6. Effect of FO administration in the 3-HK and QA levels in brain area of mice. Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test). (a) $P<.05$ when compared to the control group (CG). (b) $P<.05$ when comparing the FO/LPS with the LPS. (c) $P<.05$ when comparing the FO and FO/LPS.

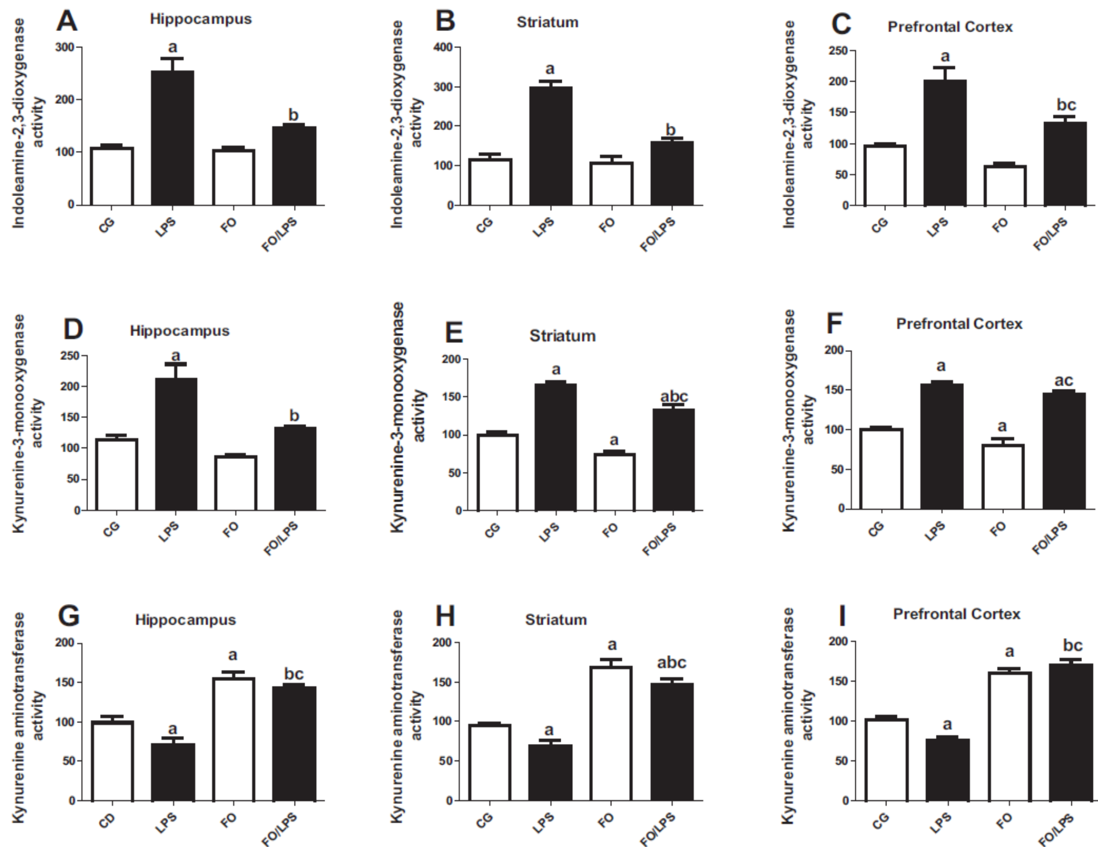

Fig. 7. The effect of FO administration in the IDO, KMO and KAT activities in brain areas of mice. Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test). (a) $P<.05$ when compared to the control group (CG). (b) $P<.05$ when comparing the FO/LPS with the LPS. (c) $P<.05$ when comparing the FO and FO/LPS.

roles of KP metabolites and enzymes to investigate sickness behavior. In addition, we also indicate that the FO supplementation is an effective intervention in pathologic process that involves infection and neuroinflammation.

Clinical symptoms of inflammation-mediated sickness are induced through infections by pathogenic organisms [2]. The pathogenesis of acute neurological complications has been studied in the past few years, with growing understanding of the role of immunity with nutraceutical supplementation [37,38]. LPS administration modifies the immune system through the elevation of plasmatic proinflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ) [39,40]. These cytokines are thought to play a key role in the link between activation of the immune system and the sickness behavior [40]. It was hypothesized that elevated proinflammatory cytokines lead to marker activation of

Table 3
The fatty acid compositions of brain areas of mice

Groups	CG			FO		
Fatty acids (%)	Hippocampus	Striatum	Prefrontal cortex	Hippocampus	Striatum	Prefrontal cortex
14:0	0.67±0.04	0.71±0.05	0.65±0.03	0.73±0.04	0.77±0.07	0.80±0.05
16:0	24.4±0.21	23.8±0.19	23.4±0.24	24.5±0.31	24.1±0.41	23.2±0.44
16:1n7	0.15±0.01	0.17±0.02	0.17±0.01	0.23±0.02 [a]	0.24±0.02 [a]	0.25±0.02 [a]
18:0	19.8±0.25	20.4±0.31	20.1±0.35	19.5±0.22	20.7±0.40	20.3±0.24
18:1n9	21.4±0.34	22.0±0.29	20.9±0.18	21.7±0.27	22.3±0.41	21.0±0.44
18:2n6	0.34±0.01	0.37±0.02	0.37±0.01	0.38±0.02	0.41±0.04	0.40±0.03
20:4n6	10.7±0.54	10.7±0.41	10.1±0.35	10.2±0.29	10.9±0.50	10.9±0.46
20:5n3	0.07±0.01	0.04±0.01	0.05±0.01	0.27±0.01 [a]	0.31±0.01 [a]	0.28±0.01 [a]
22:5n6	1.59±0.05	1.74±0.04	1.79±0.08	0.27±0.01 [a]	0.14±0.01 [a]	0.29±0.01 [a]
22:5n3	0.15±0.01	0.18±0.01	0.19±0.01	0.85±0.04 [a]	0.98±0.05 [a]	0.82±0.03 [a]
22:6n3	14.7±0.24	15.7±0.35	16.4±0.41	19.7±0.21 [a]	18.8±0.25 [a]	18.7±0.19 [a]
Total n-3 PUFA	15.7±0.24	16.2±0.21	16.1±0.19	19.7±0.35 [a]	19.2±0.24 [a]	19.3±0.17 [a]
Total n-6 PUFA	17.4±0.24	18.1±0.31	18.0±0.41	15.4±0.34 [a]	15.8±0.24 [a]	15.7±0.11 [a]

Data are expressed as means±S.E.M. of six animals per group (two-way ANOVA/Bonferroni multiple-comparison test).
[a] $P<.05$ when compared to the control group (CG).

KP in the brain [41]. In our study, we observed a sickness behavior in mice 24 h after LPS injection, which was characterized by decreased body weight, reduced locomotor activity and anhedonic-like state. This behavior changes are associated with increased proinflammatory cytokines in hippocampus, striatum and prefrontal cortex. Collectively, this behavioral pattern observed in our study is referred to as sickness behavior and includes a constellation of nonspecific symptoms [2]. These symptoms and behaviors are robustly expressed across species following either an immune challenge or cytokine administration, indicating a highly conserved connection between sickness behaviors and proinflammatory cytokines [42]. In the present study, we extend these observations by demonstrating that aged mice exhibit sickness behavior parallels with activation neuroinflammation and up-regulation of KP. On the other hand, the data of our study demonstrated that intervention with FO treatment complete recovery the sickness-related behavior, neuroinflammation and cytokines alterations in mice, corroborating beneficial findings of the FO [43,44]. Thus, we demonstrated that FO treatment blocked the neuroinflammation (attenuation the proinflammatory cytokines production in hippocampus, striatum and prefrontal cortex) and prevented the LPS-induced body weight loss, anhedonic-like state and reduced locomotor activity in aged mice.

Central KP stimulation, mediated by IDO activation in the brain [45], seems to play a key role in the modulation of sickness behavior [7,46]. Several clinical works reported an overstimulation of the KP in plasma of patients in situation similar to the sickness model [47,48]. Astrocytes express high amounts of KAT and degrade KYN to KYNA. Microglia highly expressed the enzymes responsible for the generation of 3-HK and QA [49]. In this context, large clinical studies suggested that activation of the KP is related to the expression of anhedonic and locomotor alterations [50,51]. Several studies have investigated the involvement of proinflammatory cytokines in sickness model and comorbid conditions [14,15]. In our study, sickness behavior induced by LPS administration was associated with enhanced KP metabolites in the brain areas together with enhanced brain IDO and KMO activities. These results are in line with several lines of evidence suggesting that IDO and KMO brain activation play an important role in the development of sickness symptoms [14,15,52]. In these studies, there are a number of proinflammatory cytokines that stimulated IDO (*e.g.*, INF-γ, IL-1β and TNF-α) and precipitated sickness symptoms in mice after peripheral immunological challenges. Reciprocally, KP metabolites are involved in generation of the inflammatory reaction induced by pathogens such as malaria parasites or endotoxemia [53]. Following bacterial infection in mice, it was found that cytokine mediates chronic IDO up-regulation, inducing depressive-like behavior [14,52]. Induction of sickness symptoms in our study may be explained by enhanced IDO and KMO activities due to elevated cytokines levels, thereby increasing the KP metabolites in brain areas. Our findings that FO intervention suppressed LPS-induced inflammatory cytokines and KP enzymes and metabolites support the notion that modulation of KP signaling is the underlying mechanism of anti-inflammatory properties of FO.

To explain the link between the behavior metabolic changes and the KP, we also evaluated the levels of neuroactive metabolites of KP, such as 3-HK and QA, involved in the overactivation of glutamatergic neurotransmission. In the brain, both KYN produced locally and KYN transported from periphery are metabolized along two pathways: one pathway involving KATs, which results in the formation of the glutamatergic NMDA receptor antagonist KYNA, and another pathway involving KMO, which leads to the formation of 3-HK and QA [54,55]. In addition, endogenous KYNA can exert its antiglutamatergic action by antagonizing α7 nicotinic receptors [55]. Microdialysis analysis showed that KYNA is able to reduce the extracellular content of glutamate in prefrontal cortex [56] and striatum [57], resulting in a decrease of glutamate-related excitatory effect. These data suggested that KP metabolites are involved in sickness-induced anhedonic and locomotor alterations and are in agreement with clinical studies that KP products may be related to behavior and metabolic characteristics

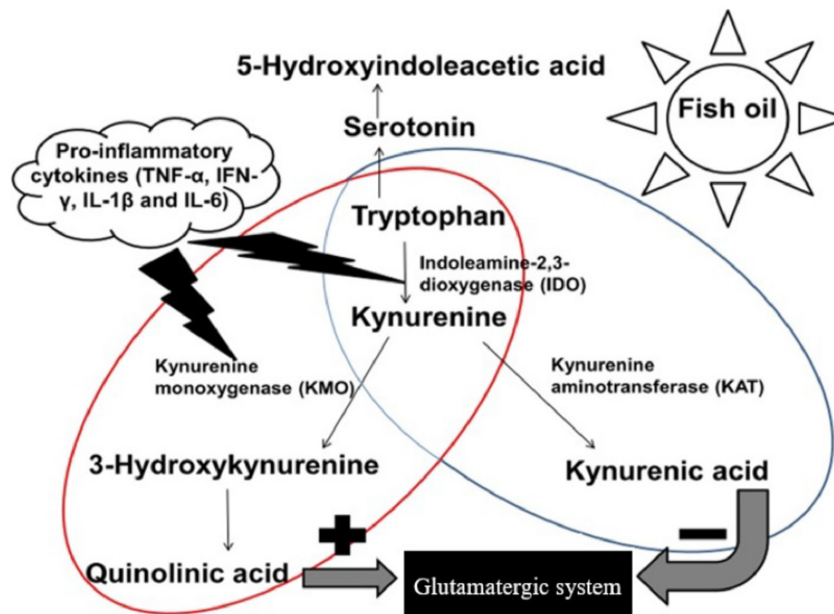

Fig. 8. Diagram showing the mechanistic links between neuroinflammation and neurobehavioral alterations induced by LPS injection and the neuroprotective effect of FO. On the one hand, the neuroinflammation response (IL-1β, IL-6, TNF-α and IFN-γ) following an LPS injection caused KP activation and subsequent increase of neurotoxic branch (3-HK and QA levels and KMO activity) and decrease of neuroprotective branch (KYNA levels and KAT activity). The levels of 5-HT and 5-HIAA were also altered in this model of sickness. Hence, KP activation within the brain may activate neuroinflammation pathways that provoke sickness symptoms. On the other hand, the FO administration prevented the behavioral and sickness disturbances induced by LPS due to the modulation effect in proinflammatory cytokines and KP in hippocampus, striatum and prefrontal cortex of mice.

in humans [14,15,52]. Thus, we suggest that LPS-induced KP imbalances may contribute to a modification to the glutamatergic homeostasis. Recent data have demonstrated evidence for a determinant role of glutamate in the pathophysiology of sickness model [58,59]. Another hypothesis is that KYN levels in brain areas increased 24 h after the LPS administration, and the balance of KP metabolism is shifted toward KMO-dependent neurotoxic branch, decreasing the neuroprotective metabolites of the KP [60]. These studies suggest an important role for neuroprotective/neurotoxic branches of the KP in mediating in LPS-induced sickness behavior in mice. Other studies need to be performed in order to clarify if brain-specific enrichment of FO can block the LPS-induced neuroinflammation and the neurotoxic branch activation of KP.

In our study, the up-regulation of KP induced by LPS was followed by increased levels of TRP in the hippocampus, striatum and prefrontal cortex. These findings are somewhat counterintuitive and certainly argue against the hypothesis that KP enzymes depleted TRP bioavailability for KP synthesis. However, this result could be explained as a compensatory mechanism of the brain against the inflammatory processes elicited by LPS. The observed increase in brain levels of TRP and 5-HT metabolism in response to LPS has already been described [61,62]. In addition, we observed alterations in 5-HIAA/5-HT ratio in the hippocampus, striatum and prefrontal cortex, indicating that serotonergic system may be involved in the body weight loss, anhedonic and locomotor behavior induced by LPS. Thus, these results reinforce that TRP, 5-HT and 5-HIAA levels were associated with behavior and metabolic changes in sickness-like behavior induced by LPS in aged mice. Taken together, these data indicate that, under pathological conditions such as sickness, the activation of KP might be associated with increase of TRP and altered 5-HIAA/5-HT ratio in in the brain. Moreover, we demonstrated that FO supplementation has a neuroprotective effect *via* modulation of the TRP metabolism and the serotonergic system. Therefore, we hypothesize that the increase of EPA+DHA and DPA/AA ratio observed in the brain of mice supplemented with FO resulted in a reduction of inflammatory responses *via* modification in the cellular membrane phospholipid concentration that finally led to improvement in the serotonergic system, contributing to the prevention of sickness behavior. A summary of the mechanistic explanations for the protective effects of FO against LPS-induced sickness behavior can be seen in Fig. 8.

5. Conclusions

Our results demonstrate a critical role for KP metabolism in mediating sickness-like behavior and neuroinflammation induced by LPS. Furthermore, we found that sickness symptoms and neuroinflammation were attenuated with FO supplementation. Consequently, the PUFAs present in FO appear as ideal candidates for nutritional interventions to promote the decrease of KP activation and neuroinflammation, conditions that are related to several neuropsychiatric and neurodegenerative diseases affecting aged people. Therefore, it is of great interest to further investigate the relation of sickness-mediated impairments and the potential benefits of dietary PUFA supplementation in humans.

Conflict of interest

The authors declare that there are no conflicts of interest in the present work.

Acknowledgment

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References

[1] Parnet P, Kelley KW, Bluthé RM, Dantzer R. Expression and regulation of interleukin-1 receptors in the brain role in cytokines-induced sickness behavior. J Neuroimmunol 2002;125:5–14.
[2] Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46–56.
[3] Dantzer R. Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am 2009;29:247–64.
[4] Ribeiro DE, Maiolini VM, Soncini R, Antunes-Rodrigues J, Elias LL, Vilela FC, et al. Inhibition of nitric oxide synthase accentuates endotoxin induced sickness behavior in mice. Pharmacol Biochem Behav 2012;103:535–40.
[5] Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988;12:123–37.
[6] Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur J Pharmacol 2004;500:399–411.
[7] Eimerbrink MJ, Kranjac D, St Laurent C, White JD, Weintraub MK, Pendry RJ, et al. Pre-treatment of C57BL6/J mice with the TLR4 agonist monophosphoryl lipid A prevents LPS-induced sickness behaviors and elevations in dorsal hippocampus interleukin-1β, independent of interleukin-4. Behav Brain Res 2016;302:171–4.
[8] Sagar SM, Price KJ, Kasting NW, Sharp FR. Anatomic patterns of Fos immunostaining in rat brain following systemic endotoxin administration. Brain Res Bull 1995;36:381–92.
[9] Gaykema RP, Goehler LE. Ascending caudal medullary catecholamine pathways drive sickness induced deficits in exploratory behavior: brain substrates for fatigue? Brain Behav Immun 2011;25:443–60.
[10] Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 2012;37:137–62.
[11] Salazar A, Gonzalez-Rivera BL, Redus L, Parrott JM, O'connor JC. Indoleamine 2,3 dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. Horm Behav 2012;62:202–9.
[12] O'Connor JC, Andre C, Wang Y, Lawson MA, Szegedi SS, Lestage J, et al. Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette Guerin. J Neurosci 2009;29:4200–9.
[13] Rana P, Sharma AK, Jain S, Deshmukh P, Bhattacharya SK, Banerjee BD, et al. Comparison of fluoxetine and 1-methyl-L-tryptophan in treatment of depression-like illness in Bacillus Calmette-Guerin-induced inflammatory model of depression in mice. J Basic Clin Physiol Pharmacol 2016(6):569–76.
[14] Cathomas F, Fuertig R, Sigrist H, Newman GN, Hoop V, Bizzozzero M, et al. CD40-TNF activation in mice induces extended sickness behavior syndrome co-incident with but not dependent on activation of the kynurenine pathway. Brain Behav Immun 2015;50:125–40.
[15] Murray C, Griffin ÉW, O'Loughlin E, Lyons A, Sherwin E, Ahmed S, et al. Interdependent and independent roles of type I interferons and IL-6 in innate immune, neuroinflammatory and sickness behaviour responses to systemic poly I:C. Brain Behav Immun 2015;48:274–86.
[16] Pascoe MC, Howells DW, Crewther DP, Constantinou N, Carey LM, Rewell SS, et al. Fish oil diet associated with acute reperfusion related hemorrhage, and with reduced stroke-related sickness behaviors and motor impairment. Front Neurol 2014;6:5–14.
[17] Orr SK, Trépanier MO, Bazinet RP. n-3 Polyunsaturated fatty acids in animal models with neuroinflammation. Prostaglandins Leukot Essent Fatty Acids 2013 (1):97–103.
[18] Chalon S. Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins Leukot Essent Fatty Acids 2006;75:259–69.
[19] Sidhu VK, Huang BX, Kim HY. Effects of docosahexaenoic acid on mouse brain synaptic plasma membrane proteome analyzed by mass spectrometry and O-16/O-18 labeling. J Proteome Res 2011;10:5472–80.
[20] Dong Y, Xu M, Kalueff AV, Song C. Dietary eicosapentaenoic acid normalizes hippocampal omega-3 and 6 polyunsaturated fatty acid profile, attenuates glial activation and regulates BDNF function in a rodent model of neuroinflammation induced by central interleukin-1β administration. Eur J Nutr 2017. https://doi.org/10.1007/s00394-017-1462-7.
[21] Shih JM, Shih YM, Pai MH, Hou YC, Yeh CL, Yeh SL. Fish oil-based fat emulsion reduces acute kidney injury and inflammatory response in antibiotic-treated polymicrobial septic mice; 2016. https://doi.org/10.3390/nu8030165.
[22] Mocellin MC, Camargo CQ, Nunes EA, Fiates GM, Trindade EB. A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer. Clin Nutr 2016(2):359–69.
[23] Food and Drug Administration. FDA announces qualified health claims for omega-3 fatty acids. https://www.fda.gov/ohrms/dockets/98fr/oc0698.pdf; 2005.
[24] Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, et al. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J 2005(10):1329–31.
[25] Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci 2011;933:222–34.
[26] Kent S, Bluthé RM, Kelley KW, Dantzer R. Sickness behavior as a new target for drug development. Trends Pharmacol Sci 1992;1:24–8.
[27] Grippo AJ, Gerena D, Huang J, Kumar N, Shah M, Ughreja R, et al. Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles. Psychoneuroendocrinology 2007;32:966–80.

