UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

EFEITOS DA CAFEÍNA E DO DICLOFENACO SOBRE O ESTRESSE OXIDATIVO E A INFLAMAÇÃO EM RATOS SUBMETIDOS AO EXERCÍCIO FÍSICO

TESE DE DOUTORADO

RÔMULO PILLON BARCELOS

Orientadora: Profª. Drª. Nilda de Vargas Barbosa

Santa Maria, RS, Brasil

2015

EFEITOS DA CAFEÍNA E DO DICLOFENACO SOBRE O ESTRESSE OXIDATIVO E A INFLAMAÇÃO EM RATOS SUBMETIDOS AO EXERCÍCIO FÍSICO

Rômulo Pillon Barcelos

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Doutor em Ciências Biológicas: Bioquímica Toxicológica.

Orientadora: Profª. Drª. Nilda de Vargas Barbosa

Santa Maria, RS, Brasil

2015

Ficha catalográfica elaborada através do Programa de Geração Automática
da Biblioteca Central da UFSM, com os dados fornecidos pelo(a) autor(a).

Barcelos, Rômulo Pillon
Efeitos da cafeína e do diclofenaco sobre o estresse oxidativo e a inflamação em ratos submetidos ao exercício físico / Rômulo Pillon Barcelos.-2015.
95 p.; 30cm

Orientadora: Nilda Berenice de Vargas Barbosa
Coorientador: Félix Alexandre Antunes Soares
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, RS, 2015

1. Exercício Físico 2. Cafeína 3. Diclofenaco 4. Estresse Oxidativo 5. Inflamação I. Barbosa, Nilda Berenice de Vargas II. Soares, Félix Alexandre Antunes III. Título.

Universidade Federal De Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Ciências Biológicas:
Bioquímica Toxicológica

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

EFEITOS DA CAFEÍNA E DO DICLOFENACO SOBRE O ESTRESSE OXIDATIVO E A INFLAMAÇÃO EM RATOS SUBMETIDOS AO EXERCÍCIO FÍSICO

elaborada por

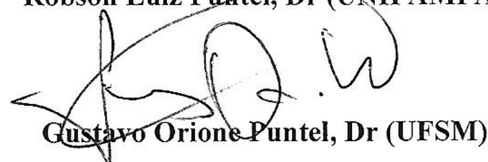
Rômulo Pillon Barcelos

Como requisito parcial para a obtenção do grau de
Doutor em Ciências Biológicas: Bioquímica Toxicológica

Comissão Examinadora

Nilda de Vargas Barbosa, Drª.
(Presidente/Orientador)

Robson Luiz Puntel, Dr (UNIPAMPA)



Gustavo Orione Puntel, Dr (UFSM)

Mauro Schenider de Oliveira, Dr (UFSM)

Lisiane de Oliveira Porciúncula, Drª (UFRGS)

Santa Maria, 1 de julho de 2015.

AGRADECIMENTOS

Definitivamente a parte mais difícil, aquela que é deixada por último, justamente pela dificuldade, aquela que proporciona sentimentos variados, entre eles nostalgia, angústia, saudade, felicidade, amor. Tudo isso por lembrar de todos que ajudaram, que participaram, que apoiaram, que ao menos se importaram em algum momento. E não há busca a literatura ou justificativas para expressar o verdadeiro agradecimento que eu gostaria de fazer aqui.

Por isso, no primeiro momento quero agradecer a minha família, pois essa é a base, é quem dá o incentivo, quem se preocupa, quem pergunta os próximos passos, que sempre diz a verdade não importa a situação. Portanto, agradeço a meu pai Canrobert Barcelos e a minha mãe Clenir Teresa Pillon Barcelos, educadores, incentivadores e exímios profissionais dos quais me espelho e tenho orgulho. Também a meus irmãos Renan Pillon Barcelos e Rossano Pillon Barcelos pela compreensão, paciência e parceria durante todo esse tempo. Um agradecimento especial a Daniele Coradini Zamberlan, minha namorada, parceira e companheira em todos os momentos, dos mais felizes aos mais perturbados, nos quais sempre contei com seu apoio.

Também quero aqui registrar meu agradecimento ao professor Dr. Félix Antunes Soares, o qual na graduação foi meu orientador, e desde o mestrado e agora no doutorado, um coorientador. Deixo aqui minha sincera admiração pelo seu trabalho e profissionalismo, espero podermos manter contato e trabalhamos quem sabe um dia como parceiros.

À minha orientadora professora Drª. Nilda Berenice de Vargas Barbosa, por ter me aceitado como aluno, por suas ideias e por se disponibilizar e dedicar seu tempo para trabalharmos e melhorarmos este trabalho.

Agradeço as instituições: Universidade Federal de Santa Maria, Universidad de León (Espanha) e a CAPES por apoiarem e financiarem nossos projetos.

Por último, mas não menos importante, aos amigos. Os amigos são os que te alegram quando nada mais parece ter saída. Os que fazem piadas, as vezes sem graça, os que jogam truco, os que jogam ou assistem a partidas de futebol, os de churrasco, os de festa, os de viagem, os de laboratório, entre muitos outros. Amigo é colega, amigo é parceiro, amigo é sinônimo de companheirismo. Por isso, agradeço aos meus amigos por participarem junto comigo deste trabalho. Sem dúvida foram muito importantes.

Um grande abraço a todos que de alguma forma participaram e contribuíram nesta caminhada. Espero poder levá-los não só na memória, mas também no coração.

EPÍGRAFE

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

Arthur Schopenhauer

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria, RS, Brasil

EFEITOS DA CAFEÍNA E DO DICLOFENACO SOBRE O ESTRESSE OXIDATIVO E A INFLAMAÇÃO EM RATOS SUBMETIDOS AO EXERCÍCIO FÍSICO

AUTOR: Rômulo Pillon Barcelos
ORIENTADORA: Nilda de Vargas Barbosa
Local e data da defesa: Santa Maria, 1 de julho de 2015.

O exercício físico pode representar um tipo de estresse por alterar a homeostase corporal. O elevado consumo de oxigênio pelo músculo esquelético durante o exercício físico aumenta a produção de espécies reativas de oxigênio (EROs) e proteínas inflamatórias, desencadeando estresse oxidativo e inflamação sistêmica. Atualmente, a cafeína, um composto presente em diversas bebidas e medicamentos, e os anti-inflamatórios não-esteroidais (AINEs), incluindo o diclofenaco, têm sido amplamente usados em competições esportivas. Considerando que os atletas treinam com o objetivo de melhorar o seu desempenho esportivo, principalmente em provas de alta intensidade e curta duração e que essas podem levar à sensação de dor e processo inflamatório, um grande número desses atletas usa tanto cafeína e AINEs como recursos ergogênicos ou até mesmo para evitar perdas de performance em suas provas. No entanto, pouco se sabe sobre os efeitos da associação entre treinamento físico e o uso concomitante de cafeína/diclofenaco nos tecidos. Levando em consideração a especificidade esporte, a maioria dos estudos são referentes a associação exercício físico e músculo esquelético. Entretanto, as respostas adaptativas ao exercício físico não são restritas ao tecido muscular. Considerando o importante papel do fígado durante a atividade física, um objetivo desta tese foi analisar os efeitos da cafeína e, em um segundo momento, do diclofenaco, sobre marcadores hepáticos de estresse oxidativo, dano tecidual e inflamação em ratos treinados. Nos artigos destacamos o papel da cafeína em modular as respostas de dano, estresse oxidativo, inflamação e adaptação causadas pelo treinamento físico em fígado, músculo e plasma. O protocolo experimental foi realizado com 4 grupos distintos: sedentário-salina, sedentário-cafeína, exercício-salina e exercício-cafeína. Os grupos exercício foram submetidos a 4 semanas de treinamento aeróbio de natação e os grupos tratados foram suplementados com cafeína (6 mg/kg), durante o treinamento. Identificamos mudanças significativas na atividade das enzimas citrato sintase (CS), superóxido desmutase (SOD), glutationa peroxidase (GPx) e acetilcolinesterase (AChE), e nos níveis da aspartato aminotransferase (AST) e substâncias reativas ao ácido tiobarbitúrico (TBARS) após treinamento. Todas essas alterações foram revertidas pelo tratamento com cafeína. No manuscrito destacamos o papel modulador do diclofenaco sobre a inflamação gerada por exercício agudo. O protocolo consistiu em uma sessão de 90 mim de exercício excêntrico agudo em esteira em ratos tratados previamente com salina ou diclofenaco (10mg/kg) (grupos: controle-salina, exercício-salina, controle-diclofenaco, exercício-diclofenaco). Após o exercício, foi identificado um aumento na expressão gênica e níveis da proteínas TLR4, MyD88, TRIF, NFκB p65, IL-6, TNF-α e iNOS. Essas respostas geradas pelo exercício excêntrico foram bloqueadas pelo tratamento com diclofenaco. Os dados obtidos nos permitem concluir que o diclofenaco e a cafeína interferem nas respostas adaptativas de cunho oxidativo/inflamatório geradas pelo exercício físico, podendo assim alterar os mecanismos de hormesis dos tecidos ao exercício físico.

Palavras-chave: Exercício Excêntrico, Diclofenaco, Cafeína, Status Redox, Inflamação, Fígado.

ABSTRACT

Doctoral Thesis
Post-Graduate Program in Biology Science: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

The effects of caffeine and diclofenac on oxidative stress and inflammation on exercised rats

Author: Rômulo Pillon Barcelos
Advisor: Nilda de Vargas Barbosa
Place and date of defense: Santa Maria, July 1st, 2015.

Exercise can represent a physical stress that disrupts the homeostasis. Elevated muscle oxygen consumption increases reactive oxygen species (ROS) and inflammatory proteins production, leading to oxidative stress and systemic inflammation. Nowadays, both caffeine, a commonly compound present in many commercial beverages and medicines, and the non-steroidalanti-inflammatory drugs (NSAIDs), including diclofenac, have been used in sports competitions events. Considering that athletes intent to improve their sports performance, mainly on high intensity competitions and short duration that can lead to inflammation and pain, a great number of athletes consume caffeine and NSAIDs due they ergogenic effects or avoid inflammation and loss of performance. However, little is known about the physical exercise and concomitant use of caffeine/diclofenac. Considering the sport specificity, most authors have focused exercise in skeletal muscle studies. In view of the important role of liver during physical activities, one of the goals of this work was to analyze the effect of caffeine and diclofenac on cell damage, hepatic inflammation and oxidative stress markers in exercised rats. In two papers, we highlight the effect of caffeine on the oxidative damage, inflammation and tissue adaptation in liver, muscle and plasma of training rats. The experimental protocol included four groups: sedentary-saline, sedentary-diclofenac, exercise-saline, and exercise-diclofenac. The exercised groups performed a 4-week aerobic swimming training protocol and were treated with saline or caffeine (6 mg/kg). We found significant changes on citrate synthase (CS), superoxide desmutase (SOD), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) enzyme activities and aspartate aminotransferase (AST) and thiobarbituric acid reactive substances (TBARS) levels after training. These modifications were reverted by caffeine treatment. In the manuscript, we highlight the effects of diclofenac (10 mg/kg) on the inflammation induced by an acute exercise. Rats were divided in 4 groups: control-saline (CS), control-diclofenac (CD), exercise-saline (ES) and exercise-diclofenac (ED). The animals from the C and E groups received saline, while the groups CD and ED received diclofenac treatment during seven days previous to the exercise bout, which consisted in an acute bout of eccentric exercise lasting 90 min. We identified an increase in both gene expression and levels of proteins TLR4, MyD88, TRIF, NFκB, p65, IL-6, TNF-α and iNOS in the exercised groups. Diclofenac treatment blunted these responses exercised-induced. Taken together, the data indicate that diclofenac and caffeine interfere on oxidative and inflammation responses induced by physical exercise, altering the adaptive mechanisms of tissues under exercise.

Key words: Eccentric exercise, Diclofenac, Caffeine, Redox Status, Inflammation, Liver.

SUMÁRIO

1. Introdução......10

1.1. Exercício físico......10

1.1.1. Aspectos gerais......10

1.1.2. Exercício físico e estresse oxidativo......13

1.1.3. Exercício físico e tecido hepático......15

1.1.4. Exercício físico e inflamação......16

1.1.5. Via dos TLRs e cascata inflamatória......18

1.2. Substâncias Ergogênicas......21

1.2.1. Cafeína......22

1.3. Anti-inflamatórios não esteroidais (AINEs)......24

1.3.1. Diclofenaco......28

2. Objetivos......31

2.1. Objetivo geral......31

2.2. Objetivos específicos......31

2.2.1. Objetivo artigo 1......31

2.2.2. Objetivo artigo 2......31

2.2.3. Objetivo manuscrito 1......31

3. Uso de animais......32

4. Artigo 1: Caffeine Supplementation Modulates Oxidative Stress Markers in the Liver of Trained Rats......33

5. Artigo 2: Caffeine Intake May Modulate Inflammation Markers in Trained Rats......40

6. Manuscrito 1: Effects of Diclofenac on the Toll-Like Receptor 4/Nuclear Factor Kappa B-Mediated Inflammatory Response to Eccentric Exercise in Rat Liver......54

7. Discussão......73

8. Conclusão......78

9. Referências......79

1. INTRODUÇÃO

1.1. Exercício Físico

1.1.1. Aspectos gerais

O treinamento físico pode ser definido com um efetivo e não-invasivo método de melhoramento da saúde. O mesmo depende diretamente de uma boa organização, estruturação e controle para que cause melhoramento do desempenho físico (GOMES, 2003). Desde 2500 AC, na china, há registros de treinamentos físicos organizados para promover a saúde (LEE; SKERRETT, 2001; LYONS; PETRUCELLI, 1978). Na época greco-romana, Hipócrates (460 – 370 AC) e depois Galen (129 – 210 DC), reconheceram a necessidade do exercício físico para o benefício da saúde e como profilaxia para atletas (SPEED; JAQUES, 2011). O mesmo foi reconhecido pelo filósofo Platão que afirmou “a falta de atividade física destrói a boa condição de saúde de qualquer ser humano, enquanto o movimento e o exercício físico metodológico a asseguram e a preservam” (FOX; HASKELL, 1968).

Exercício físico moderado regular é considerado benéfico ao ser humano, evidenciado em todas as faixas etárias: crianças (MEYER et al., 2006), jovens (CZEPLUCH et al., 2011), adultos e idosos (OGAWA et al., 2010; RODRIGUEZ-MIGUELEZ et al., 2014). É prescrito como terapia tanto para indivíduos saudáveis como para doentes. Há indiscutíveis evidências sobre seus benefícios tanto na prevenção quanto no tratamento de diversas doenças. Já foi demonstrado que homens e mulheres com uma frequência maior de atividade física têm uma redução relativa no risco de morte, que pode variar de 20 a 35% (BLAIR et al., 1989; MACERA; HOOTMAN; SNIEZEK, 2003).