[28] Tiziana R, Daniela V, Natalia R, Cinzia G, Daniela B, Valeria C, et al. Chronic δ^9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology 2008;33:2760–71.
[29] Spijker S. Dissection of rodent brain regions. In: Li Ka Wan, editor. Neuroproteomics (neuromethods). Springer Science+Business Media, LLC; 2011. p. 13–26.
[30] Williams RW. The mouse brain library. http://www.mbl.org/atlas165/atlas165_start.html; 1999.
[31] Silva NM, Rodrigues CV, Santoro MM, Reis LF, Alvarez-Leite JI, Gazzinelli RT. Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with Toxoplasma gondii: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. Infect Immun 2002;70:859–68.
[32] Harkin A, Connor TJ, Walsh M, St John N, Kelly JP. Serotonergic mediation of the antidepressant-like effects of nitric oxide synthase inhibitors. Neuropharmacology 2003(5):616–23.
[33] Sono M. Enzyme kinetic and spectroscopic studies of inhibitor and effector interactions with indoleamine 2,3-dioxygenase. 2. Evidence for the existence of another binding site in the enzyme for indole derivative effectors. Biochemistry 1989(13):5400–7.
[34] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem 1976;72:248–54.
[35] Guidetti P, Okuno E, Schwarcz R. Characterization of rat brain kynurenine aminotransferases I and II. J Neurosci Res 1997;50:457–65.
[36] Harris WS, Von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease. Prev Med 2004;39:212–20.
[37] Wang X, Ma DW, Kang JX, Kulka M. n-3 Polyunsaturated fatty acids inhibit Fc ε receptor mediated mast cell activation. J Nutr Biochem 2015(12):1580–8.
[38] Schuster GU, Bratt JM, Jiang X, Pedersen TL, Grapov D, Adkins Y, et al. Dietary long-chain omega-3 fatty acids do not diminish eosinophilic pulmonary inflammation in mice. Am J Respir Cell Mol Biol 2014(3):626–36.
[39] Basu MS, Mudgal J, Nampoothiri M, Hall S, Dukie SA, Grant G, et al. Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. Neurosci Lett 2016;632:218–23.
[40] Cai KC, Van-Mil S, Murray E, Mallet JF, Matar C, Ismail N. Age and sex differences in immune response following LPS treatment in mice. Brain Behav Immun 2016;58:327–37.
[41] Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3 dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:702–21.
[42] Dantzer R, Kelley KW. Stress and immunity: an integrated view of relationships between the brain and the immune system. Life Sci 1989;44:1995–2008.
[43] Mingam R, Moranis A, Bluthé RM, De Smedt-Peyrusse V, Kelley KW, Guesnet P, et al. Uncoupling of interleukin-6 from its signalling pathway by dietary n-3 polyunsaturated fatty acid deprivation alters sickness behaviour in mice. Eur J Neurosci 2008(9):1877–86.
[44] Liu YH, Li XY, Chen CY, Zhang HM, Kang JX. Omega-3 fatty acid intervention suppresses lipopolysaccharide-induced inflammation and weight loss in mice. Mar Drugs 2015(2):1026–36.
[45] Badawy AA. Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. Int J Tryptophan Res 2017. https://doi.org/10.1177/1178646917691938.
[46] Dantzer R, O'Connor JC, Lawson MA, Kelley KW. Inflammation associated depression: from serotonin to kynurenine. Psychoneuroendocrinology 2011;36:426–36.
[47] Capuron L, Schroecksnadel S, Feart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. Biol Psychiatry 2011;70:175–82.
[48] Myint AM, Schwarz MJ, Steinbusch HW, Leonard BE. Neuropsychiatric disorders related to interferon and interleukins treatment. Metab Brain Dis 2009(1):55–68.
[49] Alberati-Giani D, Ricciardi-Castagnoli P, Köhler C, Cesura AM. Regulation of the kynurenine metabolic pathway by interferon-gamma in murine cloned macrophages and microglial cells. J Neurochem 1996;3:996–1004.
[50] Meier TB, Drevets WC, Wurfel BE, Ford BN, Morris HM, Victor TA, et al. Relationship between neurotoxic kynurenine metabolites and reductions in right medial prefrontal cortical thickness in major depressive disorder. Brain Behav Immun 2016;53:39–48.
[51] Kim H, Chen L, Lim G, Sung B, Wang S, McCabe MF, et al. Brain indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression. J Clin Invest 2012(8):2940–54.
[52] Dantzer R. Role of the kynurenine metabolism pathway in inflammation-induced depression: preclinical approaches. Curr Top Behav Neurosci 2017;31:117–38.
[53] Hunt NH, Too LK, Khaw LT, Guo J, Hee L, Mitchell AJ, et al. The kynurenine pathway and parasitic infections that affect CNS function. Neuropharmacology 2017;112:389–98.
[54] Connick JH, Stone TW. Excitatory amino acid antagonists and endogenous aspartate and glutamate release from rat hippocampal slices. Br J Pharmacol 1988 (4):863–7.
[55] Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J Neurosci 2001(19):7463–73.
[56] Wu HQ, Pereira EF, Bruno JP, Pellicciari R, Albuquerque EX, Schwarcz R. The astrocyte derived alpha7 nicotinic receptor antagonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. J Mol Neurosci 2010;40:204–10.
[57] Carpenedo R, Meli E, Peruginelli F, Pellegrini-Giampietro DE, Moroni F. Kynurenine 3 mono oxygenase inhibitors attenuate post-ischemic neuronal death in organotypic hippocampal slice cultures. J Neurochem 2002(6):1465–71.
[58] Chaskiel L, Paul F, Gerstberger R, Hübschle T, Konsman JP. Brainstem metabotropic glutamate receptors reduce food intake and activate dorsal pontine and medullar structures after peripheral bacterial lipopolysaccharide administration. Neuropharmacology 2016;107:146–59.
[59] Frühauf PK, Ineu RP, Tomazi L, Duarte T, Mello CF, Rubin MA. Spermine reverses lipopolysaccharide-induced memory deficit in mice. J Neuroinflammation 2015;3:9–12.
[60] Walker AK, Budac DP, Bisulco S, Lee AW, Smith RA, Beenders B, et al. NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. Neuropsychopharmacology 2013(9):1609–16.
[61] Sens J, Schneider E, Mauch J, Schaffstein A, Mohamed S, Fasoli K, et al. Lipopolysaccharide administration induces sex-dependent behavioural and serotonergic neurochemical signatures in mice. Pharmacol Biochem Behav 2017;153:168–81.
[62] Schneiders J, Fuchs F, Damm J, Herden C, Gerstberger R, Soares DM, et al. The transcription factor nuclear factor interleukin 6 mediates pro-and anti-inflammatory responses during LPS-induced systemic inflammation in mice. Brain Behav Immun 2015;48:147–64.

3.2. Artigo 2

A gordura vegetal hidrogenada dietética exacerba a ativação da via da quinurenina causada pelo desafio imune periférico ao lipopolissacarídeo em camundongos idosos

Marcelo Gomes de Gomes, Leandro Cattelan Souza, Silvana Peterini Boeira, André Rossito Goes, Lucian Del Fabbro, Carlos Borges Filho, Franciele Donato, Robson Luiz Puntel, Cristiano Ricardo Jesse

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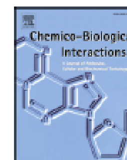

Dietary hydrogenated vegetable fat exacerbates the activation of kynurenine pathway caused by peripheral lipopolysaccharide immune challenge in aged mice

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ABSTRACT

Sickness behavior is a normal immune response of body to fight infection, accompanied by endocrine and behavioral alterations. Lipopolysaccharide (LPS) causes sickness behavior in rodents through the increase of proinflammatory cytokines, generating peripheral inflammation and thus overactivation of kynurenine pathway (KP). In the present study we investigated the effects of dietary hydrogenated vegetable fat (HVF) in sickness behavior induced by LPS in aged mice. Male C57BJ/6 aged mice received a supplementation with HVF for six months. After HVF supplementation mice were treated with LPS (0.15 mg/kg; i. p. injection). Twenty-four hours post LPS injection mice were submitted to behavioral tests and then, the hippocampus, striatum and prefrontal cortex were removed for neurochemical determinations. Our results showed that dietary HVF did not exacerbate the behavioral alterations induced by LPS. Although HVF did not modulate the proinflammatory cytokines analyzed, it caused a potentiation in the increase of brain tumor necrosis factor-alpha levels induced by LPS. Moreover, dietary HVF aggravated LPS-induced KP activation in the brain of mice, mainly by further increase of neurotoxic metabolite quinolinic acid and further decrease of kynurenic acid/kynurenine ratio, a marker of neuroprotective branch of KP. Overall, our study demonstrated that dietary HVF did not worsen the sickness behavioral induced by LPS administration. However, HVF aggravated the activation of KP and exacerbated the shift of KP metabolism towards the neurotoxic branch.

1. Introduction

Sickness behavior has been well described both experimentally and observationally, in a variety of animal species [1]. The sickness behavior, which is a normal immune response of body to fight infection, is manifested by reduced mobility, fatigue, cognitive impairment and inability to derive pleasure from otherwise enjoyable situations [2]. This endocrine and behavioral alterations is triggered by proinflammatory cytokines.

The lipopolysaccharide (LPS) is a component of the outer cell wall of gram-negative bacteria, and its administration has been used as a predictive model for sickness behavior in rodents [2]. LPS stimulates the immune system and induces a release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and interlukin-6 (IL-6) [3]. By this mechanism it generates an acute peripheral inflammation, which activates an immune response. The interaction between the immune system and central nervous system (CNS) is important for regulating the immunological, physiological, and behavioral responses to immune stimulation [4]. Likewise, an inflammatory response that is generated peripherally can affect the brain because cytokines can reach the CNS via neural and humoral pathways [5].

It has been demonstrated that proinflammatory cytokines in the CNS were able to activate the kynurenine pathway (KP). The kynurenine is a catabolite of the amino acid tryptophan, which is generated by catalytic action of the rate-limiting enzyme of KP indoleamine-2,3-dioxygenase (IDO) [6]. In the brain, IDO activation plays a key role in the development of depressive-like behavior [7]. Thus, KP metabolism

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has been related to neuroinflammation and diseases of the CNS.

The hydrogenated vegetable fat (HVF) is obtained basically from vegetable oils, by the hydrogenation process which generates trans isomers. The consumption of HVF has been steadily increasing since the 20th century and now accounts for 1.7–8% of the world dietary fat intake [8]. In fact, processed foods have been widely consumed in Western countries in recent decades [9]. Due to great increase of HVF consumption in the last decades, the number of studies began to increase as a result of the effect of this type of fat on human health and neurological diseases. It is well documented that dietary HVF can be quickly incorporated into membrane phospholipids, thus decreasing membrane fluidity and altering the biochemical properties as well the functionality of their proteins [10]. By altering membrane properties and functionality, HVF may contribute to CNS dysfunctions. Experimental evidence has reported that HVF is proinflammatory [11]. HVF can induce the increase of inflammatory eicosanoids and cytokines, which adversely influence mental health [12].

In this context, the objective of the present study is to investigate whether the consumption of HVF could exacerbates sickness behavior induced by peripheral administration of LPS in mice. Moreover, neurochemical parameters related to inflammatory status and KP activation were evaluated in the hippocampus, striatum and prefrontal cortex.

2. Materials and methods

2.1. Animals

Experiments were performed using male C57BJ/6 mice (30–40 g, 2 years old). The aged mice were used because age is the most predominant risk factor for the development of neurodegenerative diseases [13]. Our model induces a large neuroinflammation, which is involved in the etiology of several neurodegenerative diseases that affect the elderly population. Animals were maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out during light phase on the day. All efforts were made to minimize animal suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for Animal Use (CEUA protocol # 010/2016) of Federal University of Pampa, Brazil.

2.2. Mouse treatments

Food was placed in dry-diet feeder jars to avoid significant food loss that often accompanies overhead pellet feeding, and the jars were weighed daily to determine the amount of food consumed. General animal health was also monitored during the test periods. Diet consisted of defined mouse diet AIN-93 M (Puro Trato, RS). This diet was isocaloric, isonitrogenous, 60.1% carbohydrate, 18.2% protein, 10.2% fat, and approximately 90% dry matter. The animals were divided into 4 groups: Control group (Corn oil + PBS), LPS group (LPS + Corn Oil), HVF group (HVF + PBS) and LPS/HVF group (LPS + HVF). The animals were supplemented by gavage (3 g/kg; p. o.) [14] with corn oil (control) or hydrogenated vegetable fat (HVF-rich in trans fatty acids) for 180 days [14]. The period of 180 days of HVF supplementation was determined by the previous study by Trevisol et al. [14]. Thus, the animals consumed hydrogenated vegetable fat almost 1/4 of their life, reproducing the chronic consumption of fat by elderly people. Aged animals in the HVF group were treated with the diet for 180 days prior LPS injection. LPS treatment involved an intraperitoneal injection of freshly made solutions of 0.15 mg/kg LPS (L-020M4062, serotype 0127:B8; Sigma, St. Louis, MO) prepared with sterile endotoxin free isotonic PBS or PBS (equivolume). This dose of LPS was chosen to induce sickness behaviors and cytokine activation at the time point of measurement, while still providing room for the potential of elevated responses. This range of LPS doses was selected based upon previous studies demonstrating that 0.33 mg/kg LPS produced prolonged sickness behavior in aged, compared to young mice and 0.02 mg/kg being the lowest dose capable of inducing statistically significant changes in sickness behaviors when compared to saline treated mice [15].

2.3. Body weight and locomotor activity

Sickness behavior was assessed by changes in body weight and locomotor activity (LMA) at 24 h after LPS injection. Decreased LMA in a novel environment is a sensitive measure of sickness behavior [16]. For this test, mice were individually placed into a clean, novel cage (30 L X 19 W × 12 H cm) devoid of bedding or litter, and LMA was video-recorded for a 5-min period. Videos were analyzed by dividing the cage into four virtual quadrants and counting the number of quadrant entrances over the 5- min period; counting was done by a trained observer who was blind to experimental treatments. Changes in locomotor activity and depression-like behavior were assessed 24 h after drug administration. We selected behavioral endpoints that we had already demonstrated to be very sensitive to systemic inflammation-induced sickness [17].

2.4. Corticosterone levels

Blood was collected on ice and separated in a refrigerated centrifuge at 4 °C (4000 g for 10 min). Serum was stored at −20 °C until assays were performed. Serum corticosterone levels were measured using a commercial kit based on enzyme immune assay (ELISA). The corticosterone levels were expressed as ng/l.

2.5. Sucrose preference test

Separate groups of mice were evaluated in sucrose preference test. At 24 h after treatment, anhedonia was measured by preference for a sucrose solution over water, using a two-bottle free choice method [18]. Each animal was presented simultaneously to two bottles, one containing 1% sucrose solution (w/v), and the other containing tap water. Blunted sucrose intake, in this test, is proposed to reflect impaired sensitivity to reward and model anhedonia, a core symptom of major depression [19]. Tap water and 1% sucrose solution were placed in premeasured bottles in the cages, and fluid intake was monitored for 24 h. Before the start of the protocol the mice were exposed only to a 1% sucrose solution for 24 h aiming the adaptation to sucrose solution. Mice were private food and water for about 20 h before each sucrose preference test. Sucrose preference was evaluated via the sucrose uptake rate, namely, the ratio of volume of sucrose consumption to the volume of sucrose consumption plus tap water consumption (sucrose preference = sucrose consumption/(sucrose consumption + water consumption) × 100%).

2.6. Tail suspension test

Mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The immobility time was recorded for 6 min. The immobility behavior was defined according to the method described previously [20].

2.7. Tissue preparation for neurochemical determinations

After behavioral tests, mice were euthanized with barbiturate overdose (pentobarbital sodium 150 mg/kg; i. p. route) and transcardiacally perfused with 10 ml ice-cold saline via the aorta. The brain dissection was performed according to the method of Spijker [21], a method to dissect multiple brain regions from a single brain based on existing atlases [22]. Prefrontal cortex, striatum and hippocampus were bilaterally removed and rapidly homogenized in 50 mM Tris–Cl, pH 7.4.

The homogenate was centrifuged at 2400 × g for 15 min at 4 °C and a low-speed supernatant fraction (S1) was used for assays. The blood was also collected after perfusion with saline.