Os efeitos da atividade física são evidentes. Exercício regular contribui para a melhora e prevenção de doenças crônicas pulmonares e cardiovasculares (WARBURTON; NICOL; BREDIN, 2006), desordens metabólicas (diabetes tipo 2, dislipidemia, obesidade, resistência à insulina), doenças musculares, em ossos e junções (artrite reumatoide, fibromialgia, síndrome da fadiga crônica, osteoporose), câncer e depressão (PEDERSEN; SALTIN, 2006; WARBURTON; NICOL; BREDIN, 2006).

Em relação à redução dos riscos cardiovasculares é importante ressaltar melhoras nos níveis de lipídios, tolerância a glicose, gordura visceral e sensibilidade à insulina (DREXEL et al., 2008; STEPPAN et al., 2012). O treinamento físico aeróbio também é conhecido por reduzir a disfunção endotelial vascular (DEVAN et al., 2013; LUTTRELL et al., 2013), por diminuir a rigidez das artérias centrais e periféricas em adultos saudáveis e não-obesos (CURRIE;

THOMAS; GOODMAN, 2009; TANAKA et al., 2000) e em hipertensivos médios (COLLIER et al., 2008; WESTHOFF et al., 2008). Uma diminuição de marcadores ateroscleróticos, como menor quantidade de lipoproteínas no plasma e aumento da resistência da lipoproteína de baixa densidade (LDL) à oxidação (MEDLOW et al., 2015), tem sido observada após protocolos de exercício físico crônico (THOMPSON et al., 2001). Além dos efeitos no sistema cardiovascular, o exercício regular é também um componente fundamental na prevenção e tratamento do diabetes tipo 2. Existem evidências que o exercício físico melhora a glicemia e aumenta a sensibilidade dos tecidos à insulina (ROBERTS; LITTLE; THYFAULT, 2013), afetando positivamente também fatores relacionados como lipidemias e pressão sanguínea (COLBERG et al., 2010).

O exercício físico apresenta efeitos benéficos também sobre o Sistema Nervoso Central (SNC), incluindo uma melhora da função cognitiva (DISHMAN et al., 2006; VAYNMAN; GOMEZ-PINILLA, 2006), como por exemplo: melhora na capacidade de memória (VAN PRAAG et al., 1999), aprendizado, diminuição do declínio cognitivo decorrente do envelhecimento, melhora na resposta neuroimunológica, além de proteger o SNC de insultos, como o acidente vascular encefálico (HU et al., 2014; INOUE et al., 2014; SPEISMAN et al., 2013) e ajuda na recuperação de um traumatismo cranioencefálico (LIMA et al., 2009). O impacto do exercício físico sobre o SNC também está relacionado com a melhora de humor e depressão. Em humanos diagnosticados clinicamente com depressão, o exercício físico tem se mostrado eficaz em reduzir os sintomas associados a essa condição patológica (JOSEFSSON; LINDWALL; ARCHER, 2014). Também existem evidências que o exercício melhora a qualidade do sono e contra-ataca o declínio mental oriundo da idade (LAURIN et al., 2001).

Apesar de tantos benefícios a saúde, assim como para qualquer outro tratamento, a dosagem (volume e intensidade), a frequência (sessões por semana), o tipo (aeróbio ou de força), os efeitos sistêmicos e psicoativos, as contra-indicações e os efeitos adversos do exercício devem ser levados em consideração para a obtenção de uma resposta clínica adequada. Por exemplo, um exercício físico de alta intensidade pode causar danos no tecido muscular, aumentando a produção de espécies de reativas de oxigênio (EROs) e de diversos fatores inflamatórios (AOI et al., 2004; FISHER-WELLMAN; BLOOMER, 2009). Atualmente, sabe-se que a liberação de determinados mediadores da inflamação culmina com dor em diferentes tecidos, incluindo o músculo esquelético (ZELENKA; SCHÄFERS; SOMMER, 2005). Neste sentido, os relatos mais comuns de dor em atletas têm etiologia mecânica, que pode estar relacionada à distensão muscular ou lesões de origem ligamentar (ALARANTA; ALARANTA; HELENIUS, 2008).

Além da geração de EROs, estudos experimentais e clínicos relatam que determinados tipos de exercício físico, como o excêntrico, causam micro traumas na musculatura esquelética e aumentam a liberação de citocinas pró-inflamatórias no músculo (CARMICHAEL et al., 2010) e regiões do córtex cerebral (NYBO et al., 2002). Estudos com o objetivo de avaliar os efeitos do exercício físico agudo sobre o estresse oxidativo no fígado têm mostrado que o exercício físico extenuante provoca uma série de alterações metabólicas que podem prejudicar o organismo de várias formas (RASMUSSEN et al., 2001; RADAK et al., 2009), dentre as quais destaca-se a geração de EROs. Porém, apesar de alguns pesquisadores sugerirem que o aumento na geração de radicais livres em diversos tecidos biológicos coincide com a presença de danos celulares, a ligação entre estes parâmetros ainda não está totalmente estabelecida (HOENE e WEIGERT, 2010).

Uma única, aguda e extenuante sessão de exercício físico tem o efeito temporário de depressão do sistema imune; e alguns estudos associam o envolvimento de exercícios extremos (maratonas) com o aumento da incidência de infecções nas semanas seguintes após o exercício (NIEMAN et al., 1990; PETERS, 1997). Uma sessão aguda de exercício físico pode ser acompanhada por respostas que são nitidamente similares à aquelas de uma infecção, sepse ou trauma: um aumento no número de leucócitos circulantes (principalmente linfócitos e neutrófilos), cuja magnitude está relacionada com a intensidade e duração do exercício físico (GLEESON, 2007). Há também aumento das concentrações plasmáticas de várias substâncias que são conhecidas por influenciar as funções leucocitárias, incluindo citocinas como o fator de necrose tumoral (TNF-α), interleucina (IL) 1β; IL-6, IL-10, e antagonista do receptor de IL-1 (IL-1ra); e ainda proteínas de fase aguda, incluindo a proteína C reativa (PCR). Um maior aumento na concentração de IL-6 plasmática durante o exercício pode ser acompanhado da secreção desta citoquina pelas fibras musculares (STEENSBERG et al., 2000).

1.1.2. Exercício físico e estresse oxidativo

O exercício aeróbio demanda um suprimento energético que é atendido por um alto consumo de oxigênio. A mitocôndria, a qual tem uma função chave na regulação do estado redox, metabolismo energético, apoptose e sinalização intracelular (PICARD et al., 2011; RYAN; HOOGENRAAD, 2007), faz com que o consumo de oxigênio esteja diretamente acoplado à formação do radical superóxido (BOVERIS; CHANCE, 1973), o qual pode ser gerado em diferentes taxas de acordo com tecido (ST-PIERRE et al., 2002). Neste contexto, existem evidências de que o exercício extenuante causa mudanças metabólicas marcantes na função mitocondrial (RASMUSSEN et al., 2001), sendo o aumento na geração de EROs uma

das principais (RADÁK et al., 2004). A ocorrência de disfunção mitocondrial tem sido apontada como um evento importante durante um exercício exaustivo (WILLIS; JACKMAN, 1994). Por isso, o exercício é considerado um tipo de estresse físico que pode perturbar transitoriamente a homeostase celular (MASTORAKOS; PAVLATOU, 2005).