2.8. Cytokine levels

Levels of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β) and interleukin-6 (IL-6) in the hippocampus, striatum and prefrontal cortex were measured using sample aliquots of 100 μL and mouse cytokine ELISA DuoSet Kits from R&D Systems (Minneapolis, MN, USA), according to the manufacturer's instructions (protein range of 31.25–2000 pg). The level of cytokine was estimated by interpolation from a standard curve by colorimetric measurements at 450 nm (correction wavelength 540 nm) on an ELISA plate reader (Berthold Technologies-Apollo 8-LB 912, KG, Germany). Results are shown as pg/mg of protein.

2.9. Analysis of TRP, KYN, KYNA, 3-HK and QUIN concentrations by high performance liquid chromatography (HPLC)

The levels of TRP and its metabolite KYN in the hippocampus, striatum and prefrontal cortex were performed in a Shimadzu LC-10 A liquid chromatograph according to Silva et al. [23]. The chromatographic separation was achieved using a 250 × 4.6-mm (inner diameter) C18 reverse-phase column (particle size, 4 μm; Aquapore RP-300C-18). For TRP measurement, the column was eluted isocratically at flow rate of 1.0 ml/min with 0.015 M sodium acetate (pH 4.5) containing 15% methanol. For KYN determination, the column was eluted with acetonitrile at a 1:47 dilution in 0.1 M acetic acid–0.1 M ammonium acetate (pH 4.65). The absorbance of the column effluent was monitored at 280 and 365 nm for TRP and KYN, respectively. The peaks of TRP or KYN were identified by comparison with the retention times of standard compounds (Sigma), and quantification was based on the ratios of the peak areas of compound to the internal standard. The tissue levels were expressed in ng/mg tissue. KYN/TRP ratio was calculated by ratio between KYN and TRP concentrations. KYNA, 3-HK and QUIN levels were measured in the hippocampus, striatum and prefrontal cortex using HPLC. The mobile phase contained 50 nM glacial acetic acid, 100 mM zinc acetate and 3% acetonitrile dissolved in double-distilled NANO pure water HPLC grade H2O. The pH was adjusted to 4.9 using 5 M NaOH. S1 of PFC and HP were sonicated in 1 ml of mobile phase containing 7% perchloric acid spiked with 50 ng/20 μl of N-methyl 5-HT as an internal standard. The resultant solution was centrifuged at 44,800 g for 20 min and the supernatants were placed into new Eppendorf tubes using a syringe fitted with a 0.45-μm filter (Phenomenex). Approximately 20 μl of the filtered supernatant was injected using a Waters auto sampler and a Reverse Phase analytical column (Kinetex™ Core Shell Technology column with specific area of 4.6 mm and particle size of 2.6 μl, Phenomenex) was used for the separation of metabolites. A PDA-UV detector (Shimadzu SPD-M10A VP), calibrated to integrate at 230 and 250 nm, as well as a fluorescent detector (Shimadzu RF- 20 A XS prominence fluorescence detector), set to excitation wavelength 254 nm and emission wavelength 404 nm, were used to detect the metabolites. Chromatographs were generated by CLASS-VP software (Shimadzu). The results are expressed as ng/g tissue.

2.10. 5-HT and 5-HIAA levels and 5-HIAA/5-HT ratio

The levels of 5-HT and its metabolite 5-Hydroxyindoleacetic acid (5-HIAA) in the hippocampus, striatum and prefrontal cortex were analyzed by HPLC with electrochemical detection, as previously described [24]. The mobile phase, used at a flow rate of 0.8 ml/min, consisted of 0.02 M phosphate/citrate buffer and 90/10 methanol (v/v), 0.12 mMNa2 EDTA, and 0.0556% heptane sulphonic acid as ion pair. The pH was adjusted to 2.64 with H3PO4 at 22 °C. A 5-μm (220 × 4.6) Spheri-5 RP-18 column from Brownlee Laboratory was used. Electrochemical detection was performed with a Shimadzu L-ECD-6A electrochemical detector with a potential of 0.75 V. The peak area of the internal standard, dihydroxybenzylamine (DHBA) was used to quantify the sample peaks. The tissue levels were expressed in ng/g tissue. 5-HIAA/5-HT ratio was calculated by ratio between 5-HIAA and 5-HT concentrations.

2.11. Determination of IDO, KMO and KAT activities

IDO activity in the hippocampus, striatum and prefrontal cortex was determined as previously described [25], with minor modifications: the tissue used in the original study was rabbit small intestine and in our study it was prefrontal cortex (PFC) and hippocampus (HP) of mice; the amount of the enzyme as expressed in terms of its heme content based on the absorbance at 406 nm and in our method, the protein concentration was measured by the method of Bradford [26], using bovine serum albumin as the standard; the temperature was 25 °C and in our study was 37 °C; the original method did not describe the block with trichloroacetic acid and the rotations of the centrifugation. The S1 (0.2 ml) were added to 0.8 ml of the reaction mixture containing 400 μM L-tryptophan, 20 mM ascorbate, 10 μM methylene blue, and 100 μg catalase in 50 mM potassium phosphate buffer pH 6.5. The reaction was carried out at 37 °C under agitation for 60 min. Then, it was blocked by adding 0.2 ml of 30% trichloroacetic acid and further incubated at 50 °C for 30 min to convert the N-formylkynurenine to L-kynurenine. Samples were centrifuged at 13,000 g for 10 min at 4 °C. The supernatants were filtered through microspin ultrafiltrates with a cut-off of 10,000 Mr before being taken for measurement of IDO. The amount of L-kynurenine formed from TRP was determined by reversed phase high performance liquid chromatography (HPLC). One hundred μl of the reaction product was injected onto a Merck LiChrospher column (150 mm long, 4.6 mm diameter, packed with 5 l m silica beads holding 18C long-carbon chains). A cartridge guard column containing the same material as the analytical column was used. The mobile phase consisted of 0.1 M ammonium acetate buffer (pH 4.65) with 5% acetonitrile. Flow rate was 1 ml/min. KYN was detected using a spectrometer measuring absorbency at a wavelength of 365 nm and was quantified using known amounts of L-kynurenine. The retention time of KYN was around 5.35 min. All determinations were performed in duplicate. One unit of the activity was defined as 1 nmol KYN/h/mg protein at 37 °C.

To measurement the KMO activity, the hippocampus tissue was homogenized 1:5 (wt/vol) in ultrapure water and further diluted 1:5 (vol/vol) in 100 mM Tris–HCl buffer (pH 8.1) containing 10 mM KCl and 1 mM EDTA. Eighty μl of the tissue preparation were incubated for 40 min at 37 °C in a solution containing 1 mM NADPH, 3 mM glucose-6-phosphate, 1 U/ml glucose-6 phosphate dehydrogenase, 100 μM kynurenine, 10 mM KCl and 1 mM EDTA in a total volume of 200 μL. The reaction was stopped by the addition of 50 μl of 6% perchloric acid. Blanks were obtained by adding the specific enzyme inhibitor Ro 61–8048 (100 μM) in the incubation solution. After centrifugation (16,000 × g, 15 min), 20 μl of the supernatant were applied to HPLC to measure 3-HK.

The KAT activity was made using the method previously described by Guidetti et al. [27]. Briefly, the hippocampus was harvested and homogenized in distilled water. After centrifugation (12,000 × g, 10 min), KAT activity was measured in a total volume of 200 μl, containing 80 μl of supernatant fluid, 150 mm Tris-acetate buffer, pH 7.4, 2 l m kynurenine, 1 mm pyruvate and 80 l m pyridoxal-5-phosphate. Samples were incubated for 24 h at 37 °C, and the reaction was terminated by adding 50% (w/v) trichloroacetic acid. After successive washes with 0.1 m HCl and distilled water, KYNA was eluted from the column with 2 × 1 mL of distilled water and quantified by HPLC.

2.12. Protein determination

Protein concentration was measured by the method Bradford [26], using bovine serum albumin as the standard.

2.13. Statistical analysis

The data distribution was verified by applying the Kolmogorov-Smirnov test. Results are presented as means ± standard error medium (SEM). Comparisons between the experimental and the control groups were performed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test or two-way ANOVA, followed by Bonferroni post hoc test, when appropriate. A value of $P < 0.05$ was considered to be statistically significant. All tests were carried out using the GraphPad Prism software 5.0 (San Diego, California, USA).

3. Results

3.1. Sucrose preference test (SPT), Open field test (OFT), tail suspension test (TST), body weight (BW) and corticosterone levels

The statistical analysis revealed no significant differences in basal sucrose preference test, sucrose preference test and OFT (HVF X LPS, $F_{1,24} = 0.07$, $p < 0.78$; Fig. 1A), (HVF X LPS, $F_{1,24} = 0.04$, $p < 0.83$; Fig. 1B) and (HVF X LPS, $F_{1,24} = 0.07$, $p < 0.79$; Fig. 1C) respectively.

Two-way ANOVA of TST, body weight and corticosterone serum levels showed no significant differences in HVF × LPS interaction ($F_{1,24} = 0.59$, $p < 0.45$; Fig. 1D), ($F_{1,24} = 0.00$, $p < 0.94$; Fig. 1E) and ($F_{1,24} = 2.02$, $p < 0.18$; Fig. 1F) respectively. Sickness behavior induced by LPS was not aggravated by dietary HVF in the SPT, OFT, TST, body weight and corticosterone serum levels compared to LPS group (Fig. 1A, B, 1C, 1D, 1E and 1F **respectively**).

3.2. Cytokine levels

Statistical analysis of IL-1β levels in the hippocampus, striatum and prefrontal cortex showed no significant HVF × LPS interaction ($F_{1,24} = 0.06$, $p < 0.80$; Table 1), ($F_{1,24} = 0.43$, $p < 0.52$; Table 1) and ($F_{1,24} = 2.18$, $p < 0.16$; Table 1), respectively.

In a similar manner, two-way ANOVA of IL-6 levels in the hippocampus, striatum and prefrontal cortex demonstrated no significant HVF × LPS interaction ($F_{1,24} = 1.77$, $p < 0.20$; Table 1), ($F_{1,24} = 0.22$, $p < 0.65$; Table 1) and ($F_{1,24} = 0.13$, $p < 0.72$; Table 1). *Post hoc* comparisons showed that HVF supplementation did not modulate the increase of IL-1β and IL-6 levels in brain structures (Table 1).

Statistical analysis of TNF-α levels in the hippocampus, striatum and prefrontal cortex revealed a no significant HVF × LPS interaction ($F_{1,24} = 2.82$, $p < 0.11$; Table 2), ($F_{1,24} = 4.37$, $p < 0.05$; Table 2) and ($F_{1,24} = 2.28$, $p < 0.15$; Table 2), respectively. In the hippocampus, striatum and prefrontal cortex, *post hoc* showed a HVF supplementation potentiated the increase of TNF-α levels induced by LPS (Table 2).

A two-way ANOVA of IFN-γ levels demonstrated no significant HVF × LPS interaction in hippocampus ($F_{1,24} = 0.01$, $p < 0.94$; Table 2) and striatum ($F_{1,24} = 1.99$, $p < 0.18$; Table 2). Statistical analysis of IFN-γ levels in the prefrontal cortex showed a significant HVF × LPS interaction ($F_{1,24} = 5.17$, $p < 0.04$; Table 2). *Post hoc* comparisons revealed that HVF supplementation did not modulate the increase of IFN-γ levels caused by LPS in prefrontal cortex of the mice (Table 2).

3.3. Serotonin (5-HT) and 5-Hydroxyindoleacetic acid (5-HIAA) levels

Statistical analysis of 5-HT levels in the hippocampus, striatum and prefrontal cortex showed no significant HVF × LPS interaction ($F_{1,24} = 0.87$, $p < 0.36$; Table 3), ($F_{1,24} = 0.18$, $p < 0.68$; Table 3) and ($F_{1,24} = 0.71$, $p < 0.41$; Table 3), respectively. *Post hoc* analysis yielded that HVF supplementation did not decrease 5-HT levels in brain structures (Table 3).

Two-way ANOVA of 5-HIAA levels in the hippocampus, striatum and prefrontal cortex revealed no significant HVF × LPS interaction ($F_{1,24} = 2.47$, $p < 0.14$; Table 3), ($F_{1,24} = 2.09$, $p < 0.17$; Table 3) and ($F_{1,24} = 1.56$, $p < 0.23$; Table 3), respectively. In the hippocampus, striatum and prefrontal cortex *post hoc* showed a HVF supplementation did not modulate to the decrease 5-HIAA levels caused by LPS (Table 3).

Statistical analysis of 5-HIAA/5-HT ratio in hippocampus, striatum and prefrontal cortex was not altered significantly by LPS injection ($F_{1,24} = 3.24$, $p < 0.09$; Table 3), ($F_{1,24} = 0.35$, $p < 0.56$; Table 3), ($F_{1,24} = 0.50$, $p < 0.61$; Table 3). In the brain structures *post hoc* demonstrated that dietary HVF did not alter 5-HIAA/5-HT ratio (Table 3).

3.4. Tryptophan (TRP) and kynurenine (KYN) levels

Statistical analysis of TRP levels in the hippocampus, striatum and prefrontal cortex revealed no significant HVF × LPS interaction ($F_{1,24} = 0.16$, $p < 0.69$; Table 4), ($F_{1,24} = 2.66$, $p < 0.12$; Table 4) and ($F_{1,24} = 1.62$, $p < 0.22$; Table 4), respectively. In the brain structures, *post hoc* analysis showed that dietary HVF was not able to further decrease of TRP levels induced by LPS in hippocampus of the mice (Table 4).

A two-way ANOVA of KYN levels in the hippocampus and prefrontal cortex demonstrated no significant HVF × LPS interaction ($F_{1,24} = 1.74$, $p < 0.21$; Table 4) and ($F_{1,24} = 1.17$, $p < 0.30$; Table 4). *Post hoc* comparisons revealed that HVF supplementation potentiated the increase of KYN levels caused by LPS in striatum of the mice (Table 4).

Statistical analysis of KYN/TRP ratio in the hippocampus and prefrontal cortex demonstrated no significant HVF × LPS interaction ($F_{1,24} = 0.83$, $p < 0.38$ and $F_{1,24} = 0.00$, $p < 0.97$, respectively; Table 4). Two-way ANOVA of KYN/TRP ratio in the striatum showed a significant HVF × LPS interaction ($F_{1,24} = 4.90$, $p < 0.04$; Table 4). *Post hoc* comparisons revealed that dietary HVF was potentiated the KYN/TRP ratio caused by LPS in striatum of the mice (Table 4).

3.5. Kynurenic acid levels (KYNA) and kynurenic acid/kynurenine (KYNA/KYN) ratio

Two-way ANOVA of KYNA levels in the hippocampus, striatum and prefrontal cortex did not reveal significant HVF × LPS interaction ($F_{1,24} = 2.45$, $p < 0.14$; Fig. 2A), ($F_{1,24} = 2.50$, $p < 0.14$; Fig. 2B) and ($F_{1,24} = 0.01$, $p < 0.94$; Fig. 2C), respectively.

Statistical analysis of KYNA/KYN ratio in the hippocampus and prefrontal cortex did not demonstrate significant HVF × LPS interaction ($F_{1,24} = 4.74$, $p < 0.06$; Fig. 2D) and ($F_{1,24} = 0.06$, $p < 0.80$; Fig. 2F). A two-way ANOVA of KYNA/KYN ratio in the striatum showed a significant HVF × LPS interaction ($F_{1,24} = 8.12$, $p < 0.01$; Fig. 2E). *Post hoc* comparisons revealed that HVF supplementation potentiated the decrease of KYNA/KYN ratio caused by LPS in striatum of the mice (Fig. 2E).

3.6. 3-Hydroxykynurenine (3-HK), quinolinic acid (QUIN) levels and 3-hydroxykynurenine/kynurenine (3-HK/KYN) ratio

Statistical analysis of 3-HK levels in the hippocampus, striatum and prefrontal cortex did not reveal significant HVF × LPS interaction ($F_{1,24} = 3.84$, $p < 0.07$; Fig. 3A), ($F_{1,24} = 0.01$, $p < 0.93$; Fig. 3B) and ($F_{1,24} = 2.00$, $p < 0.18$; Fig. 3C).

In a similar manner, two-way ANOVA of 3-HK/KYN ratio in the hippocampus, striatum and prefrontal cortex showed a not significant HVF × LPS interaction ($F_{1,24} = 3.84$, $p < 0.07$; Fig. 3D), ($F_{1,24} = 0.01$,

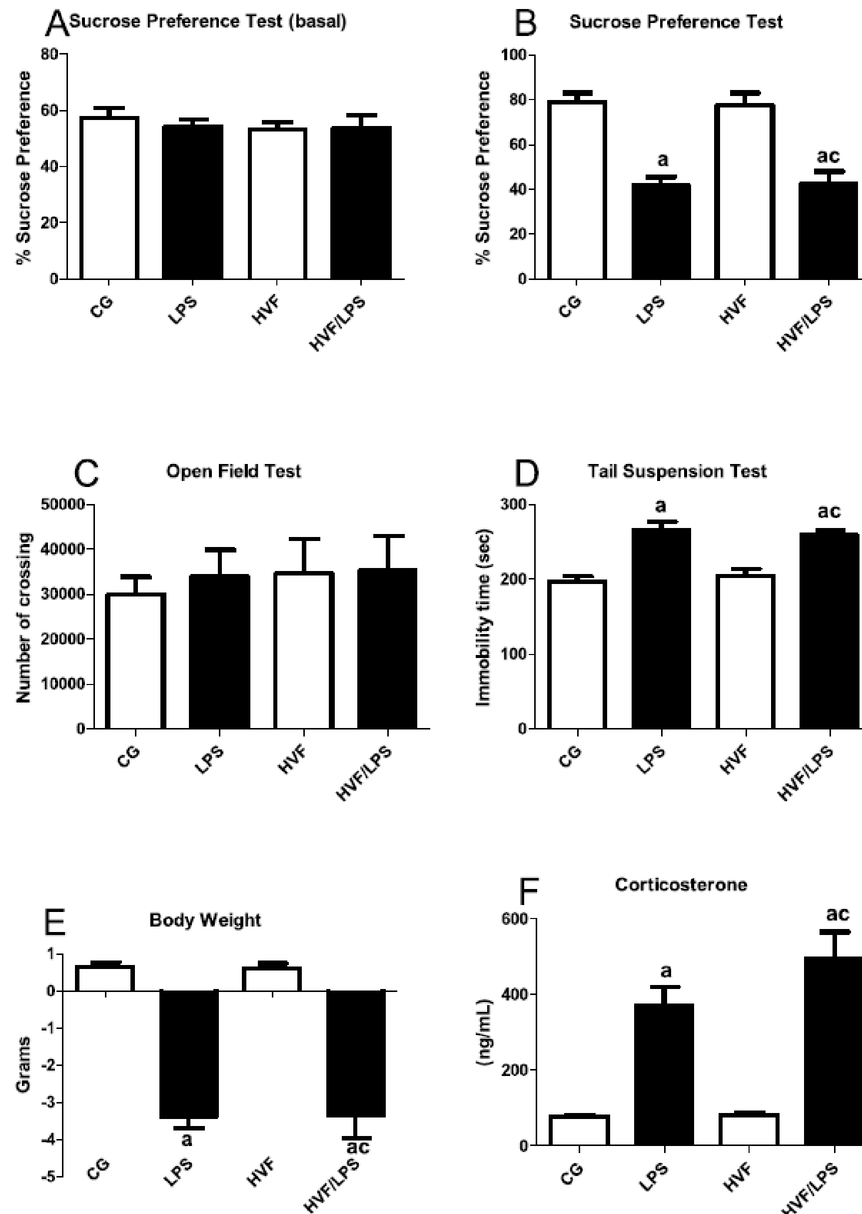

Fig. 1. Effect of HVF supplementation and LPS administration in the SPT, OFT, TST, body weight and corticosterone serum levels in aged mice. Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Bonferroni Multiple Comparison Test"). [a] $p < 0.05$ when compared to the control group (CG). [b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS). [c] $p < 0.05$ when compared the (HVF) and (HVF/LPS).