O estresse oxidativo pode ser definido como um estado de desequilíbrio celular entre as defesas antioxidantes e a produção de EROs (SEDDON; LOOI; SHAH, 2007); sendo caracterizado pelo aumento da produção de EROs à níveis considerados danosos para biomoléculas como DNA, proteínas e lipídios. Sob condições fisiológicas, até 5% do oxigênio consumido na cadeia respiratória mitocondrial pode ser direcionado para a formação EROs, que em condições saudáveis são neutralizadas/decompostas pela maquinaria antioxidante do organismo (FINAUD; LAC; FILAIRE, 2006). Níveis basais de EROs são requeridos para manter o *status redox* normal da célula, defesa e sinalização celulares (JEŽEK; HLAVATÁ, 2005; VINCENT; TAYLOR, 2006).

Durante a atividade física intensa, a produção exacerbada de EROs pode ser resultado de fatores como: o aumento do fluxo de oxigênio na cadeia respiratória (VINCENT; TAYLOR, 2006), uma vez que a quantidade total de oxigênio consumida pode ser aumentada entre dez a vinte vezes, aumentando, assim, a produção de EROs (BLOOMER, 2008); o processo de isquemia/reperfusão; a ativação de leucócitos em resposta aos danos musculares; e a maior taxa de oxidação de mioglobina (DAVIES et al., 1982; FINAUD; LAC; FILAIRE, 2006). Esse aumento na formação de EROs é influenciado pelo volume e a intensidade do exercício tanto em humanos (PINHO et al., 2010; TURNER et al., 2011) quanto em animais (SELMAN et al., 2002). Portanto, dependendo do tipo e intensidade, o exercício pode levar a fadiga (HOLLANDER et al., 1999; REID, 2001). Neste sentido, estudos têm demonstrado que o tratamento com antioxidantes pode evitar a fadiga (MOOPANAR; ALLEN, 2006; SUPINSKI et al., 1997).

Em contrapartida, como já foi abordado, sabe-se que o exercício físico regular apresenta um papel protetor contra doenças relacionadas ao estilo de vida, principalmente por promover resistência a diversos estímulos estressores (RADAK; CHUNG; GOTO, 2005; RADÁK et al., 2004). Um grande número de evidências sugere que esse tipo de atividade tem um importante papel na prevenção e tratamento de doenças associadas ao estresse oxidativo, incluindo isquemia cardíaca, diabetes tipo 2 e Alzheimer (LAZAREVIC et al., 2006). O exercício físico regular é capaz de induzir várias adaptações nos sistemas cardio-respiratório, muscular, metabólico e nervoso. No entanto, as razões pelas quais estas adaptações acontecem ainda não estão completamente elucidadas (GIBALA et al., 2012; VINA et al., 2012). Estudos sugerem

que essas adaptações relacionadas ao exercício físico regular, podem ser causadas pela contínua e moderada geração de EROs durante a sua prática. Neste cenário, os radicais livres atuariam como sinais para a ativação e melhoria dos sistemas de defesas celular (VINA et al., 2012). Níveis moderados de EROs podem ativar vias de transdução de sinais que culminam com aumento na expressão de enzimas antioxidantes e modulação de processos oxidativos (GOMEZ-CABRERA; DOMENECH; VIÑA, 2008; JACKSON, 2008; JI, 1993; RADAK; CHUNG; GOTO, 2005), induzindo resistência ao estresse e proteção ao organismo contra danos oxidativos.

Parte dessa adaptação deve-se à modulação do metabolismo oxidativo, afetando diretamente o estado redox celular (GOMEZ-CABRERA; DOMENECH; VIÑA, 2008; JACKSON, 2008; RADAK et al., 2008). Como resultado pressupõe-se que as células se tornam menos susceptíveis aos danos associados a episódios agudos de estresse oxidativo, sendo este um efeito do processo de adaptação ao treinamento (GOMES; SILVA; OLIVEIRA, 2012; KAROLKIEWICZ et al., 2009; NIESS; SIMON, 2007; PAULSEN et al., 2014). Tanto em animais como em humanos, as adaptações teciduais induzidas pelo exercício e que incluem aumento da capacidade de resistência, estão associadas com aumento da biogênese mitocondrial, redução da produção de agentes oxidantes e aumento das defesas antioxidantes (PACKER; CADENAS, 2007; SACHDEV; DAVIES, 2008). Estudos mostram que animais expostos ao exercício crônico apresentam menor dano oxidativo depois de um exercício exaustivo do que os não treinados (PARKER; MCGUCKIN; LEICHT, 2014; SALMINEN; VIHKO, 1983). Isto é amplamente atribuído ao aumento da expressão de enzimas antioxidantes tais como a glutationa peroxidase (GPx), superóxido dismutase mitocondrial (MnSOD) e a γ-glutamil-cisteína-sintetase (SALMINEN; VIHKO, 1983). Um estudo realizado por Jessica e cols (2009) constatou que a corrida voluntária em roda diminui o estresse oxidativo em artérias de camundongos idosos através da redução da expressão da NADPH oxidase, a qual, juntamente com a mitocôndria, é umas das principais fontes geradoras de EROs.

Por causa das amplas implicações das EROs em praticamente todas as funções biológicas, é difícil definir as vias e alvos moleculares afetados pela sinalização redox durante o exercício. No entanto, as mais relevantemente moduladas pelo exercício incluem: PPAR-ɣ coativador-1a e b (PGC-1a e PGC-1b) (GOMEZ-CABRERA et al., 2008; RISTOW et al., 2009), p53 (BORRÁS; GÓMEZ-CABRERA; VIÑA, 2011), fator induzível por de hipóxia 1 (HIF-1) (HUANG et al., 1996), *heat shock factor* (HSF) (PALOMERO et al., 2008) e as vias de sinalização NFκB e MAPK (GOMEZ-CABRERA et al., 2005; JI et al., 2004). Importantes adaptações no músculo esquelético como a biogênese mitocondrial, defesa antioxidante,

inflamação, hipertrofia, citoproteção e transformação de fibras são reguladas primariamente por estas vias (GOMEZ-CABRERA; VIÑA; JI, 2009).

1.1.3. Exercício físico e tecido hepático

As adaptações metabólicas do exercício não estão restritas ao músculo em atividade, uma vez que este constitui um esforço generalizado para outros órgãos como o músculo cardíaco, estômago, fígado e cérebro (ÇAKIR et al., 2010; VENEROSO et al., 2009). Neste cenário, salienta-se a relevância do exercício físico para o fígado devido ao papel central que este órgão tem na manutenção do fornecimento de energia ao músculo em atividade (HOENE; WEIGERT, 2010). Além disso, o fígado por ser o principal órgão envolvido na síntese de glutationa reduzida (GSH), fornecendo 90% da GSH circulante, tem papel efetivo na regulação redox, uma vez que este peptídeo é um dos mais importantes antioxidantes endógenos (MÃRTENSSON; MEISTER, 1991; NIKOLAIDIS et al., 2008). O treinamento físico induz notáveis mudanças no metabolismo (GUL et al., 2002; LIU et al., 2000) e no status oxidante/antioxidante do fígado (BOTEZELLI et al., 2011; HOENE; WEIGERT, 2010; KAKARLA; VADLURI; REDDY KESIREDDY, 2005; RADÁK et al., 2000; WILSON; JOHNSON, 2000). Nesse sentido, alguns estudos têm demonstrado que o exercício físico crônico causa adaptações oxidativas favoráveis no tecido hepático, potencializando o status antioxidante do tecido (HOENE; WEIGERT, 2010; LIMA et al., 2013).