$p < 0.93$; Fig. 3E) and ($F_{1,24} = 2.00$, $p < 0.18$; Fig. 3F), respectively. In the hippocampus, striatum and prefrontal cortex, *post hoc* analysis showed that HVF supplementation did not cause a decrease in 3-HK/KYN ratio (Fig. 3E).

The statistical analysis of QUIN levels in the hippocampus, striatum and prefrontal cortex did not demonstrate significant HVF × LPS interaction ($F_{1,24} = 0.83$, $p < 0.37$; Fig. 3G), ($F_{1,24} = 0.02$, $p < 0.90$; Fig. 3H) and ($F_{1,24} = 1.24$, $p < 0.28$; Fig. 3I). In the hippocampus, striatum and prefrontal cortex, *post hoc* analysis showed that HVF supplementation potentiated the QUIN levels (Figs. 3G, H and I).

3.7. Indoleamine-2,3-dioxygenase (Ido), kynurenine 3-monoxygenase (KMO) and kynurenine aminotransferase (KAT) activities

Two-way ANOVA of IDO activity in hippocampus, striatum and prefrontal cortex did not demonstrate significant HVF × LPS

Table 1
Effect of supplementation with HVF on IL-1β and IL-6 levels in brain structures of mice in a sickness behavior model.

Groups	IL-1β (pg/mg)			IL-6 (pg/mg)		
	Hippocampus	Striatum	Prefrontal Cortex	Hippocampus	Striatum	Prefrontal Cortex
CG	78.75 ± 5.63	79.50 ± 6.22	85.50 ± 8.70	65.50 ± 7.32	24.00 ± 4.34	43.75 ± 2.98
LPS	735.3 ± 98.73[a]	687.0 ± 105.0[a]	683.3 ± 50.25[a]	220.8 ± 24.88[a]	219.5 ± 29.07[a]	267.3 ± 28.06[a]
HFV	120.0 ± 13.29	150.5 ± 22.07	125.3 ± 12.71	85.50 ± 2.78	39.75 ± 4.64	53.25 ± 9.69
HFV/LPS	807.3 ± 67.83[ac]	662.3 ± 100.1[ac]	510.3 ± 134.1[ac]	181.5 ± 36.05[a]	251.5 ± 18.42[ac]	264.5 ± 15.40[a]

Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Tukey's Multiple Comparison Test").
[a] $p < 0.05$ when compared to the control groups (CG).
[b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS).
[c] $p < 0.05$ when compared the (HVF) or (HVF/LPS) interactions.

Table 2
Effect of supplementation with HVF on TNF-α and IFN-γ levels in brain structures of mice in a sickness behavior model.

Groups	TNF-α (pg/mg)			IFN-γ (pg/mg)		
	Hippocampus	Striatum	Prefrontal Cortex	Hippocampus	Striatum	Prefrontal Cortex
CG	58.50 ± 11.38	34.75 ± 3.81	28.50 ± 5.33	34.00 ± 2.73	29.25 ± 2.78	58.75 ± 6.95
LPS	515.8 ± 68.38[a]	439.5 ± 40.81[a]	436.8 ± 37.67[a]	330.5 ± 38.57[a]	337.3 ± 44.82[a]	307.3 ± 55.80[a]
HVF	58.75 ± 11.12	71.00 ± 10.98	42.00 ± 8.58	42.25 ± 15.20	69.75 ± 12.75	107.3 ± 15.35
HVF/LPS	727.5 ± 104.6[abc]	634.8 ± 63.15[abc]	593.5 ± 86.58[abc]	334.8 ± 35.38[ac]	288.5 ± 42.67[ac]	207.0 ± 29.70[a]

Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Tukey's Multiple Comparison Test").
[a] $p < 0.05$ when compared to the control groups (CG).
[b] $p < 0.05$ when compared the (HFV/LPS) with the (LPS).
[c] $p < 0.05$ when compared the (HFV) or (HVF/LPS) interactions.

Table 3
Effect of supplementation with HVF on 5-HT and 5-HIAA metabolite levels in brain structures of mice in a sickness behavior model.

Groups	5-HT (ng/mg)			5-HIAA (ng/mg)			5-HIAA/5-HT ratio		
	Hippocampus	Striatum	Prefrontal Cortex	Hippocampus	Striatum	Cortex	Hippocampus	Striatum	Prefrontal Cortex
CG	406.3 ± 40.07	318.3 ± 20.95	268.3 ± 13.06	234.5 ± 13.70	188.0 ± 15.58	190.8 ± 17.11	0.60 ± 0.08	0.60 ± 0.07	0.71 ± 0.07
LPS	529.8 ± 36.19	434.0 ± 41.72	376.0 ± 13.64[a]	380.5 ± 14.57[a]	285.3 ± 10.11[a]	289.8 ± 6.21[a]	0.72 ± 0.05	0.67 ± 0.08	0.77 ± 0.03
HVF	445.0 ± 55.19	332.8 ± 34.44	304.8 ± 16.32	276.3 ± 35.09	240.5 ± 10.62[a]	242.8 ± 15.58	0.62 ± 0.04	0.74 ± 0.06	0.80 ± 0.06
HVF/LPS	645.8 ± 30.06[ac]	419.5 ± 37.66	386.3 ± 18.48[ac]	348.8 ± 23.56[ac]	297.8 ± 17.56[ac]	289.5 ± 33.0[a]	0.54 ± 0.02	0.72 ± 0.07	0.74 ± 0.07

Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Tukey's Multiple Comparison Test").
[a] $p < 0.05$ when compared to the control groups (CG).
[b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS).
[c] $p < 0.05$ when compared the (HVF) or (HVF/LPS) interactions.

interaction ($F_{1,24} = 1.69$, $p < 0.21$; Table 5), ($F_{1,24} = 0.00$, $p < 0.96$; Table 5) and ($F_{1,24} = 0.16$, $p < 0.69$; Table 5).

Similarly, statistical analysis of KMO activity in hippocampus, striatum and prefrontal cortex did not yield HVF × LPS interaction ($F_{1,24} = 0.32$, $p < 0.58$; Table 5), ($F_{1,24} = 0.33$, $p < 0.57$; Table 5) and ($F_{1,24} = 1.26$, $p < 0.28$; Table 5). In the striatum, *post hoc* analysis showed that HVF supplementation modulated the increase of KMO activity induced by LPS (Table 5).

A two-way ANOVA of KAT activity in hippocampus, striatum and prefrontal cortex did not reveal HVF × LPS interaction ($F_{1,24} = 0.06$, $p < 0.80$; Table 5), ($F_{1,24} = 4.46$, $p < 0.06$; Table 5) and ($F_{1,24} = 2.68$, $p < 0.12$; Table 5). In the hippocampus, striatum and prefrontal cortex, *post hoc* analysis showed that HVF supplementation was not modulate the KAT activity (Table 5).

4. Discussion

In the study, we investigated the effects of supplementation with HVF in the aggravation of sickness behavior induced by peripheral administration of LPS in aged mice. We found that sickness behavior caused by LPS is mediated by the increase of proinflammatory cytokines. In the behavioral parameters, LPS caused depressive-like behavior due to the excessively activation of KP in the brain of aged mice. On the other hand, dietary HVF was unable to modulate neuroinflammation and behavioral alterations in aged mice. However, HVF aggravated the KP activation induced by LPS.

Research has reported that IDO activation plays a key role in the precipitation of depressive states both in rodents and humans [28]. SPT is considered an indicator of a key symptom of depression, i.e., anhedonia, indicating loss of interest or pleasure [29]. The TST is a behavior paradigm predictive for depression, which is based on immobility time [20]. In addition, the depressive-like state in rodents is a feature of the pathophysiology of sickness behavior [30]. In our study, dietary HVF did not aggravate the alterations in SPT and TST caused by peripheral administration of LPS. The LPS-induced sickness behavior is probably mediated by IDO activation, in response to increase of proinflammatory cytokines in the brain [31].

A classic feature of sickness behavior is the loss of body weight [30]. It is known that sickness behavior can cause a great weight loss in rodents, reaching values higher than 5% [32]. In the present study, LPS caused a loss of 10–13% in the body weight of mice, but HVF supplementation did not change it. Corticosterone is a hormone that at elevated levels can generate a high catabolism or degradation of body mass. Here, we observed that the immune response caused by LPS

Table 4
Effect of supplementation with HVF on tryptophan and kynurenine levels in brain structures of mice in a sickness behavior model.

Groups	TRP (ng/mg)			KYN (ng/mg)			KYN/TRP ratio (*100)		
	Hippocampus	Striatum	Prefrontal Cortex	Hippocampus	Striatum	Cortex	Hippocampus	Striatum	Prefrontal Cortex
CG	4473 ± 208.5	3753 ± 12.8	3981 ± 146.4	79.50 ± 5.04	63.25 ± 5.20	66.75 ± 8.51	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
LPS	5717 ± 273.9^{a}	5685 ± 408.3^{a}	5011 ± 156.6	301.0 ± 43.69^{a}	266.3 ± 16.40^{a}	305.5 ± 19.12^{a}	5.00 ± 0.00^{a}	4.00 ± 0.00^{a}	6.00 ± 0.00^{a}
HVF	4945 ± 343.3	4252 ± 196.9	3954 ± 140.4	102.5 ± 16.54	91.50 ± 11.43	77.50 ± 10.57	2.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00
HVF/LPS	6399 ± 199.3^{ac}	5102 ± 467.2^{a}	5775 ± 565.0^{ac}	422.8 ± 58.22^{abc}	361.5 ± 19.29^{abc}	354.0 ± 25.90^{ac}	6.00 ± 1.00^{ac}	7.00 ± 0.00^{abc}	6.00 ± 0.00^{ac}

Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Tukey's Multiple Comparison Test").
[a] $p < 0.05$ when compared to the control groups (CG).
[b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS).
[c] $p < 0.05$ when compared the (HVF) or (HVF/LPS) interactions.

resulted in an increase of circulating corticosterone levels [32]. In addition, it is known that increased corticosterone levels are directly related to the elevation of proinflammatory cytokines [33]. In this context, the dietary HVF contributed to a greater increase in brain TNF-α levels, aggravating KP activation. Therefore, increased levels of corticosterone should mediate the results found for the weight loss in aged mice, as a result of energetic-protein depletion.

It is well described that one of the mechanisms by which LPS induces sickness behavior is through the increase of proinflammatory cytokines, mainly IL-1β, TNF-α, and IL-6 [34]. In our study, we found that HVF supplementation did not exacerbate the increase in the levels of IL-1β, IL-6 and IFN-γ in the hippocampus, striatum and prefrontal cortex of mice treated with LPS. On the contrary, HVF further increase TNF-α levels in these brain regions of LPS-treated mice. These results corroborate that increased levels of proinflammatory cytokines in response to LPS could be mediated by KP activation [30]. Thus, dietary HVF aggravates the elevation of TNF-α levels in the brain of aged mice, contributing to overstimulation of KP.

When the KP is overactivated, the TRP is used as substrate for pathway [16,35]. It has known that serotonergic system is involved with the depressive and anhedonic-like behaviors, where the TRP is required for 5-HT synthesis. An essential role of brain serotonergic system was related to anorexia, which is another behavioral phenomenon observed in a few hours after LPS administration [36]. In our study, we observed that 5-HT levels in the hippocampus and prefrontal cortex in conjunction with its metabolite 5-HIAA in all brain regions were higher when LPS was administered. On the other hand, HVF supplementation did not increased the levels of 5-HT and 5-HIAA in the brain of mice. These results are consistent with previous data where LPS-induced neuroinflammation provoked an increase in brain 5-HIAA levels, indicating an increase of 5-HT turnover [37]. Therefore, dietary HVF did not cause or aggravate serotonergic dysfunction in response to LPS administration in aged mice.

The IDO induction has been linked to depressive-like complications in rodents and humans [38]. A previous work of our laboratory has demonstrated that IDO is an important mediator of neuroinflammation and neurobehavioral disturbances in a model of Alzheimer's disease induced by amyloid-beta (1–42) peptide in mice [35]. IDO is activated by proinflammatory cytokines and provides the initial enzymatic activity in the degradation of TRP in KP. In the present study, we demonstrated that LPS induced an increase in IDO activity in the hippocampus, striatum and prefrontal cortex of mice. This increase in IDO activity could be responsible for the increase in the levels of TRP, KYN and its KYN/TRP ratio in all brain regions analyzed of the LPS-treated mice. Once again, dietary HVF did not cause IDO induction neither TRP and KYN alterations.

It has been described that inflammation-related KYN pathway activation results in KYN production and its neuroactive metabolites [39]. The metabolite KYN can undergo the action of the enzyme kynurenine amino-transferase (KAT) generating kynurenic acid (KYNA). KYNA is an endogenous TRP derivative, which multiple central actions have emerged within last years, including its neuroprotective effect [40]. In addition, kynurenine 3-monooxygenase (KMO) is used to cleave KYN to 3-hydroxykynurenine (3-HK), which subsequently generates quinolinic acid (QUIN). Increased QUIN concentrations have also been described in *postmortem* brains of patients with inflammatory-infectious disorders (bacterial, viral, autoimmune diseases and septicemia) [41]. It is known that increased levels of QUIN in the brain means a potent neuroinflammation. In our study, dietary HVF did not modulate KAT activity and KYNA levels in the brain regions studied. However, dietary HVF aggravated the decrease of KYNA/KYN ratio in the brain of mice, an important marker of neuroprotective branch of KP. In addition, the dietary HVF aggravated the increase of brain KMO activity and 3-HK and QUIN levels induced by LPS. Our results are in line with previous studies reporting that QUIN is related to the development of depressive symptomatology in both laboratory animals and depressed patients

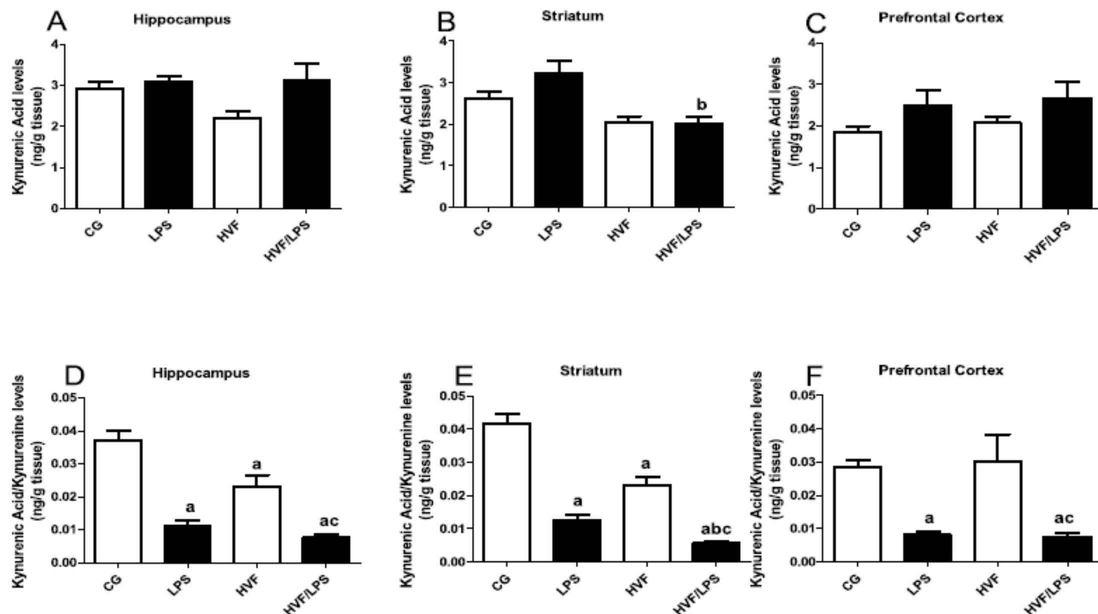

Fig. 2. Effect of HVF supplementation in KYNA and KYNA/KYN ratio levels in the brain structures of aged mice. Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Bonferroni Multiple Comparison Test"). [a] $p < 0.05$ when compared to the control group (CG). [b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS). [c] $p < 0.05$ when compared the (HVF) and (HVF/LPS).