Entretanto, sabe-se que o exercício físico extenuante altera a função mitocondrial de muitas maneiras (RASMUSSEN et al., 2001). A formação de EROs mitocondrial, devido à alta taxa de consumo de oxigênio durante o exercício intenso, está entre os principais fatores de dano tecidual (RADÁK et al., 2000). Sun e cols. (2010) encontraram níveis elevados de GHS mitocondrial em fígado de ratos depois de 4 semanas de treinamento, que foi atribuído ao aumento da atividade antioxidante. Navarro e cols. (2004) também reportou que o exercício moderado aumenta a atividade da MnSOD e diminui os produtos de oxidação em mitocôndrias (TBARS e proteínas carboniladas) de fígado de ratos treinados.

Protocolos de exercício exaustivo têm sido relacionados com elevação nos níveis de TBARS (KORIVI et al., 2012) e aumento nos níveis de SOD em fígado de ratos (KAKARLA; VADLURI; REDDY KESIREDDY, 2005). Protocolos experimentais usando exercício físico agudo também têm evidenciado um aumento na produção de EROs, na peroxidação lipídica (GUL et al., 2002; HUANG; TSAI; LIN, 2008; VILLA et al., 1993), na carbonilização de proteínas (KORIVI et al., 2012; LIU et al., 2000); porém uma diminuição nos níveis de

antioxidantes hepáticos (LEEUWENBURGH; JI, 1995; LEW; PYKE; QUINTANILHA, 1985).

Com relação ao estresse oxidativo causado pelo exercício físico, pouca informação existe sobre a sua influência no tecido hepático após exercício físico (HUANG, TSAI e LIN, 2008; GONZALEZ e MANSO, 2004; TURGUT et al., 2003). O fígado é um órgão que possui um papel central na regulação dos estoques de carboidratos e lipídios durante a prática de exercício físico crônico e agudo (WAHREN e EKBERG, 2007; FRITSCHE et al., 2008). Dessa forma, estudos sobre a influência de um período de treinamento aeróbio no aparecimento de estresse oxidativo no fígado e a sua relação com exercícios exaustivos agudos ainda são necessários.

1.1.4. Exercício físico e inflamação

Um estilo de vida sedentário leva ao acúmulo de gordura visceral, e isto é acompanhado pela infiltração de células imunes pró-inflamatórias no tecido adiposo, aumentando a liberação de adipocinas e causando o desenvolvimento de um estado de inflamação sistêmica de baixo grau (OUCHI et al., 2011). Esta inflamação sistêmica de baixo grau tem sido associada ao desenvolvimento da resistência à insulina, aterosclerose, neurodegeneração e crescimento tumoral (LEONARD, 2007; PEDERSEN; SALTIN, 2006; PRADHAN et al., 2001). Contudo, indivíduos que são fisicamente ativos têm uma redução dos níveis de biomarcadores inflamatórios. Por exemplo, um exercício físico intenso e regular está associada com uma produção reduzida de citocinas inflamatórias e de proteínas inflamatórias musculares, baixa produção de adipocina e baixos níveis sanguíneos de proteína C-reativa (CRP) (GLEESON; MCFARLIN; FLYNN, 2006).

A hipótese do efeito anti-inflamatório do treinamento físico esta sendo cada vez mais estudada nos últimos anos (GLEESON et al., 2011). O treinamento físico de moderado-a-alta intensidade tem propriedades anti-inflamatórias via vários mecanismos, incluindo a supressão de citocinas pró-inflamatórias (BYRKJELAND et al., 2011) e a potencialização da função anti-inflamatória do endotélio (LAMINA; OKOYE; DAGOGO, 2009).

O efeito anti-inflamatório do exercício (KASAPIS; THOMPSON, 2005; MATHUR; PEDERSEN, 2008; PETERSEN; PEDERSEN, 2005) é mediado não somente via a redução de gordura visceral (com subsequente diminuição da produção e liberação de adipocinas pró-inflamatórias) mas também pela indução de um ambiente anti-inflamatório a cada set de exercício (MATHUR; PEDERSEN, 2008; PETERSEN; PEDERSEN, 2005). As citocinas que exercem efeitos predominantemente anti-inflamatório (exemplo: IL-4, IL-10 e IL-13)

(PROKOPCHUK et al., 2007) também aumentam a miogênese (DENG et al., 2012; HEREDIA et al., 2013). Desta forma, pelo aumento da produção de citocinas anti-inflamatórias no músculo esquelético, o exercício físico pode induzir hipertrofia muscular – ou pelo menos limitar a atrofia muscular (DELLA GATTA et al., 2014).

Estudos relacionados atribuem o efeito anti-inflamatório do exercício principalmente a três fatores: redução da gordura visceral, produção e liberação de citocinas anti-inflamatórias do músculo (PEDERSEN; FEBBRAIO, 2008; PETERSEN; PEDERSEN, 2005) e redução da expressão de receptores Toll Like (TLRs) (e subsequentemente toda a cascata, culminando na redução da produção de citocinas pró-inflamatórias) (GLEESON; MCFARLIN; FLYNN, 2006). No entanto, a participação de outros fatores como infiltração de macrófagos no tecido adiposo bem a como a diminuição de monócitos circulantes e a troca fenotípica destes em macrófagos (KAWANISHI et al., 2010) também é reconhecida.

Quanto a liberação das citocinas anti-inflamatórias pelo músculo, cabe destacar o papel da IL-6. Esta interleucina apresenta ação paradoxal, pois pode ser classificada tanto como pró-inflamatória quanto como anti-inflamatória. A IL-6 pode ser descrita como uma citoquina pró-inflamatória (PEDERSEN; FEBBRAIO, 2008), pelo fato de que seus níveis plasmáticos estão aumentados após a sepse inflamatória e por estar envolvida na estimulação da produção de outras citocinas pró-inflamatórias, oriundas de diferentes fontes (WILUND, 2007). No entanto, a IL-6 derivada da contração muscular tem função anti-inflamatória. Essa diferenciação ocorre possivelmente pelo mecanismo de sinalização. Em um estado de inflamação crônica a IL-6 é responsável pela ativação de monócitos e macrófagos ativados pelo TNF-α, ao passo que a contração muscular induz a ativação de IL-6 independente da resposta anterior ao TNF-α, sugerindo que a IL-6 tem um papel mais metabólico que inflamatório (BENATTI; PEDERSEN, 2015; PEDERSEN; FEBBRAIO, 2008). A concentração de IL6 plasmática aumenta exponencialmente com a duração do exercício (KELLER et al., 2005; PEDERSEN; FISCHER, 2007), porém este aumento é transitório e normalmente retorna aos de descanso (basais) dentro de uma hora após o término do exercício (PEDERSEN, 2009). Frente a essa elevação, outros hormônios e citocinas anti-inflamatórias também são liberadas, tais como a IL-10, IL1ra, o que também aumenta a secreção de cortisol das glândulas adrenais (STEENSBERG et al., 2003). Sugere-se que a IL-6 seja o centro de mediação dos efeitos agudos provocados pelo exercício, sinalizando uma cascata de ativação de outras citocinas, e assim o efeito anti-inflamatório (BENATTI; PEDERSEN, 2015; GLEESON et al., 2011).