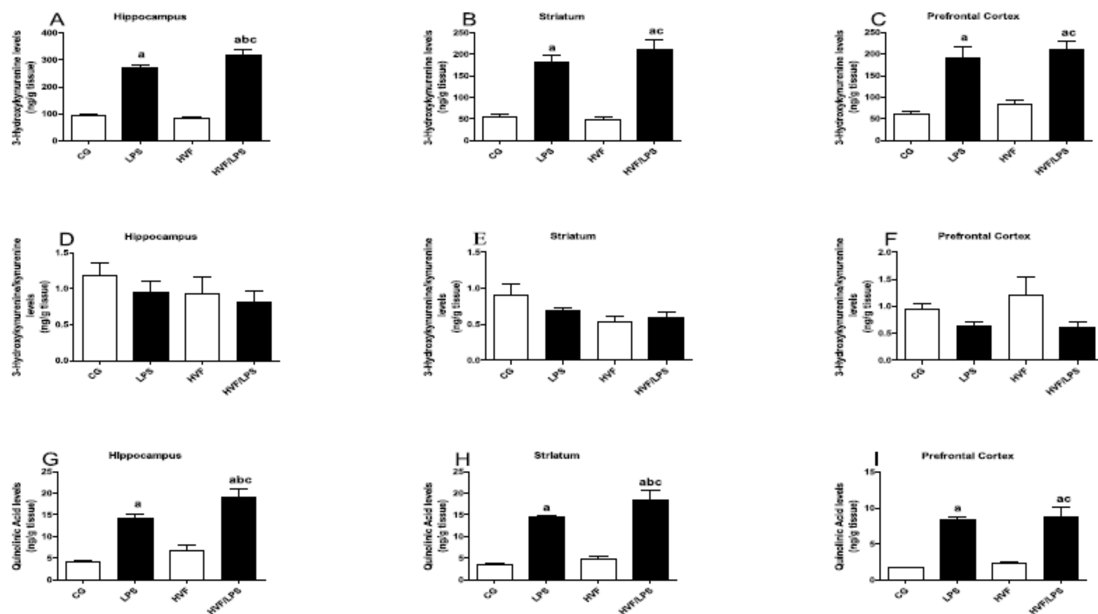

Fig. 3. Effect of HVF supplementation in 3-HK, 3-HK/KYN ratio and QUIN levels in the brain structures of aged mice. Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Bonferroni Multiple Comparison Test"). [a] $p < 0.05$ when compared to the control group (CG). [b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS). [c] $p < 0.05$ when compared the (HVF) and (HVF/LPS).

Table 5
Effect of supplementation with HVF on IDO, KMO and KAT activities in brain structures of mice in a sickness behavior model.

Groups	IDO (Pmol/h/mg protein)			KMO (Pmol/h/mg protein)			KAT (Pmol/h/mg protein)		
	Hippocampus	Striatum	Prefrontal Cortex	Hippocampus	Striatum	Cortex	Hippocampus	Striatum	Prefrontal Cortex
CG	107.8 ± 6.12	115.0 ± 13.02	95.25 ± 4.66	114.3 ± 7.15	98.75 ± 5.15	100.3 ± 3.54	99.00 ± 7.64	94.00 ± 4.20	102.0 ± 3.39
LPS	252.0 ± 25.11[a]	296.8 ± 18.46[a]	199.3 ± 22.15[a]	212.0 ± 24.42[a]	164.8 ± 4.88[a]	156.8 ± 4.09[a]	107.8 ± 3.03	119.5 ± 5.5[a]	122.8 ± 6.19[a]
HVF	135.5 ± 7.07	139.5 ± 7.06	91.75 ± 3.19	129.5 ± 6.31	135.8 ± 7.97[a]	138.0 ± 6.64[a]	79.50 ± 5.04	73.00 ± 4.41[a]	89.50 ± 5.20
HVF/LPS	238.5 ± 17.05[ac]	300.0 ± 48.26[ac]	194.5 ± 18.55[ac]	244.5 ± 15.39[ac]	219.8 ± 11.96[abc]	181.0 ± 21.74[a]	85.50 ± 4.87[b]	84.25 ± 2.86[b]	84.75 ± 8.29[b]

Data are expressed as means ± S.E.M of 6 animals per group (Two-way ANOVA/"Tukey's Multiple Comparison Test").
[a] $p < 0.05$ when compared to the control groups (CG).
[b] $p < 0.05$ when compared the (HVF/LPS) with the (LPS).
[c] $p < 0.05$ when compared the (HVF) or (HVF/LPS) interactions.

[42,43]. Thus, this study provides evidence tha HVF consumption further increase the shift towards neurotoxic branch of KP elicited by LPS, mainly due to the excessive generation of QUIN.

In conclusion, our study reinforces a critical role for KP metabolism in mediating sickness-behavior induced by LPS in aged mice. Despite dietary HVF did not aggravate sickness symptomatology, it has been exacerbated KP activation probably due to the further increase in TNF-α levels in the brain of LPS-treated mice. Thus, although did not cause any additional behavioral deficits, prolonged consumption of HVF may aggravate neuroinflammation and the generation of KP-derived neurotoxic metabolites that can generate brain damages associated to CNS disorders.

Conflicts of interest

The authors declare that there are no conflicts of interest in the present work.

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References

[1] N.T. Ashley, Q.R. Hays, G.E. Bentley, J.C. Wingfield, Testosterone treatment diminishes sickness behavior in male songbirds, Horm. Behav. 56 (2009) 169–176.
[2] R. Dantzer, J.C. O'Connor, G.G. Freund, R.W. Johnson, K.W. Kelley, From inflammation to sickness and depression: when the immune system subjugates the brain, Nat. Rev. Neurosci. 9 (2008) 46–56.
[3] M. Rosas-Ballina, P.S. Olofsson, M. Ochani, S.I. Valdés-Ferrer, Y.A. Levine, C. Reardon, M.W. Tusche, V.A. Pavlov, U. Andersson, S. Chavan, Acetylcholine synthesizing T cells relay neural signals in a vagus nerve circuit, Science 334 (2011) 98–101.
[4] L. Steinman, Elaborate interactions between the immune and nervous systems, Nat. Immunol. 5 (2004) 575–581.
[5] E.E. Benarroch, Circumventricular organs receptive and homeostatic functions and clinical implications, Neurology 77 (2011) 1198–1204.
[6] E. Haroon, C.L. Raison, A.H. Miller, Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior, Neuropsychopharmacology 37 (2012) 137–162.
[7] A. Salazar, B.L. Gonzalez-Rivera, L. Redus, J.M. Parrott, J.C. O'Connor, Indoleamine 2,3-dioxygenase mediates anedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide imune challenge, Horm. Behav. 62 (2012) 202–209.
[8] F.S. Osso, A.S. Moreira, M.T. Teixeira, R.O. Pereira, M.D. Tavares do Carmo, A.S. Moura, Trans fatty acids in maternal milk lead to cardiac insulin resistance in adult offspring, Nutrition 24 (2008) 727–732.
[9] L.A.T. Santos, R. Cruz, S. Casal, Trans fatty acids in commercial cookies and biscuits: an update of Portuguese market, Food Contr. 47 (2015) 141–146.
[10] E. Larqué, P.A. García-Ruiz, F. Perez-Lamas, S. Zamora, A. Gil, Dietary trans fatty acids alter the compositions of microsomes and mitochondria and the activities of microsome delta 6-fatty acid desaturase and glucose-6 phosphatase in livers of pregnant rats, J. Nutr. 133 (2003) 2526–2531.
[11] D.J. Baer, J.T. Judd, B.A. Clevidence, R.P. Tracy, Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study, Am. J. Clin. Nutr. 79 (2004) 969–973.
[12] A. Stahl, D. Begg, R. Welsinger, A. Sinclair, The role of omega-3 fatty acids in mood disorders, Curr. Opin. Invest. Drugs 9 (2008) 57–64.
[13] L.C. Souza, C.R. Jesse, L. Del Fabbro, M.G. de Gomes, N.S. Gomes, C.B. Filho, A.T.R. Goes, E.A. Wilhelm, C. Luchese, S.S. Roman, S.P. Boeira, Aging exacerbates cognitive and anxiety alterations induced by an intracerebroventricular injection of amyloid-β1-42 peptide in mice, Mol. Cell. Neurosci. 88 (2018) 93–106.
[14] F. Trevizol, V.T. Dias, K. Roversi, R.C.S. Barcelos, F.T. Kuhn, K.R. Roversi, C.S. Pase, R. Golombieski, J.C. Veit, J. Piccolo, T. Emanuelli, J.B.T. Rocha, M.E. Bürger, Cross-generational trans fat intake modifies BDNF mRNA in the hippocampus: impact on memory loss in a mania animal model, Hippocampus 5 (2014) 556–565.
[15] J.P. Godbout, J. Chen, J. Abraham, A.F. Richwine, B.M. Berg, K.W. Kelley, R.W. Johnson, Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system, Faseb. J. 10 (2005) 1329–1331.

[16] R. Dantzer, Cytokine-induced sickness behavior: mechanisms and implications, Ann. N. Y. Acad. Sci. 933 (2011) 222–234.
[17] S. Kent, R.M. Bluthé, K.W. Kelley, R. Dantzer, Sickness behavior as a new target for drug development, Trends Pharmacol 1 (1992) 24–28.
[18] A.J. Grippo, D. Gerena, J. Huang, N. Kumar, M. Shah, R. Ughreja, C.S. Carter, Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles, Psychoneuroendocrinology 32 (2007) 966–980.
[19] R. Tiziana, V. Daniela, R. Natalia, G. Cinzia, B. Daniela, C. Valeria, C. Chiara, C. Francesca, R. Patrizia, C. Sanzio, S. Mariaelvina, P. Daniela, Chronic δ9 tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates, Neuropsychopharmacology 33 (2008) 2760–2771.
[20] L. Steru, R. Chermat, B. Thierry, P. Simon, The tail suspension test: a new method for screening antidepressants in mice, Psychopharmacology 85 (1985) 367–370.
[21] S. Spijker, Dissection of rodent brain regions, in: Ka Wan Li (Ed.), Neuroproteomics (Neuromethods), Springer Science+Business Media, New York, 2011, pp. 13–26.
[22] R.W. Williams, The Mouse Brain Library, (1999) http://www.mbl.org/atlas165/atlas165_start.html.
[23] N.M. Silva, C.V. Rodrigues, M.M. Santoro, L.F. Reis, J.I. Alvarez-Leite, R.T. Gazzinelli, Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with Toxoplasma gondii: induction by endogenous gamma interferon and requirement of interferon regulatory factor1, Infect. Immun. 70 (2002) 859–868.
[24] A. Harkin, T.J. Connor, M. Walsh, N. St John, J.P. Kelly, Serotonergic mediation of the antidepressant-like effects of nitric oxide synthase inhibitors, Neuropharmacology 5 (2003) 616–623.
[25] M. Sono, Enzyme kinetic and spectroscopic studies of inhibitor and effector interactions with indoleamine 2,3-dioxygenase, Evidence for the existence of another binding site in the enzyme for indole derivative effectors, Biochemistry 13 (1989) 5400–5407.
[26] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[27] P. Guidetti, E. Okuno, R. Schwarcz, Characterization of rat brain kynurenine aminotransferases I and II, J. Neurosci. Res. 50 (1997) 457–465.
[28] J.C. O'Connor, C. Andre, Y. Wang, M.A. Lawson, S.S. Szegedi, J. Lestage, N. Castanon, K.W. Kelley, R. Dantzer, Interferon-gamma and tumor necrosis factor alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette Guerin, J. Neurosci. 29 (2009) 4200–4209.
[29] B. Kumar, A. Kuhad, K. Chopra, Neuropsychopharmacological effect of sesamol in unpredictable chronic mild stress model of depression: behavioral and biochemical evidences, Psychopharmacology 214 (2011) 819–828.
[30] R. Dantzer, Cytokine, sickness behavior, and depression, Neurol. Clin. 24 (2006) 441–460.
[31] R. Dantzer, J.C. O'Connor, M.A. Lawson, K.W. Kelley, Inflammation associated depression: from serotonin to kynurenine, Psychoneuroendocrinology 36 (2011) 426–436.
[32] H.R. Leite, O.C. Oliveira-Lima, L.M. Pereira, V.E.M. Oliveira, V.F. Prado, G.S. Pereira, M.A.M. Prado, A.R. Massensini, Vesicular acetylcholine transporter knock down-mice are more susceptible to inflammation, c Fos expression and sickness behavior induced by lipopolysaccharide, Brain Behav. Immun. 57 (2016) 282–292.
[33] M. Maes, B.E. Leonard, A.M. Myint, M. Kubera, R. Verkerk, The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamina 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the on set of depression, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35 (2011) 702–721.
[34] M.J. Eimerbrink, D. Kranjac, C. St Laurent, J.D. White, M.K. Weintraub, R.J. Pendry, R. Madigan, S.L. Hodges, L.N. Sadler, M.J. Chumley, G.W. Boehm, Pre treatment of C57BL6/J mice with the TLR4 agonist monophosphoryl lipid A prevents LPS-induced sickness behaviors and elevations in dorsal hippocampus interleukin-1β, independent of interleukin-4, Behav. Brain Res. 302 (2016) 171–174.
[35] L.C. Souza, C.R. Jesse, M.S. Antunes, J.R. Ruff, D.O. Espinosa, N.S. Gomes, F. Donato, R. Giacomeli, S.P. Boeira, Indoleamine-2,3-dioxygenase mediates neurobehavioral alterations induced by an intracerebroventricular injection of amyloid-β1-42 peptide in mice, Brain Behav. Immun. 56 (2016) 363–377.
[36] L. Asarian, W. Langhans, A new look on brain mechanisms of acute illness anorexia, Physiol. Behav. 100 (2010) 464–471.
[37] M.P. Heyes, B.J. Quearry, S.P. Markey, Systemic endotoxin increases L tryptophan, 5-hydroxyindoleacetic acid, 3-hydroxykynurenine and quinolinic acid content of mouse cerebral cortex, Brain Res. 491 (1989) 173–179.
[38] L. Capuron, S. Schroecksnadel, C. Feart, A. Aubert, D. Higueret, P. Barberger Gateau, S. Laye, D. Fuchs, Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms, Biol. Psychiatr. 70 (2011) 175–182.
[39] T.W. Stone, L. Darlington, Endogenous kynurenines as targets for drug discovery and development, Nat. Ver. Drug Discov 1 (2002) 609–620.
[40] E. Luchowska, R. Kloc, B. Olajossy, S. Wnuk, M. Wielosz, B. Owe-Larsson, E.M. Urbanska, B adrenergic enhancement of brain kynurenic acid production mediated via cAMP-related protein kinase A signaling, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33 (2009) 519–529.
[41] V.P. La Cruz, M. Königsberg, A. Santamaría, Kynurenine pathway and disease: an overview, CNS Neurol. Disord. - Drug Targets 6 (2007) 398–410.
[42] A. Salazar, B.L. Gonzalez-Rivera, L. Redus, J.M. Parrott, J.C. O'Connor, Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge, Horm. Behav. 62 (2012) 202–209.
[43] C.L. Raison, R. Dantzer, K.W. Kelley, M.A. Lawson, B.J. Woolwine, G. Vogt, J.R. Spivey, K. Saito, A.H. Miller, CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and Depression, Mol. Psychiatr. 15 (2010) 393–403.

4. DISCUSSÃO

Este estudo mostrou o efeito neuroprotetor da suplementação com AGPI n-3 e a potencialização dos efeitos neuroinflamatórios da suplementação com GVH em um modelo de comportamento doentio induzido por LPS em camundongos idosos. Assim, os camundongos foram expostos a suplementação diária pelo período de 24 semanas consecutivas. Nesse sentido, somos os pioneiros na literatura científica a demonstrar o efeito farmacológico da suplementação crônica com AGPI n-3 e os efeitos deletérios da suplementação crônica com GVH no modelo de comportamento doentio. Ainda, demonstramos os efeitos comportamentais causados pelas suplementações nos animais. Além disso, mostramos como diferentes ácidos graxos, oriundos de fonte dietética são capazes de mediar a ativação da via da quinurenina em camundongos idosos, os quais já se apresentavam imunocomprometidos, devido ao processo fisiológico de envelhecimento.

Indução do comportamento doentio pelo LPS causando alterações hormonais e comportamentais

Nesse contexto, a injeção de LPS induziu ao comportamento doentio nos camundongos idosos. Um dos principais sintomas do comportamento doentio ao organismo, é rápida e grande perda de peso corporal que a doença ocasiona. Em nosso estudo, o comportamento doentio causou uma perda de peso corporal de 10-13% nos animais. A suplementação com AGPI n-3 foi capaz de proteger contra essa drástica perda de peso corporal causada pelo LPS nos camundongos idosos, enquanto a suplementação com GVH não modificou a perda de peso corporal causada no comportamento doentio. Sabe-se, que o comportamento doentio pode causar uma grande perda de peso em roedores, geralmente superior a 5% (DANTZER, 2006), e que a perda de peso é uma característica clássica do comportamento doentio (LEITE et al., 2016).

Nosso estudo, está de acordo com a resposta clássica inicial causada no comportamento doentio. No intuído de corroborar com esse achado, verificamos os níveis de corticosterona (artigo 2). Pois, a corticosterona é um hormônio que em alto níveis, gera um alto catabolismo energético, que culmina em uma grande perda de peso corporal. Assim, a injeção intraperitoneal de LPS causou um aumento nos

níveis séricos de corticosterona, níveis os quais a suplementação com GVH não alterou o aumento causado pelo LPS. Além disso, sabe-se que o aumento dos níveis de corticosterona está diretamente relacionado à elevação das citocinas pró-inflamatórias (MAES et al., 2011). Portanto, acreditamos que a perda de peso está relacionada diretamente com aumento nas citocinas pró-inflamatórias. Como por exemplo, o grande aumento nos níveis de TNF-α causados pela suplementação com GVH, agravando a ativação da via da quinurenina. Ainda nesse contexto, não fizemos a mensuração dos níveis de corticosterona no estudo com a suplementação com AGPI n-3. Porém, a suplementação com AGPI n-3 foi eficaz em proteger contra o aumento das citocinas pró-inflamatórias causado pelo comportamento doentio. Sendo que a suplementação evitou a perda de peso corporal nos animais. Assim, a modulação dos níveis das citocinas pró-inflamatórias por parte das suplementações é essencial para que ocorra a ativação da via da quinurenina e toda a coleção de sintomas não específicos causados pelo comportamento doentio.

Nesse contexto, a alteração da função locomotora após 24 horas da injeção de LPS é um dos primeiros sintomas comportamentais da doença. A redução da atividade locomotora é realizada pois é uma maneira de se comprovar o comportamento doentio induzido pelo LPS (DANTZER, 2011). Assim, a injeção com LPS reduziu drasticamente a atividade locomotora dos animais, sendo essa mensuração realizada no teste do campo aberto (artigo 1). Esse resultado em conjunto com a perda de peso, corrobora com os achados de um estudo similar realizado por DANTZER, 2006. A suplementação com AGPI n-3, foi capaz de impedir a diminuição da atividade locomotora no teste do campo aberto induzida pelo LPS, bloqueando um dos principais sintomas do comportamento doentio. Esse resultado, em conjunto com a inibição da perda de peso causada pela suplementação com AGPI n-3, demonstram a efetividade em bloquear os principais sintomas, que validam o modelo de comportamento doentio induzido pelo LPS.