Sabe-se também que há uma relação entre o sistema colinérgico e a resposta imunológica. A ativação imunológica de linfócitos T e B, através da interação de antígenos

presentes nas células CD3, CD4 e CD8 com as moléculas de superfície de células endoteliais vasculares e células inflamatórias, aumentam a síntese e extravasamento de acetilcolina (ACh) pelas células T e aumentam a expressão de receptores para ACh em ambas células T e B (RINNER; KAWASHIMA; SCHAUENSTEIN, 1998; WATANABE et al., 2002). Evidências também destacam a relação entre o sistema colinérgico e o processo inflamatório, onde a ACh parece ter um papel importante no combate à inflamação por inibir a produção de TNF-α, IL-1β por macrófagos, os quais possuem expressão de receptores nicotínicos de ACh (BOROVIKOVA et al., 2000).

Sabe-se que a produção de citocinas é controlada por neurônios via reflexo inflamatório (TRACEY, 2002, 2007). A atividade do nervo vago eferente, o qual inclui o reflexo inflamatório, regula a produção de citocinas especificamente via o sinal nomeado "via anti-inflamatória colinérgica" (BOROVIKOVA et al., 2000; PAVLOV; TRACEY, 2005; TRACEY, 2007; WANG et al., 2003). Esta via é mediada primariamente por receptores nicotínicos de ACh dos macrófagos (PAVLOV; TRACEY, 2006). Neste contexto, Pavlov e cols. demonstraram que um agonista de receptor muscarínico, suprime os níveis de TNF-α na endotoxemia de ratos e que o nervo vago eferente deve ser o mediador deste efeito (PAVLOV et al., 2006). Somado a isso, que a galantamina, um inibidor clínico da acetilcolinesterase (AChE), suprime os níveis de citocinas durante uma endotoxemia (PAVLOV et al., 2009). Além disso, ACh inibe a síntese e liberação de TNF-α tanto *in vitro* como *in vivo* (BOROVIKOVA et al., 2000) e aumenta a produção de óxido nítrico (BLAISE; STEWART; GUÉRARD, 1993; CZURA; FRIEDMAN; TRACEY, 2003). Esta via colinérgica anti-inflamatória mediada pela ACh atua inibindo a produção de TNF-α e IL-1 e suprimindo a ativação de NFκB (BOROVIKOVA et al., 2000; PAVLOV; TRACEY, 2006; WANG et al., 2004).

Considerando a ACh uma molécula anti-inflamatória, pode-se inferir que em várias condições onde haja inflamação sistêmica de baixo grau, como por exemplo: Alzheimer, diabetes mellitus, hipertensão, hiperlipidemia, resistência insulínica, doenças coronárias e na síndrome metabólica, a atividades da enzimas AChE esteja alterada (TAGLIARI et al., 2011). Devido ao importante papel na modulação da inflamação, a atividade de tais enzimas bem como os níveis de ACh podem ser usados como indicadores de inflamação sistêmica de baixo grau (KAWASHIMA; FUJII, 2003).

1.1.5. Via dos TLRs e cascata inflamatória

Os TLRs são expressos em vários tecidos (MUZIO et al., 2000) e possuem papéis importantes no reconhecimento e indução de respostas tanto do sistema imune inato quanto do adquirido (MUZIO; MANTOVANI, 2000; TAKEDA; AKIRA, 2004, 2005). Atualmente, 11 membros da família TLRs foram identificados em humanos e 13 membros dessa família em camundongos (POLTORAK et al., 1998). O TLR2 e o TLR4 são encontrados em vários tipos celulares incluindo adipócitos, hepatócitos e miócitos (LANG et al., 2003; LIN et al., 2000).

Os TLRs são glicoproteínas transmembranas do tipo I expressas em diferentes tipos celulares de resposta imune inata e em fibroblastos e células epiteliais (AKIRA; UEMATSU; TAKEUCHI, 2006). Os TLRs mediam o reconhecimento de padrões moleculares associados à patógenos e possuem um papel importante nas respostas imune e anti-inflamatória (GLEESON; MCFARLIN; FLYNN, 2006). Os TLRs de monócitos, macrófagos, e células dentríticas contribuem significantemente para o desenvolvimento da resposta imune adaptativa (PASARE; MEDZHITOV, 2004), sendo que a produção de citocinas IL-1β, IL-6, IL-8, e TNF-α (BANCHEREAU; STEINMAN, 1998; MEDZHITOV, 2001; PASARE; MEDZHITOV, 2003) também levam a geração de uma resposta imune adaptativa (ALEXOPOULOU et al., 2001; YAMAMOTO et al., 2003).

Há vários ligantes endógenos de TLRs que podem ter um papel na regulação da expressão desses receptores ou permitirem o sistema imune responder ao dano ou a sinais de dano (KILMARTIN; REEN, 2004). O reconhecimento de antígenos pelos TLRs dispara uma sinalização intracelular (TAKEUCHI; AKIRA, 2001) que resulta na indução de um conservado programa de defesa que inclui a produção de citocinas (ALEXOPOULOU et al., 2001). A família dos TLRs utiliza motivos proteicos de grande quantidade de leucina para a ligação e um domínio citoplasmático que é homólogo ao do receptor da IL-1. Frente a um estímulo, o TLR recruta adaptadores como o “myeloid differentiation primary response gene 88” (MyD88) e o “Toll/interleukin-1 receptor domain containing adapter protein” (TIR-AP) (WEST; KOBLANSKY; GHOSH, 2006). A cascata de sinalização leva a ativação de “mitogen-activated protein kinase” (MAPKs), proteína quinase C (PKC), ativação de fatores de transcrição como o NFκB e PGC-1 e de fatores pró-inflamatórios (AKIRA; SATO, 2003).

A regulação dos TLRs como fator chave na resposta inflamatória mediada pelo exercício físico tem recebido grande interesse da comunidade científica (FLYNN; MCFARLIN, 2006; GLEESON; MCFARLIN; FLYNN, 2006). Estudos envolvendo exercício excêntrico destacam o impacto desse tipo de exercício na resposta inflamatória (PEAKE; NOSAKA; SUZUKI, 2005). Uma sessão de exercício excêntrico induz uma resposta pró-inflamatória marcante, enquanto que um programa de treinamento com exercício excêntrico atenua a ativação de

diferentes vias de sinalização envolvidas no processo inflamatório (FERNANDEZ-GONZALO et al., 2012; GARCÍA-LÓPEZ et al., 2007; JIMÉNEZ-JIMÉNEZ et al., 2008). Sabe-se que indivíduos fisicamente ativos têm uma menor expressão de TLR4 na superfície celular e menor capacidade de produção de citocinas inflamatórias de monócitos que os sujeitos fisicamente inativos (FLYNN et al., 2003; MCFARLIN et al., 2004, 2006; STEWART et al., 2005). Recentemente, foi demonstrado que um programa de treinamento de 12 semanas tanto de exercício de endurance ou de força reduz a expressão de mRNA de TLR4, TNF-a e de IL-6 no músculo esquelético de pacientes obesos (LAMBERT et al., 2008). Os exatos mecanismos pelos quais a expressão de TLR4 é reduzida pelo treinamento físico são ainda desconhecidos; no entanto, várias hipóteses já foram propostas (FLYNN; MCFARLIN, 2006).