Ainda, via mensuração do comportamento doentio, a injeção com LPS não causou uma redução na atividade locomotora dos animais (artigo 2). Porém, causou uma grande perda de peso dos animais, assim os animais exibiram um sintoma dos dois os quais são usados para a validação e mensuração do comportamento doentio. Em relação, ao LPS não causar alteração na atividade locomotora no teste do campo aberto, nosso estudo corroborou com os resultados encontrados no estudo de DANG et al., 2018, o qual mimetizou o comportamento tipo-depressivo em

ratos por repetidas injeções com LPS. Diante do exposto, a suplementação com GVH não alterou também a atividade locomotora dos camundongos no teste do campo aberto. Porém, sabíamos que a suplementação crônica com GVH gera um quadro de ansiedade em roedores (TREVIZOL et al., 2014) e que o modelo de comportamento doentio causa comportamento tipo-depressivo em roedores (DANTZER, 2006).

Assim, realizamos o teste de suspenção de cauda (artigo 2), o qual é um teste preditivo para depressão (STERU et al., 1985). A injeção com LPS aumentou o tempo de imobilidade dos animais no teste de suspenção de cauda, indicando um comportamento tipo-depressivo o qual também faz parte dos sintomas causados pelo comportamento doentio. Quando suplementados com GVH, os animais apresentaram um aumento na imobilidade no teste de suspenção de cauda, não sendo capaz de modular o comportamento tipo-depressivo causado pelo LPS. Deste modo, nossos resultados corroboram com os estudos de DANTZER 2006 e 2011, demonstrando o comportamento tipo-depressivo causado no modelo de comportamento doentio.

Ainda, investigando o comportamento tipo-depressivo causado pela administração de LPS a qual induz o comportamento doentio, realizamos o teste de preferência da sacarose. O teste de preferência da sacarose é um modelo que mimetiza o comportamento tipo-anedônico em animais, sendo a anedonia um sintoma central da depressão (TIZIANA et al., 2008). Dessa maneira, a administração do LPS causou uma diminuição no consumo de sacarose pelos camundongos (artigo 1), caracterizando o comportamento tipo-anedônico nos animais. Já quando os animais foram suplementados com AGPI n-3, a suplementação impediu a diminuição do consumo de sacarose causado pelo LPS e consequentemente impediu a manifestação do comportamento tipo-anedônico. Novamente, a suplementação com AGPI n-3 impediu a manifestação comportamental relacionada aos sintomas de depressão em roedores. Resultados similares também foram obtidos no estudo de DANG et al., 2018, em que a suplementação com AGPI n-3 impediu parcialmente a diminuição no consumo de sacarose em ratos induzida por LPS.

Também seguimos essa linha de raciocínio e investigamos o comportamento tipo-anedônico no artigo 2. Conforme o esperado, a administração do LPS reduziu o consumo de sacarose nos animais, no teste de preferência da sacarose. Quando os

animais foram suplementados com GVH, a suplementação foi incapaz de impedir a diminuição do consumo de sacarose causada pelo LPS. Assim, a suplementação com GVH não alterou a manifestação do comportamento tipo-anedônico nos animais. Junto com o resultado do teste de suspenção de cauda, fica claro que a suplementação com GVH não é capaz de evitar os sintomas depressivos causados pelo comportamento doentio. Em um estudo realizado por DANTZER et al., 2008, demonstra a relação das citocinas inflamatórias nos sintomas depressivos, apresentados em reposta ao comportamento doentio induzido pelo LPS. Diante do exposto, ressaltamos a relação entre as suplementações e o efeito direto nos níveis das citocinas pró-inflamatórias, que acaba por modular a ativação da via da quinurenina, com ação direta nos sintomas comportamentais causados pelo comportamento doentio.

Envolvimento do sistema serotoninérgico nas alterações comportamentais no modelo de comportamento doentio

Ainda, relacionado as alterações comportamentais causadas pelo comportamento doentio induzido pela administração de LPS nos camundongos idosos, sabemos que o sistema serotoninérgico está envolvido com os comportamentos tipo-depressivo e anedônico, onde o TRP é necessário para a síntese de 5-HT. Sendo que, alguns estudos já observaram o aumento nos níveis cerebrais do TRP e do metabolismo da 5-HT em resposta ao LPS (SCHNEIDERS et al., 2015; SENS et al., 2017). Um papel essencial do sistema serotoninérgico está relacionado à anorexia, que é outro fenômeno comportamental observado em poucas horas após a administração de LPS em roedores (ASARIAN et al., 2010). De maneira geral, a injeção de LPS causou um aumento nos níveis de 5-HT e seu metabólito ácido 5-hidroxi-indolacético (5-HIAA) nas três estruturas cerebrais (hipocampo, estriado e córtex pré-frontal) dos camundongos idosos (artigo 1). Quando os animais foram expostos a suplementação com AGPI n-3, acarretou na proteção contra o aumento dos níveis de 5-HT e 5-HIAA causado pelo LPS nas estruturas cerebrais.

Dessa maneira, quando administrada a injeção com LPS, acarretou em um aumento nos níveis de 5-HT no córtex pré-frontal e aumento nos níveis de 5-HIAA no hipocampo, estriado e córtex pré-frontal dos animais (artigo 2). A suplementação

com GVH não modulou o aumento nos níveis de 5-HT e 5-HIAA causados pelo LPS e ainda potencializou o aumento nos níveis de 5-HT no hipocampo dos camundongos idosos. Tomados em conjunto, esses dados indicam que a suplementação com AGPI n-3 está exercendo um efeito neuroprotetor e impedindo as alterações comportamentais causadas pelo comportamento doentio via a modulação do sistema serotoninérgico. Para a suplementação com GVH, não houve uma modulação nos níveis de 5-HT e 5-HIAA. Assim, a suplementação com GVH foi incapaz de proteger contra as alterações comportamentais induzidas pelo LPS, pois não mediou a modulação do sistema serotoninérgico o qual está diretamente relacionado com o comportamento tipo-depressivo apresentado pelos animais idosos.

A ação das citocinas pró-inflamatórias mediando o estabelecimento do comportamento doentio e a ativação da enzima IDO

Em adição, a administração de LPS modifica o sistema imunológico através do aumento dos níveis plasmáticos de citocinas pró-inflamatórias (IL-1β, IL-6, TNF-α e IFN-γ) (BASU et al., 2016; CAI et al., 2016). Acredita-se que essas citocinas desempenham um papel fundamental na ligação entre a ativação do sistema imunológico e o comportamento doentio (CAI et al., 2016). Corroborando, em nosso estudo 24 horas após a administração do LPS nos camundongos idosos gerou um aumento expressivo nos níveis das citocinas pró-inflamatórias IL-1β, IL-6, TNF-α e IFN-γ, no hipocampo, estriado e córtex pré-frontal (artigo 1). Diante da suplementação crônica com AGPI n-3 nos animais, acarretou em uma atenuação do aumento dos níveis dessas citocinas (IL-1β, IL-6, TNF-α e IFN-γ), nas três estruturas cerebrais estudas.

Novamente, no artigo 2, quando administrado LPS nos camundongos idosos gerou um grande aumento nos níveis das citocinas (IL-1β, IL-6, TNF-α e IFN-γ), no hipocampo, estriado e córtex pré-frontal. A suplementação com GVH, foi incapaz de impedir o aumento nos níveis das citocinas pró-inflamatórias IL-1β, IL-6 e IFN-γ no hipocampo, estriado e córtex pré-frontal dos animais causados pela administração do LPS. Ainda, a suplementação com GVH potencializou um aumento ainda maior nos níveis de TNF-α alterados pelo LPS, nas três estruturas cerebrais (hipocampo, estriado e córtex pré-frontal). Assim, de maneira geral a suplementação com AGPI n-

3 protegeu contra o aumento dos níveis das citocinas pró-inflamatórias nas estruturas cerebrais estudadas. Resultados similares foram encontrados nos estudos de MINGAM et al., 2008; LIU et al., 2015 e DANG et al., 2018. Corroborando com os dados comportamentais, essa modulação das citocinas atenuou o comportamento doentio e consequentemente houve uma menor ativação da via da quinurenina, acreditamos que esses mecanismos estão envolvidos com a neuroproteção apresentada pela suplementação com AGPI n-3. A suplementação com GVH, não foi capaz de modular o aumento nos níveis das citocinas pró-inflamatórias alterados pela administração de LPS. Pelo contrário a suplementação com GVH potencializou o aumento nos níveis de TNF-α no hipocampo, estriado e córtex pré-frontal dos animais. Esse aumento excessivo nos níveis de TNF-α e a não modulação das demais citocinas pró-inflamatórias está diretamente relacionado com o aumento nos níveis de corticosterona encontrados. Assim, tomados em conjunto os dados indicam que a suplementação com GVH, foi incapaz de proteger contra a neuroinflamação causada pelo LPS nas estruturais cerebrais e acabou contribuindo para aumentar neuroinflamação via o aumento nos níveis de TNF-α. Acreditamos que essa incapacidade de proteção contra o aumento das citocinas pró-inflamatórias e potencialização (TNF-α), contribuiu para uma exacerbação do comportamento doentio nos animais.

Hipotetizou-se que as citocinas pró-inflamatórias elevadas servem como marcadoras da ativação da via da quinurenina no cérebro (MAES et al., 2011). Quando a via de quinurenina sofre uma ativação excessiva, o TRP é utilizado como substrato para via (DANTZER, 2011; SOUZA et al., 2016). Assim, quando administrado LPS nos camundongos idosos gerou um drástico aumento nos níveis de TRP, junto com aumento do seu metabólito a quinurenina (artigo 1). De maneira similar, o LPS também gerou um aumento na razão do aminoácido e seu metabólito no hipocampo, estriado e córtex pré-frontal. A suplementação com AGPI n-3 foi capaz de proteger contra o aumento dos níveis de TRP, quinurenina e razão TRP/quinurenina nas três estruturas cerebrais estudadas. Resultado similar foi encontrado no estudo de DANG et al., 2018, porém nesse estudo foi analisado o conteúdo de TRP e quinurenina apenas no córtex pré-frontal. Já para o estudo com GVH, quando administrado o LPS novamente gerou um aumento nos níveis de TRP e quinurenina e na razão TRP/quinurenina nas estruturas cerebrais estudadas. A suplementação crônica com GVH foi incapaz de proteger contra o aumento dos

níveis de TRP, quinurenina e aumento da razão TRP/quinurenina alterados pela administração de LPS no hipocampo, estriado e córtex pré-frontal dos animais idosos. Dessa maneira, demonstramos que o LPS induziu um aumento nos níveis de TRP e quinurenina, nos levando a inferir que a via da quinurenina estava sofrendo uma ativação excessiva. Diante disso, a suplementação com AGPI n-3 foi capaz de proteger contra o aumento dos níveis de TRP e quinurenina e a suplementação com GVH não teve esse efeito. Assim, acreditamos que um dos mecanismos pelo os quais os AGPI n-3 exercem neuroproteção, seria justamente por modular a neuroinflamação gerada pelo LPS no modelo de comportamento doentio. Via a menor ativação da via de quinurenina, pois diminuiu os níveis do substrato para ativação da via da quinurenina, isso é confirmado pela menor formação do seu metabólito. Para confirmar nossa hipótese seguimos com a investigação na modulação ou não da atividade da enzima IDO.

A estimulação central da via da quinurenina é mediada pela ativação da enzima IDO no cérebro (BADAWY, 2017). A indução da IDO tem sido associada a complicações depressivas em roedores e humanos (CAPURON et al., 2011). Em um trabalho anterior do nosso laboratório demonstrou que a IDO é um importante mediador de neuroinflamação e distúrbios neurocomportamentais em um modelo de doença de Alzheimer induzida pelo peptídeo beta-amilóide (1-42) em camundongos (SOUZA et al., 2016). Ainda, a ativação da IDO parece desempenhar um papel fundamental na modulação do comportamento doentio (EIMERBRINK et al., 2016). A injeção de LPS gerou um aumento considerável na atividade da enzima IDO dos camundongos idosos no hipocampo, estriado e córtex pré-frontal (artigo 1). Nossos resultados corroboram com estudos clínicos que relataram uma superestimulação da via da quinurenina no plasma de pacientes em situação semelhante ao comportamento doentio (MYINT et al., 2009; CAPURON et al., 2011). Diante da suplementação crônica com AGPI n-3 houve modulação da atividade da IDO, impedindo o aumento de sua atividade frente a alteração causada pelo LPS nas estruturas cerebrais estudadas.

De maneira similar, a administração de LPS causou novamente um aumento na atividade da enzima IDO em todas estruturas cerebrais analisadas em nosso estudo (artigo 2). A suplementação com GVH, não bloqueou o aumento na atividade de IDO gerado pelo LPS no hipocampo, estriado e córtex pré-frontal. Acreditamos que esse resultado é devido a GVH não modular as citocinas pró-inflamatórias e

ainda contribuir no aumento dos níveis de TNF-α, devido as citocinas pró-inflamatórias serem as principais mediadoras da ativação da via da quinurenina. Em estudos anteriores do no nosso laboratório já havíamos demonstrado a importância da modulação da atividade enzimática da IDO em modelos animais que mimetizaram a depressão e Alzheimer (SOUZA et al., 2016; SOUZA et al., 2018). Assim, novamente destacamos a importância da suplementação com AGPI n-3, pois acabou modulando a atividade da enzima IDO e consequentemente atenuando a ativação da via da quinurenina causada pelo comportamento doentio. Dessa maneira, acreditamos que essa modulação da IDO por parte dos AGPI n-3 está relacionada com o efeito neuroprotetor encontrado em nosso estudo.

Metabólitos neuroativos da via da quinurenina e sua ação no comportamento doentio

Nesse contexto, sabe-se que a inflamação está relacionada com a ativação da via da quinurenina que resulta na produção de quinurenina e seus metabólitos neuroativos (STONE e DARLINGTON, 2002). A quinurenina pode sofrer a ação da enzima QAT gerando o ácido quinurênico. Sendo que os astrócitos expressam grandes quantidades de QAT degradando a quinurenina em ácido quinurênico. O ácido quinurênico foi bastante estudado nos últimos anos e foi sugerido seu efeito neuroprotetor (LUCHOWSKA et al., 2009). Outra, rota que a quinurenina pode sofrer na via é a ação da enzima QMO, sendo clivada em 3-HQ e AQ. Sabe-se que a micróglia expressa bastante QMO, responsável direta pela geração de 3-HQ e AQ (ALBERATI-GIANI et al., 1996). Concentrações aumentadas de AQ foram encontradas em cérebros *post-mortem* de pacientes com distúrbios inflamatórios-infecciosos (bacterianos, virais, doenças autoimunes e septicemia) (La Cruz et al., 2007). Assim, sabe-se que níveis aumentados de AQ no cérebro significam uma neuroinflamação potente.

Diante do exposto, procuramos investigar o braço neuroprotetor da via da quinurenina. Assim, quando administramos LPS houve uma diminuição na atividade QAT e consequentemente nos níveis do ácido quinurênico e na razão ácido quinurênico/quinurenina no hipocampo, estriado e córtex pré-frontal dos animais idosos (artigo 1). A suplementação crônica com AGPI n-3 foi efetiva em proteger contra a alterações na atividade QAT, níveis do ácido quinurênico e na razão ácido quinurênico/quinurenina em todas estruturas cerebrais estudadas, induzidas pelo

LPS no modelo de comportamento doentio. Após a injeção de LPS nos camundongos idosos, não houve alteração na atividade de QAT e níveis de ácido quinurênico, porém aconteceu uma drástica diminuição na razão ácido quinurênico/quinurenina (artigo 2). Similarmente, a suplementação com GVH não modulou a atividade de QAT e não alterou os níveis de ácido quinurênico, mas acabou agravando a diminuição na razão ácido quinurênico/quinurenina gerada pelo LPS no hipocampo, estriado e córtex pré-frontal dos animais idosos. Destacamos novamente o efeito neuroprotetor da suplementação com AGPI n-3, agindo em favor do braço neuroprotetor da via da quinurenina. Além disso, o ácido quinurênico endógeno pode exercer sua ação anti-glutamatérgica antagonizando os receptores nicotínicos α7 (HILMAS et al., 2001). Alguns estudos ainda demonstraram que o ácido quinurênico é capaz de reduzir o conteúdo extracelular de glutamato no córtex pré-frontal e hipocampo, resultando em diminuição do efeito excitatório relacionado ao glutamato (CARPENEDO et al., 2002; WU et al., 2010). Sabendo, que dados recentes demonstraram evidências de um determinante papel do glutamato na fisiopatologia do comportamento doentio (FRÜHAUF et al., 2015; CHASKIEL et al., 2016). Inferimos que a suplementação com AGPI n-3 mediou sua neuroproteção diante das alterações neurocomportamentais causadas pelo comportamento doentio, via modulação nos níveis de ácido quinurênico e sua ação direta no sistema glutamatérgico.

Seguindo para investigação do braço neurotóxico da via da quinurenina, a administração de LPS causou um aumento na atividade da enzima QMO e consequentemente nos níveis de 3-HQ e AQ no hipocampo, estriado e córtex pré-frontal dos animais idosos (artigo 1). A suplementação com AGPI n-3 foi capaz de proteger contra essas graves alterações neurometabólicas causadas pelo LPS, via a modulação enzimática de QMO e diminuição nos níveis de 3-HQ e AQ em todas estruturas cerebrais estudadas. Diante do exposto, destacamos o efeito neuroprotetor apresentado pelos AGPI n-3 nesse estudo mediando as citocinas pró-inflamatórias, por sua vez diminuindo assim a ativação da via da quinurenina com efeito direto na via, deslocando o equilíbrio a favor de braço neuroprotetor (Fig. 1). Além disso, acreditamos que boa parte dos resultados farmacológicos apresentados pelos AGPI n-3, foram devidos ao aumento da concentração de EPA e DHA na membrana celular. Essa alteração de fosfolipídios da membrana celular, resultou na redução da resposta inflamatória com resposta direta sobre o sistema

serotoninérgico, contribuindo diretamente para a prevenção do comportamento doentio.