Os TLRs tem um importante papel nos efeitos anti-inflamatórios associados a uma vida fisicamente ativa (MCFARLIN et al., 2004), sendo que diferentes TLRs interagem com uma combinação diferente de domínios TIR. O TLR4 é o que mais utiliza a maioria deles: MyD88, TIRAP, TRIF (TIR domain-containing adaptor inducing IFN-b) e TRAM (TRIF-related adaptor molecule) (O'NEILL; BOWIE, 2007). Dentre todos os TLRs, o TLR4 é o que responde a diferentes tipos de protocolos de exercício (CRISTI-MONTERO et al., 2012; FERNANDEZ-GONZALO et al., 2012; PHILLIPS et al., 2012; ZBINDEN-FONCEA et al., 2012). Juntamente com o CD14, TLR4 orquestra vários processos na cascata inflamatória (AKIRA; UEMATSU; TAKEUCHI, 2006) e pode ativar diferentes vias de sinalização que aumentam ou inibem a atividade de muitos fatores de transcrição. Três fatores de transcrição principais são controlados, pelo menos em grande parte, pela via de sinalização do TLR4: o fator de transcrição κB (NFκB), a proteína de ativação-1 (AP-1), e o fator de transcrição regulatório interferon 3 (IRF3) (INTO et al., 2012).

De fato, os TLRs induzem um aumento da expressão de citocinas através da via do NFκB, o qual está envolvido na modulação da expressão dos próprios TLRs (MIETTINEN et al., 2001; SEIBL et al., 2003). Frente a uma estimulação, estes receptores induzem o recrutamento de adaptadores MyD88 e TRIF. Por isso, a sinalização da cascada do TLR é dividida em uma via dependente e outra independente de MyD88 (CRISTOFARO; OPAL, 2006). Os dois processos resultam na ativação de uma série de sinalização, incluindo o NFκB e o MAPK (AKIRA; SATO, 2003), os quais controlam respostas inflamatórias e imunológicas pela indução da expressão de várias citocinas pró-inflamatórias tais como o TNF-α e o interferon tipo I (IFN) (CONNOLLY; O'NEILL, 2012; OSHIUMI et al., 2003).

As subunidades de NFκB, p50 e p65, formam o mais frequente heterodímero de NFκB (MCFARLIN et al., 2006). A ativação de NFκB, tanto pela via dependente ou independente de

MyD88, leva o recrutamento da sua subunidade p50 no citoplasma e a translocação da subunidade p65 para o núcleo (KAWAI; AKIRA, 2006). Uma das mais importantes vias de sinalização ativada durante um exercício envolve o NFκB. Este fator de transcrição faz um link entre múltiplas vias de sinalização que orquestram diversos processos em virtualmente todos os tipos celulares. A translocação da subunidade p65 do NFκB ao núcleo altera diretamente a produção de um grande número de genes, incluindo aqueles que geram citocinas, receptores apresentadores e reguladores do status redox, proteínas de resposta de fase aguda, apoptose e atrofia (PAHL, 1999). Muitos dos genes ativados por NFκB são conhecidos por serem pró-inflamatórios (HOFFMANN; BALTIMORE, 2006; MA; HE; QIANG, 2013). Estes genes, incluindo a óxido nítrico sintase induzível (iNOS) e a ciclooxigenase-2 (COX-2), são induzidos nas células inflamatórias e seus produtos trabalham em conjunto para causar inflamação tecidual por diversos mecanismos, incluindo a ativação da guanilil ciclase solúvel, proteína de nitração e nitrosilação, e produção de prostanóide pró-inflamatórios como a prostaglandina E2 (CIPOLLONE et al., 2001; ISCHIROPOULOS, 1998).

No geral, os dados na literatura com relação a expressão dos TLR depois de exercício agudo são bastante controversos. Alguns indicam que há uma diminuição da expressão de TLR4 após uma sessão de exercício agudo (LANCASTER et al., 2005; LIAO et al., 2010; STEWART et al., 2005) enquanto outros não detectam nenhuma modificação (MCFARLIN et al., 2004) ou ainda mostram um aumento na expressão de TLR4 (FERNANDEZ-GONZALO et al., 2012; ROSA et al., 2011).

1.2. Substâncias ergogênicas

Na busca por melhor performance, estética ou ainda na busca do sucesso em esportes de alto nível, apenas a prática regular e treinamento físico podem ser insuficientes para que os objetivos sejam alcançados conforme o planejamento. Com isso, em muitos casos, treinadores, nutricionistas, médicos e cientistas têm lançado mão de inúmeros recursos no intuito de potencializar a performance atlética ou atenuar os mecanismos associados a fadiga de seus atletas (JUHN, 2002; MAUGHAN; KING; LEA, 2004).

Dois fatores vêm chamando a atenção e sendo levados em consideração nos últimos recordes esportivos: a dieta e os chamados recursos ergogênicos (RODRIGUEZ; DI MARCO; LANGLEY, 2009; WIENS et al., 2014). Os ergogênicos são procedimentos ou recursos utilizados para aprimorar a capacidade de realizar um trabalho físico ou desempenho atlético, e podem ser de vários tipos: mecânicos, psicológicos, fisiológicos, farmacológicos ou nutricionais (RODRIGUEZ; DI MARCO; LANGLEY, 2009). O uso de recursos nutricionais,

por exemplo, é cada vez maior entre atletas e praticantes de exercícios físicos regulares. Existe uma grande oferta destes recursos ergogênicos, que podem ser macronutrientes como os carboidratos, proteínas e alguns tipos de ácidos graxos ou também alguns tipos específicos de vitaminas e minerais (MOMAYA; FAWAL; ESTES, 2015).

No geral, a utilização de suplementos nutricionais e substâncias com potencial ergogênico tem se mostrado eficiente por resultar em benefícios como aumento das reservas energéticas, aumento da mobilização de substratos para os músculos ativos durante os exercícios físicos, aumento do anabolismo proteico, diminuição da percepção subjetiva de esforço, reposição hidroeletrolítica adequada. Tais eventos retardam o aparecimento da fadiga e aumentam o poder contrátil do músculo esquelético e/ou cardíaco, aprimorando assim a capacidade de realizar trabalho físico, ou seja, a performance atlética (COYLE, 2004; JUHN, 2003; MAUGHAN, 2002; PIPE; AYOTTE, 2002).

1.2.1. Cafeína

A cafeína está entre as substâncias ergogênicas mais utilizadas para fins desportivos e, por ser de fácil acesso e já socialmente incluída no consumo da população mundial, é a que desperta mais interesse (TARNOPOLSKY, 2010). O consumo da cafeína é realizado de forma natural, afinal está presente em diversos alimentos populares, tais como: chás, café, refrigerantes, chocolates, bebidas esportivas e em alguns medicamentos (TARNOPOLSKY, 1994, 2010).

A cafeína, uma das substâncias estimulantes mais utilizadas no mundo, é um alcaloide pertencente à família das metilxantinas, cuja fórmula é 1,3,7- trimetilxantina. A cafeína e as 3 dimetilxantinas teofilina, teobromina e paraxantina são biologicamente ativas e o impacto dessas xantinas no metabolismo tem recebido muita atenção nas últimas décadas (GRAHAM et al., 2008; MCLEAN; GRAHAM, 2002; MOISEY et al., 2008). É uma substância lipossolúvel, sendo rapidamente absorvida pelo trato gastrointestinal (aproximadamente 100%), atingindo seus níveis de pico no plasma entre 30 e 90 minutos após a ingestão oral (TARNOPOLSKY, 1994, 2010).