De maneira similar a injeção de LPS gerou um aumento na atividade enzimática da QMO e nos níveis de 3-HQ e AQ nas três estruturas cerebrais dos camundongos idosos. A suplementação com GVH agravou as alterações neurometabólicas causadas pelo comportamento doentio, via aumento na atividade de QMO e maior formação dos metabólitos 3-HQ e AQ. Tomados em conjunto, nossos resultados corroboram com a literatura científica, pois o aumento de AQ está relacionado ao desenvolvimento da sintomatologia depressiva tanto em animais de laboratório quanto em pacientes deprimidos (RAISON et al., 2010; SALAZAR et al., 2012). Resultados também que encontramos nos parâmetros comportamentais nesse estudo com GVH. Assim, fornecemos dados pioneiros que o consumo crônico de GVH diante uma potente doença neuroinflamatória, pode exacerbar os efeitos patológicos do comportamento doentio, por aumentar os níveis de TNF-α e induzir a uma excessiva ativação da via da quinurenina deslocando o equilíbrio da via para seu ramo neurotóxico (Fig. 8).

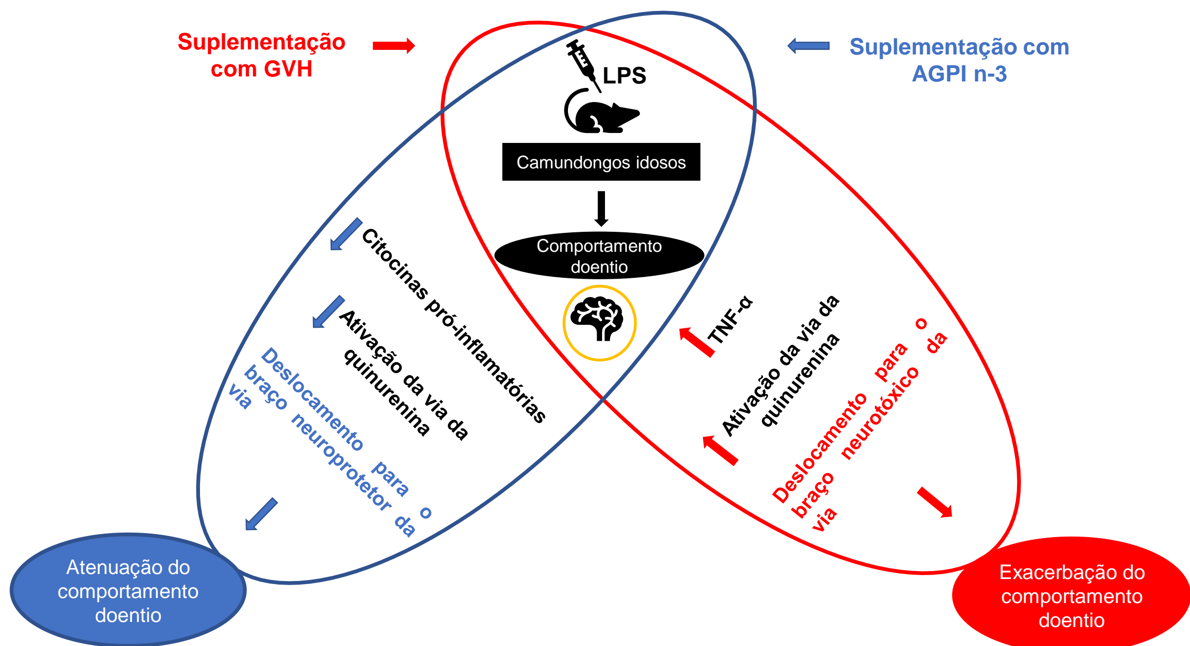

Figura 8. Principais efeitos das suplementações com AGPI n-3 e GVH no modelo de comportamento doentio induzido por LPS em camundongos idosos.

5. CONCLUSÕES

O presente trabalho demonstrou o efeito da suplementação com AGPI n-3 e GVH no modelo de comportamento doentio induzido pelo LPS em animais idosos junto com a influência da ativação da via da quinurenina nas alterações neurocomportamentais causadas pelo modelo.

Os dados do presente trabalho mostraram que a suplementação com AGPI n-3 foi capaz de proteger contra as alterações neurocomportamentais e neurometabólicas causadas pelo comportamento doentio. Por prevenir contra o aumento das citocinas inflamatórias e excessiva ativação da via da quinurenina com deslocamento do equilíbrio da via para o ramo neuroprotetor. Ainda, houve uma incorporação dos ácidos graxos EPA e DHA na membrana celular, protegendo contra os efeitos deletérios causados pelo comportamento doentio.

Ademais, também demonstramos que a suplementação com GVH não foi capaz de proteger contra as alterações causadas pelo comportamento doentio. Pelo contrário, aumentou a neuroinflamação via TNF-α que ativou mais a via da quinurenina e ainda deslocou o equilíbrio da via em direção ao ramo neurotóxico, potencializado as alterações neurocomportamentais e neurometabólicas causadas no modelo de comportamento doentio.

Por fim, demonstramos os efeitos do consumo crônico de AGPI n-3 e GVH no modelo de comportamento doentio em animais idosos, os quais tem o sistema imune mais comprometido devido ao processo fisiológico normal de envelhecimento. Fica evidente a ação direta de cada tipo de suplementação aos animais. Portanto, contribuímos para elucidação dos efeitos farmacológicos do AGPI n-3 e também de maneira pioneira demonstramos os efeitos deletérios da GVH no modelo de comportamento doentio. Esperamos, que os resultados do presente estudo possam servir de embasamento para estudos epidemiológicos e de saúde pública na população idosa que é gravemente acometida por doenças neurológicas.

6. PERSPECTIVAS

- Pretende-se dar continuidade no presente estudo, buscando corroborar com os resultados encontrados com a administração de LPS, usaremos modelos de indução direta de comportamento doentio via a administração de ácido policitidílico (Poly I:C) e bacilo *Calmette-Guérin* (BCG).

- Com esse propósito, verificaremos se o aminoácido triptofano está sendo desviado para via da melatonina, além da via da quinurenina e sistema serotoninérgico.

- Também se pretende, verificar os resultados usando um modelo alternativo a experimentação de animais na pesquisa. O modelo seria com *Drosophila melanogaster*.

7. REFERÊNCIAS BIBLIOGRÁFICAS

AKBARALY, T.N.; SABIA, S.; SHIPLEY, M.J.; BATTY, G.D.; KIVIMAKI, M. Adherence to healthy dietary guidelines and future depressive symptoms: evidence for sex differentials in the Whitehall II study. **The American Journal Of Clinical Nutrition**, v. 97, n. 2, p.419-427, 2013.

ALBERATI-GIANI, D.; RICCIARDI-CASTAGNOLI, P.; KÖHLER, C.; CESURA, A.M. Regulation of the Kynurenine Metabolic Pathway by Interferon-γ in Murine Cloned Macrophages and Microglial Cells. **Journal Of Neurochemistry**, v. 66, n. 3, p.996-1004,1996.

ANDRE, C.; O'CONNOR, J.C.; KELLEY, K.W. Spatio-temporal differences in the profile of murine brain expression of proinflammatory cytokines and indoleamine 2,3 dioxygenase in response to peripheral lipopolysaccharide administration. **J Neuroimmunol**, v. 200, n. 2, p. 90–99, 2008.

ASARIAN, L.; LANGHANS, W. A new look on brain mechanisms of acute illness anorexia. **Physiology & Behavior**, v. 100, n. 5, p.464-471, 2010.

BASU M.S.; MUDGAL, J.; NAMPOOTHIRI, M.; HALL, S.; DUKIE, S.A.; GRANT, G. Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. **Neuroscience Letters**, v. 632, p.218-223, 2016.

BAZINET, R.P.; LAYÉ, S. Polyunsaturated fatty acids and their metabolites in brain function and disease. **Nature Reviews Neuroscience**, v. 15, n. 12, p.771-785, 2014.

BHARDWAJ, S.; PASSI S.; MISRA A. Overview of trans fatty acids: Biochemistry and health effects. **Diabetes & Metabolic Syndrome**: Clinical Research & Reviews, v. 5, n. 3, p.161-164, 2011.

BONDI, C.O.; TAHA, A. Y.; TOCK, J. L.; TOTAH, N.K.B.; CHEON, Y.; TORRES, G.E. Adolescent Behavior and Dopamine Availability Are Uniquely Sensitive to

Dietary Omega-3 Fatty Acid Deficiency. **Biological Psychiatry**, v. 75, n. 1, p.38-46, 2014.
BRADBURY, J. Docosahexaenoic Acid (DHA): An Ancient Nutrient for the Modern Human Brain. **Nutrients**, v. 3, n. 5, p.529-554, 2011.

CAI, K.C.; VAN-MIL, S.; MURRAY, E.; MALLET, J.F.; MATAR, C.; ISMAIL, N. Age and sex differences in immune response following LPS treatment in mice. **Brain, Behavior, And Immunity**, v. 58, p.327-337, 2016.

CAPURON, L.; RAVAUD, A.; MILLER, A.H. Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. **Brain Behav Immun**, v. 18, n. 3, p. 205–213, 2004.

CAPURON, L.; SCHROECKSNADEL, S.; FEART, C.; AUBERT, A.; HIGUERET, D.; BARBERGER-GATEAU, P. Chronic Low-Grade Inflammation in Elderly Persons Is Associated with Altered Tryptophan and Tyrosine Metabolism: Role in Neuropsychiatric Symptoms. **Biological Psychiatry**, [s.l.], v. 70, n. 2, p.175-182, jul. 2011.

CARPENEDO, R.; MELI, E.; PERUGINELLI, F.; PELLEGRINI-GIAMPIETRO, D.E.; MORONI F. Kynurenine 3-mono-oxygenase inhibitors attenuate post-ischemic neuronal death in organotypic hippocampal slice cultures. **Journal Of Neurochemistry**, v. 82, n. 6, p.1465-1471, 2002.

CHASKIEL, L.; PAUL, F.; GERSTBERGER, R.; HÜBSCHLE, T.; KONSMAN, JP. Brainstem metabotropic glutamate receptors reduce food intake and activate dorsal pontine and medullar structures after peripheral bacterial lipopolysaccharide administration. **Neuropharmacology**, v. 107, p.146-159, 2016.

COSTA, R. P.; SILVA, C. C. Doenças cardiovasculares: In: Cuppari L. Guias de Medicina Ambulatorial e Hospitalar / Nutrição Clínica no Adulto. Barueri: Manole, p. 263-288, 2002.

COULOMBE, K.; KERDILES, O.; TREMBLAY, C.; EMOND, V.; LEBEL, M.; BOULIANNE, A.S. Impact of DHA intake in a mouse model of synucleinopathy. **Experimental Neurology**, v. 301, p.39-49, 2017.

CUNNANE, S.C.; PLOURDE, M.; PIFFERI, F.; BÉGIN, M., FÉART, C., BARBERGER-GATEAU, P. Progress in lipid research fish, docosahexaenoic acid and Alzheimer's disease. **Prog.Lipid Res**, v. 48, n. 5, p. 239–256, 2009.

DANG, R.; ZHOU, X.; TANG, M.; XU, P.; GONG, X.; LIU, Y.; JIAO, H.; JIANG, P. Fish oil supplementation attenuates neuroinflammation and alleviates depressive-like behavior in rats submitted to repeated lipopolysaccharide. **European Journal Of Nutrition**, v. 57, n. 3, p.893-906, 2018.

DANTZER, R. Cytokine, Sickness Behavior, and Depression. **Neurologic Clinics**, v. 24, n. 3, p.441-460, 2006.

DANTZER, R. Cytokine-Induced Sickness Behavior: Mechanisms and Implications. **Annals Of The New York Academy Of Sciences**, v. 933, n. 1, p.222-234, 2011.

DANTZER, R.; AUBERT, A.; BLUTHÉ, R.M.; GHEUSI, G.; CREMONA, S.; LAYÉ, S. Mechanisms of the behavioural effects of cytokines. **Adv Exp Med Biol**, v. 461, n.2, p. 83–105, 1999.

DANTZER, R.; KONSMAN, J.P.; BLUTHE, R.M.; KELLEY, K.W. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? **Auton. Neurosci**, v. 85, n. 3, p. 60–65, 2000.

DANTZER, R.; O'CONNOR, J.C.; FREUND, G.G.; JOHNSON, R.W.; KELLEY, K.W. From inflammation to sickness and depression: when the immune system subjugates the brain. **Nature Reviews Neuroscience**, v. 9, n. 1, p.46-56, 2008.

DANTZER, R.; O'CONNOR, J.C.; LAWSON, M.A.; KELLEY, K.W. Inflammation-associated depression: From serotonin to kynurenine. **Psychoneuroendocrinology**, v. 36, n. 3, p.426-436, 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. **Bulletin Of The World Health Organization**, v. 91, n. 4, p.262-269, 2013.

EIMERBRINK, M.J.; KRANJAC, D.; LAURENT, S.T.; WHITE, J.D.; WEINTRAUB, M.K.; PENDRY, R.J. Pre-treatment of C57BL6/J mice with the TLR4 agonist monophosphoryl lipid A prevents LPS-induced sickness behaviors and elevations in dorsal hippocampus interleukin-1β, independent of interleukin-4 expression. **Behavioural Brain Research**, v. 302, p.171-174, 2016.

EK, M.; KUROSAWA, M.; LUNDEBERG, T. Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. **J Neurosci**, v. 18, n. 22, p. 9471–9479, 1998.

ERICSSON, A.; KOVACS, K.J.; SAWCHENKO, P.E. A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. **J Neurosci**, v. 14, n. 2, p. 897–913 1994.

FRENOIS, F.; MOREAU, M.; O'CONNOR, J. Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. **Psychoneuroendocrinology**, v. 32, n. 5, p. 516–531, 2007.

FRÜHAUF, P.; INEU, R.P.; TOMAZI, L.; DUARTE, T.; MELLO, C.F.; RUBIN, MA. Spermine reverses lipopolysaccharide-induced memory deficit in mice. **Journal Of Neuroinflammation**, v. 12, n. 1, p.3-12, 2015.

GAYKEMA, R.P.; GOEHLER, L.E. Ascending caudal medullary catecholamine pathways drive sickness-induced deficits in exploratory behavior: brain substrates for fatigue? **Brain Behav. Immun**, v. 25, n. 3, p. 443–460, 2011.

GILES, G.I.; COLLINS, C.A.; STONE, T.W.; JACOB, C. Electrochemical and in vitro evaluation of the redox-properties of kynurenine species. **Biochemical And Biophysical Research Communications**, v. 300, n. 3, p.719-724, 2003.

GODBOUT, J.P.; CHEN, J.; ABRAHAM, J.; RICHWINE, A.F.; BERG, B.M.; KELLEY, K.W.; JOHNSON, R.W. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. **The Faseb Journal**, v. 19, n. 10, p.1329-1331, 2005.

GOEHLER, L.E.; GAYKEMA, R.P.; NGUYEN, K.T. Interleukin-1 beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? J Neurosci, v. 19, n. 7, p. 2799–2806, 1999.

GOGUS, U.; SMITH, C. N-3 Omega fatty acids: a review of current knowledge. **International Journal Of Food Science & Technology**, v. 45, n. 3, p.417-436, 2010.

GORJÃO, R; AZEVEDO-MARTINS, A.K.; RODRIGUES, H.G.; ABDULKADER, F.; ARCISIO-MIRANDA, M.; PROCOPIO, J. Comparative effects of DHA and EPA on cell function. **Pharmacol.Ther**, v. 122, n. 1, p. 56–64, 2009.

HAROON, E.; RAISON, C.L.; MILLER, A.H. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. **Neuropsychopharmacology**, v. 37, n. 1, p. 137–162, 2012.

HIBBELN, J.R. Fish consumption and major depression. **The Lancet**, v. 351, n. 9110, p.1213, 1998.

HILMAS, C.; PEREIRA, E.F.; ALKONDON, M.; RASSOULPOUR, A.; SCHWARCZ, R.; ALBUQUERQUE, EX. The Brain Metabolite Kynurenic Acid Inhibits α7 Nicotinic Receptor Activity and Increases Non-α7 Nicotinic Receptor Expression: Physiopathological Implications. **The Journal Of Neuroscience**, v. 21, n. 19, p.7463-7473, 2001.

INNIS, S.M. Dietary (n-3) fatty acids and brain development. **J. Nutr**, v. 137, n. 5, p. 855–859, 2007.

JESSE, C.R.; WILHELM, E.A.; BORTOLATTO, C.F.; NOGUEIRA, C.W. Evidence for the involvement of the serotonergic 5-HT2A/C and 5-HT3 receptors in the antidepressant-like effect caused by oral administration of bis selenide in mice. **Progress In Neuro-psychopharmacology And Biological Psychiatry**, v. 34, n. 2, p.294-302, 2010.

JUENGLING, F.D.; EBERT, D.; GUT, O. Prefrontal cortical hypometabolism during low-dose interferon alpha treatment. **Psychopharmacology (Berl**), v. 152, n. 4, p. 383–389, 2000.

KONSMAN, J.P.; LUHESHI, G.N.; BLUTHE, R.M. et al. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. **Eur J Neurosci**, v. 12, n. 12, p. 4434–4446, 2000.

KONSMAN, J.P.; PARNET, P.; DANTZER, R. Cytokine-induced sickness behaviour: mechanisms and implications. **Trends In Neurosciences**, v. 25, n. 3, p.154-159, 2002.

KUHN, F.T.; ROVERSI, K.; ANTONIAZZI, C.T.; PASE, C.S.; TREVIZOL, F.; BARCELOS, R.C.; DIAS, V.T.; ROVERSI, K.; BOUFLEUR, N.; BENVEGNÚ, D.M.; PICCOLO, J.; EMANUELLI, T.; BÜRGER, ME. Influence of trans fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats. **Pharmacology Biochemistry And Behavior**, v. 110, p.58-65, 2013.

LA CRUZ, V.; SANTAMARIA, A.; KONIGSBERG, M. Kynurenine Pathway and Disease: An Overview. **Cns & Neurological Disorders - Drug Targets**, v. 6, n. 6, p.398-410, 2007.

LACRUZ, V. P.; CARRILLO-MORA, P.; SANTAMARÍA, A. Quinolinic Acid, an Endogenous Molecule Combining Excitotoxicity, Oxidative Stress and Other Toxic Mechanisms. **International Journal Of Tryptophan Research**, v. 5, p.51-54, 2012.

LAFOURCADE, M.; LARRIEU, T.; MATO, S.; DUFFAUD, A.; SEPERS, M.; MATIAS, I. Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. **Nature Neuroscience**, v. 14, n. 3, p.345-350, 2011.

LAPIN, I.P. Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice. **Journal Of Neural Transmission**, v. 42, n. 1, p.37-43, 1978.

LARQUÉ, E.; GARCÍA-RUIZ, P.A.; PEREZ-LLAMAS, F.; ZAMORA, S.; GIL, A. Dietary Trans Fatty Acids Alter the Compositions of Microsomes and Mitochondria and the Activities of Microsome Δ6-Fatty Acid Desaturase and Glucose-6-Phosphatase in Livers of Pregnant Rats. **The Journal Of Nutrition**, v. 133, n. 8, p.2526-2531, 2003.

LARRIEU, T.; LAYÉ, S. Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety. **Frontiers In Physiology**, v. 9, p.1047, 2018.

LARRIEU, T; MADORE, C.; JOFFRE, C.; LAYÉ, S. Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice. **Journal Of Physiology And Biochemistry**, v. 68, n. 4, p.671-681, 2012.

LARSON, S.J.; DUNN, A.J. Behavioral effects of cytokines. **Brain Behav Immun**, v. 15, n. 4, p. 371–387, 2001.
LARSSON, S.C.; KUMLIN, M.; INGELMAN-SUNDBERG, M.; WOLK, A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. **Am. J. Clin. Nutr**, v. 79, n. 6, p. 935–945, 2004.

LEITE, H.R.; OLIVEIRA-LIMA, O.C.; PEREIRA, L.M.; OLIVEIRA, V.E.M.; PRADO, V.F.; PEREIRA, G.S.; PRADO, M.A.M.; MASSENSINI, A.R. Vesicular acetylcholine transporter knock down-mice are more susceptible to inflammation, c- Fos expression and sickness behavior induced by lipopolysaccharide. **Brain, Behavior, And Immunity**, v. 57, p.282-292, 2016.

LENCZOWSKI, M.J.; VAN DAM, A.M.; POOLE, S.; LARRICK, J.W.; TILDERS, F.J. Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS. **Am. J. Physiol**, v. 273, (6 Pt 2), p. 1870–1877, 1997.

LESTAGE, J.; VERRIER, D.; PALIN, K. The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. **Brain Behav Immun**, v. 16, n. 5, p. 596–601, 2002.

LIU, Y.; LI, X.Y.; CHEN, C.Y.; ZHANG, H.M.; KANG, J.X. Omega-3 Fatty Acid Intervention Suppresses Lipopolysaccharide-Induced Inflammation and Weight Loss in Mice. **Marine Drugs**, v. 13, n. 2, p.1026-1036, 2015.

LUCHOWSKA, E.; KLOC, R.; OLAJOSSY, B.; WNUK, S.; WIELOSZ, M.; OWE-LARSSON, B.; URBANSKA, E.M.B. β-adrenergic enhancement of brain kynurenic acid production mediated via cAMP-related protein kinase A signaling. **Progress In Neuro-psychopharmacology And Biological Psychiatry**, v. 33, n. 3, p.519-529, 2009.

LUCHTMAN, D.W.; SONG, C. Cognitive enhancement by omega- 3 fatty acids from child-hood toold age: findings from animal and clinical studies. **Neuropharmacology**, v. 64, p. 550–565, 2013.
LUGO-HUITRÓN, R.; BLANCO-AYALA, T.; UGALDE-MUNIZ, P.; CARRILLO-MORA, P.; PEDRAZA-CHAVERRI, J.; SILVA-ADAYA, D. On the antioxidant properties of kynurenic acid: Free radical scavenging activity and inhibition of oxidative stress. **Neurotoxicology And Teratology**, v. 33, n. 5, p.538-547, 2011.

MADDISON, D.C.; GIORGINI, F. The kynurenine pathway and neurodegenerative disease. **Seminars In Cell & Developmental Biology**, v. 40, p.134-141, 2015.

MAES, M.; LEONARD, B.E.; MYINT, A.M.; KUBERA, M.; VERKERK, R. The new ‘5-HT’ hypothesis of depression: Cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased

synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. **Progress In Neuro-psychopharmacology And Biological Psychiatry**, v. 35, n. 3, p.702-721, 2011.

MCNAMARA, R.K.; CARLSON, S.E. Role of omega-3 fatty acids in brain development and function: potential implications for the Pathogenesis and prevention of psychopathology. **Prostaglandins Leukot. Essent.Fat.Acids**, v. 75, n. 5, p. 329–349, 2006.

MCNAMARA, R.K.; LIU, Y. Reduced expression of fatty acid biosynthesis genes in the prefrontal cortex of patients with major depressive disorder. **Journal Of Affective Disorders**, v. 129, n. 1-3, p.359-363, 2011.

MENSINK, R.P.; KATAN, M.B. Effect of Dietary trans Fatty Acids on High-Density and Low-Density Lipoprotein Cholesterol Levels in Healthy Subjects. **New England Journal Of Medicine**, v. 323, n. 7, p.439-445, 1990.

MINGAM, R.; MORANIS, A.; BLUTHÉ, R.M.; DE SMEDT-PEYRUSSE, V.; KELLEY, K.W.; GUESNET, P. Uncoupling of interleukin-6 from its signalling pathway by dietary n-3-polyunsaturated fatty acid deprivation alters sickness behaviour in mice. **European Journal Of Neuroscience**, v. 28, n. 9, p.1877-1886, 2008.

MITCHELL, S.A.; SPRIGGS, K.A.; COLDWELL, M.J.; JACKSON, R.J.; WILLIS, A.E. The Apaf-1 internal ribosome entry segment attains the correct structural conformation for function via interactions with PTB and unr. **Mol. Cell**, v. 11, n. 3, p. 757-771, 2003.

MOREAU, M.; ANDRE, C.; O'CONNOR, J.C. Inoculation of Bacillus Calmette-Guerin to mice induces an acute episode of sickness behavior followed by chronic depressive-like behavior. **Brain Behav Immun**, v. 22, n. 7, p. 1087–1095, 2008.

MOZAFFARIAN, D.; ARO, A.; WILLETT, W.C. Health effects of trans-fatty acids: experimental and observational evidence. **European Journal Of Clinical Nutrition**, v. 63, n. 2, p.5-21, 2009.

MOZAFFARIAN, D.; PISCHON, T.; HANKINSON, S.E.; RIFAI, N.; JOSHIPURA, K.; WILLETT, W.C.; RIMM, E.B. Dietary intake of trans fatty acids and systemic inflammation in women. **The American Journal Of Clinical Nutrition**, v. 79, n. 4, p.606-612, 2004.

MOZAFFARIAN, D.; STAMPFER, M.J. Removing industrial trans fat from foods. **Bmj**, v. 340, n. 151, p.1826-1826, 2010.

MÜLLER, C.P.; REICHEL, M.; MÜHLE, C.; RHEIN, C.; GULBINS, E.; KORNHUBER, J. Brain membrane lipids in major depression and anxiety disorders. **Biochimica Et Biophysica Acta (bba) - Molecular And Cell Biology Of Lipids**, v. 1851, n. 8, p.1052-1065, 2015.

MYINT, A.; KIM, Y.K.; VERKERK, R.; SCHARPE, S.; STEINBUSCH, H.; LEONARD, B. Kynurenine pathway in major depression: Evidence of impaired neuroprotection. **Journal Of Affective Disorders**, v. 98, n. 1-2, p.143-151, 2007.

MYINT, A.M.; SCHWARZ, M.J.; STEINBUSCH, H.W.; LEONARD, B.E. Neuropsychiatric disorders related to interferon and interleukins treatment. **Metabolic Brain Disease**, v. 24, n. 1, p.55-68, 2009.
OKEN, E.; BELFORT, M.B. Fish, Fish Oil, and Pregnancy. **JAMA**, v.304, n. 15, p. 1717-1718, 2010.

OKUDA, S.; NISHIYAMA, N.; SAITO, H.; KATSUKI, H. 3-Hydroxykynurenine, an Endogenous Oxidative Stress Generator, Causes Neuronal Cell Death with Apoptotic Features and Region Selectivity. **Journal Of Neurochemistry**, v. 70, n. 1, p.299-307,1998.

PARLETTA, N.; ZARNOWIECKI, D.; CHO, J.; WILSON, A.; PROCTER, N.; GORDON, A. People with schizophrenia and depression have a low omega-3 index. **Prostaglandins, Leukotrienes And Essential Fatty Acids (plefa)**, v. 110, p.42-47, 2016.

PASE, C.S.; ROVERSI, K.; VEY, L.T.; DIAS, V.T.; VEIT, J.C.; MAURER, L.H.; DUARTE, T.; EMANUELLI, T.; DUARTE, M.; BÜRGER, M.E7. 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. **Physiology & Behavior**, v. 169, p.114-123, 2017.

PERKINS, M.N.; STONE, T.W. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. **Brain Research**, v. 247, n. 1, p.184-187,1982.

PERRY, V. H.; NEWMAN, T.A.; CUNNINGHAM, C. The impact of systemic infection on the progression of neurodegenerative disease. **Nature Reviews Neuroscience**, v. 4, n. 2, p.103-112, 2003.

PINNER, R.W.; TEUTSCH, S.M.; SIMONSEN, L.; KLUG, L.A.; GRABER, J.M.; CLARKE, M.J.; BERKELMAN, R.L. Trends in Infectious Diseases Mortality in the United States. **Jama**: The Journal of the American Medical Association, v. 275, n. 3, p.189-193, 1996.

PLÁTENÍK, J.; STOPKA, P.; VEJRAZKA, M.; STIPEK, S. Quinolinic acid — Iron (II) complexes: Slow autoxidation, but enhanced hydroxyl radical production in the Fenton reaction. **Free Radical Research**, v. 34, n. 5, p.445-459, 2001.

POLLMACHER, T.; HAACK, M.; SCHULD, A.; REICHENBERG, A.; YIRMIYA, R. Low levels of circulating inflammatory cytokines—Do they affect human brain functions? **Brain, Behavior, And Immunity**, v. 16, n. 5, p.525-532, 2002.

RAISON, C.L.; BROADWELL, S.D.; BORISOV, A.S.; MANATUNGA, A.K.; CAPURON, L.; WOOLWINE, B.J.; JACOBSON, I.M.; NEMEROFF, C.B.; MILLER, A.H. Depressive symptoms and viral clearance in patients receiving interferon-α and ribavirin for hepatitis C. **Brain, Behavior, And Immunity**, v. 19, n. 1, p.23-27, 2005.

RAISON, C.L.; DANTZER, R.; KELLEY, K.W.; LAWSON, M.A.; WOOLWINE, B.J.; VOGT, G.; SPIVEY, J.R.; SAITO, K.; MILLER, A.H. CSF concentrations of brain

tryptophan and kynurenines during immune stimulation with IFN-α: relationship to CNS immune responses and depression. **Molecular Psychiatry**, v. 15, n. 4, p.393-403, 2009.

RAISON, C.L.; RUTHERFORD, R.E.; WOOLWINE, B.J.; SHUO, C.; SCHETTLER, P.; DRAKE, D.F.; HAROON, E.; MILLER, A.H. A Randomized Controlled Trial of the Tumor Necrosis Factor Antagonist Infliximab for Treatment-Resistant Depression. **Jama Psychiatry**, v. 70, n. 1, p.31-41, 2013.

RÉUS, G.Z.; JANSEN, K.; TITUS, S.; CARVALHO, A.F.; GABBAY, V.; QUEVEDO, J. Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: Evidences from animal and human studies. **Journal Of Psychiatric Research**, v. 68, p.316-328, 2015.

ROMANOVSKY, A.; ALMEIDA, M.C.; ARONOFF, D.M. Fever and hypothermia in systemic inflammation: recent discoveries and revisions. **Front Biosci**, v. 10, n. 1, p. 2193–2216, 2005.
SALAZAR, A.; GONZALEZ-RIVERA, B.L.; REDUS, L.; PARROTT, J.M.; O'CONNOR, J.C. Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. **Hormones And Behavior**, v. 62, n. 3, p.202-209, 2012.

SALEM JR, N.; LITMAN, B.; KIM, H.Y.; GAWRISCH, K. Mechanisms of action of docosahexaenoic acid in the nervous system. **Lipids**, v. 36, n. 9, p. 945-979, 2001.

SANTAMARÍA, A.; RÍOS, C. MK-801, an receptor antagonist, blocks quinolinic acid-induced lipid peroxidation in rat corpus striatum. **Neuroscience Letters**, v. 159, n. 1-2, p.51-54, 1993.

SASTRY, P.S. Lipids of nervous tissue: Composition and metabolism. **Progress In Lipid Research**, v. 24, n. 2, p.69-176, 1985.

SCHNEIDERS, J.; FUCHS, F.; DAMM, J.; HERDEN, C.; GERSTBERGER, R.; SOARES, D.M. The transcription factor nuclear factor interleukin 6 mediates pro- and

anti-inflammatory responses during LPS-induced systemic inflammation in mice. **Brain, Behavior, And Immunity**, v. 48, p.147-164, 2015.

SCHWARCZ, R.; WHETSELL, W.; MANGANO, R. Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. **Science**, v. 219, n. 4582, p.316-318, 1983.

SENS, J.; SCHNEIDER, E.; MAUCH, J.; SCHAFFSTEIN, A.; MOHAMED, S.; FASOLI, K. Lipopolysaccharide administration induces sex-dependent behavioural and serotonergic neurochemical signatures in mice. **Pharmacology Biochemistry And Behavior**, v. 153, p.168-181, 2017.

SHEFFIELD, L.G.; BERMAN, N.E. Microglial Expression of MHC Class II Increases in Normal Aging of Nonhuman Primates. **Neurobiology Of Aging**, v. 19, n. 1, p.47-55, 1998.

SMITH, A.J.; SMITH, R.A.; STONE, T.W. 5-Hydroxyanthranilic Acid, a Tryptophan Metabolite, Generates Oxidative Stress and Neuronal Death via p38 Activation in Cultured Cerebellar Granule Neurones. **Neurotoxicity Research**, v. 15, n. 4, p.303-310, 2009.

SMITH, R.S. The macrophage theory of depression. **Medical Hypotheses**, v. 35, n. 4, p.298-306, 1991.

SOUZA, L.C.; JESSE, C.R.; ANTUNES, M.S.; RUFF, J.R.; ESPINOSA, D.O.; GOMES, N.S.; DONATO, F.; GIACOMELI, R.; BOEIRA, S.P. Indoleamine-2,3-dioxygenase mediates neurobehavioral alterations induced by an intracerebroventricular injection of amyloid-β 1-42 peptide in mice. **Brain, Behavior, And Immunity**, v. 56, p.363-377, 2016.

SOUZA, L.C.; JESSE, C.R.; DE GOMES, M.G.; VIANA, C.E.; MATTOS, E.; SILVA, N.C.; BOEIRA, S.P. Intracerebroventricular Administration of Streptozotocin as an Experimental Approach to Depression: Evidence for the Involvement of Proinflammatory Cytokines and Indoleamine-2,3-Dioxygenase. **Neurotoxicity Research**, v. 31, n. 4, p.464-477, 2017.

SOUZA, L.C.; JESSE, C.R.; DEL FABBRO, L.; DE GOMES, M.G.; GOMES, N.S.; FILHO, C.B.; GOES, A.T.R.; WILHELM, E.A.; LUCHESE, C.; ROMAN, S.S.; BOEIRA, S.P. Aging exacerbates cognitive and anxiety alterations induced by an intracerebroventricular injection of amyloid-β 1–42 peptide in mice. **Molecular And Cellular Neuroscience**, v. 88, p.93-106, 2018.

SPECTOR, A.A.; KIM, H. Discovery of essential fatty acids. **Journal Of Lipid Research**, v. 56, n. 1, p.11-21, 2015.

STERU, L.; CHERMAT, R.; THIERRY, B.; SIMON, P. The tail suspension test: A new method for screening antidepressants in mice. **Psychopharmacology**, v. 85, n. 3, p.367-370, 1985.

STONE, T.W.; DARLINGTON, L. G. Endogenous kynurenines as targets for drug discovery and development. **Nature Reviews Drug Discovery**, v. 1, n. 8, p.609-620, 2002.

TAVARES, R.G.; TASCA, C.I.; SANTOS, C.E.; ALVES, L.B.; PORCIUNCULA, L.O.; EMANUELLI, T. Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. **Neurochemistry International**, v. 40, n. 7, p.621-627, 2002.

TEEGALA, S.M.; WILLETT, W.C.; MOZAFFARIAN, D. Consumption and health effects of trans fatty acids: A review. **J. AOAC Int**, v. 92, p. 1250–1257, 2009.

TIZIANA R.; DANIELA, V.; NATALIA, R.; CINZIA, G.; DANIELA, B.; VALERIA, C.; CHIARA, C.; FRANCESCA, C.; PATRIZIA, R.; SANZIO, C.; MARIAELVINA, S.; DANIELA, P. Chronic Δ9-Tetrahydrocannabinol During Adolescence Provokes Sex-Dependent Changes in the Emotional Profile in Adult Rats: Behavioral and Biochemical Correlates. **Neuropsychopharmacology**, v. 33, n. 11, p.2760-2771, 2008.

TREVIZOL, F.; DIAS, V.T.; ROVERSI, K.; BARCELOS, R.C.S.; KUHN, F.T.; ROVERSI, K.R.; PASE, C.S.; GOLOMBIESKI, R.; VEIT, J.C. PICCOLO, J.;

EMANUELLI, T.; ROCHA, J.B.T.; BÜRGER, M.E. Cross-generational trans fat intake modifies BDNF mRNA in the hippocampus: Impact on memory loss in a mania animal model. **Hippocampus**, v. 25, n. 5, p.556-565, 2014.

WHETSELL, W.O.; SCHWARCZ, R. Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. **Neuroscience Letters**, v. 97, n. 3, p.271-275, 1989.

WILSON, C.J.; FINCH, C.E.; COHEN, H.J. Cytokines and Cognition-The Case for A Head-to-Toe Inflammatory Paradigm. **Journal Of The American Geriatrics Society**, v. 50, n. 12, p.2041-2056, 2002.

WU, H.; PEREIRA, E.F.; BRUNO, J.P.; PELLICCIARI, R.; ALBUQUERQUE, E.X.; SCHWARCZ, R. The Astrocyte-Derived α7 Nicotinic Receptor Antagonist Kynurenic Acid Controls Extracellular Glutamate Levels in the Prefrontal Cortex. **Journal Of Molecular Neuroscience**, v. 40, n. 1-2, p.204-210, 2010.

ANEXO

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

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CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **010/2016**

Título: **Avaliação de efeitos dos ácidos graxos poliinsaturados nos efeitos neurocomportamentais causados pelo comportamento doentio de camundongos**

Data da aprovação: **06.05.2016**

Período de vigência do projeto: 01.05.2019

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