A cafeína é lentamente catabolizada, apresentando uma meia-vida de 4 a 6 horas, sendo o fígado o principal responsável por sua metabolização via sistema enzimático do citocromo P-450 (CYP1A2). Em humanos adultos, a biotransformação primária leva a formação de paraxantina (83,9%), teobromina (12,2%) e em menor extensão teofilina (3,7%) (MCLEAN;

GRAHAM, 2002). O rim é o órgão responsável por sua eliminação (KALOW; TANG, 1991) sendo que entre 1-3% da cafeína excretada na urina é na forma livre. Este é fato foi importante para o monitoramento em testes de doping (TARNOPOLSKY, 2010).

O principal mecanismo de ação desta droga já está bem esclarecido. A cafeína aumenta a ação do SN Simpático, pelo bloqueio dos receptores de adenosina, um psico-estimulante que atua de forma oposta a cafeína (FREDHOLM et al., 1999; GRAHAM et al., 2008; TARNOPOLSKY, 2010). Devido à semelhança na estrutura das duas moléculas, a cafeína, por ser lipossolúvel, pode atravessar facilmente a barreira hematoencefálica e inibir o efeito da adenosina, aumentando a excitabilidade neuronal e o aumento da liberação de dopamina e adrenalina. Com isso, suas ações em nível de SNC incluem geralmente: aumento da atenção mental, melhora do humor, diminuição do tempo de reação e aumento da liberação de catecolaminas (adrenalina e noradrenalina) (ARCIERO et al., 1995; BACKHOUSE et al., 2011; GRAHAM; SPRIET, 1995; ROBERTSON et al., 1981). Os receptores de adenosina são encontrados em diversos tecidos, incluindo o cérebro, o coração, o músculo esquelético e os adipócitos (GRAHAM, 2001; SÖKMEN et al., 2008). Além disso, sabe-se que a cafeína pode alterar a utilização de substratos energéticos (DAVIS et al., 2003; GOLDSTEIN et al., 2010; TUNNICLIFFE et al., 2008; YANG; CHEN; FREDHOLM, 2009), aumentando a mobilização de ácidos graxos durante um exercício físico, e diminuindo assim a dependência de glicogênio durante a performance física (GRAHAM et al., 2008; TARNOPOLSKY; CUPIDO, 2000). Tais efeitos têm sido associados com a melhora do desempenho em exercícios aeróbios por aumentar a força de contração, tanto do músculo esquelético quanto do cardíaco, e consequentemente retardando o início da fadiga (GOLDSTEIN et al., 2010; SIMMONDS; MINAHAN; SABAPATHY, 2010). Embora ainda incerto, o mecanismo mais proposto e que recebe mais apoio para explicar o efeito ergogênico da cafeína é o seu antagonismo aos receptores de adenosina (GOLDSTEIN et al., 2010; GRAHAM, 2001; TARNOPOLSKY, 2010).

A cafeína também acelera o metabolismo do músculo esquelético e por isso tem sido amplamente usada em competições esportivas (GOLDSTEIN et al., 2010; KALMAR; CAFARELLI, 2004; TARNOPOLSKY, 2008) e em exercícios de diferentes naturezas (ASTORINO; ROBERSON, 2010; PALUSKA, 2003). Nos últimos anos, a ingestão de cafeína tem sido utilizada como estratégia ergogênica no esporte (PALUSKA, 2003), previamente à realização de exercícios físicos aeróbios (DOHERTY; SMITH, 2004; GRAHAM, 2001) e anaeróbios (ASTORINO; ROBERSON, 2010; DAVIS; GREEN, 2009), com o intuito de protelar a fadiga e, consequentemente, melhorar o desempenho físico.

O consumo de duas a três xícaras de café resulta em níveis plasmáticos entre 20 – 40 mmol/L de cafeína (assim como uma ingestão de aproximadamente 5 mg/Kg de cafeína), acompanhada de baixos níveis de dimetilxantinas. A cafeína em concentrações de 5 – 50 mmol/L age como uma antagonista de receptores de adenosina (GRAHAM et al., 2008). Ressalta-se, entretanto, que a dosagem de cafeína é um fator determinante na melhora do desempenho físico, pois o desencadeamento das respostas fisiológicas e metabólicas parece estar atrelado à quantidade ingerida (GRAHAM, 2001; SINCLAIR; GEIGER, 2000). Consumida em pequenas quantidades (2mg/kg), a cafeína provoca aumento do estado de vigília, diminuição da sonolência, alívio da fadiga, aumento da liberação de catecolaminas, aumento da frequência cardíaca e aumento do metabolismo. Doses entre 3 a 9 mg/kg são consideradas efetivas para estabelecer os efeitos ergogênicos da cafeína em competições atléticas (DAVIS et al., 2003) e melhorar o desempenho físico em exercícios físicos. No entanto, é sugerido como ideal de 3 a 6 mg/kg de cafeína independente do momento que estiver sendo consumido: antes ou durante o exercício (GRAHAM et al., 2008; SPRIET et al., 1992). Embora a administração desta substância possa ser feita de diversas formas, a forma oral tem sido a preferida por atletas e em estudos científicos devido a fácil aplicabilidade.

A utilização indiscriminada de cafeína por parte de atletas, no início da década de 80, com objetivo de melhorar o desempenho atlético, fez com que esta substância fosse incluída na lista de substâncias proibidas do Comitê Olímpico Internacional (COI), que estipulou valor limítrofe de 15µg/mL de cafeína na urina para casos positivos de doping (DELBEKE; DEBACKERE, 1984). Posteriormente, a partir dos Jogos Olímpicos de Los Angeles (1984), o COI alterou esse limite para 12µg/mL de cafeína na urina para casos positivos de doping (GRAHAM, 2001; SINCLAIR; GEIGER, 2000). Em 2004 a Agência Mundial Antidoping (WADA) removeu a cafeína da lista de substâncias proibidas, a qual foi incluída junto com outras substâncias em um programa de monitoramento da WADA. Uma razão para isso é que os efeitos ergogênicos da cafeína podem ser alcançados em baixas doses, as quais podem ser obtidas através do consumo habitual de vários alimentos comuns. Além disso, os valores de cafeína na urina usada para os testes antidoping variam bastante de indivíduo para indivíduo a partir da mesma dose ingerida (TARNOPOLSKY, 2010). A não inclusão da cafeína na lista de substâncias proibidas é uma condição que perdura até os dias atuais (WORLD ANTI DOPING, 2015).

1.3. Anti-inflamatórios não esteroidais (AINES)

A produção de prostaglandinas é geralmente muito baixa em tecidos não-inflamados, mas aumenta imediatamente durante a inflamação aguda através do recrutamento de leucócitos e sua infiltração de células imunológicas. Sua produção depende da atividade das prostaglandina G/H sintases, mais conhecidas com COXs, enzimas bifuncionais que contém atividade ciclooxigenase e peroxidase e consistem em três isoformas: COX-1, COX-2 e COX-3 (CHANDRASEKHARAN et al., 2002; SMITH; DEWITT; GARAVITO, 2000). As COXs causam a oxidação do ácido araquidônico, liberado da membrana celular pela ação da fosfolipase 2 (PLA_2) em resposta a estímulos térmicos, mecânicos e/ou químicos (LEES et al., 1991), tendo como produto final as prostaglandinas. A COX-1, expressa constitutivamente na maioria das células, tem uma concentração relativamente estável, e está relacionada com funções como normalização da função plaquetária, regulação do fluxo sanguíneo renal e citoproteção da mucosa do epitélio gástrico via produção da prostaglandina I2 (DUBOIS et al., 1998; VANE; BOTTING, 1996). A expressão de COX-2 pode ser aumentada em resposta a estímulos inflamatórios, hormônios e fatores de crescimento e é uma importante fonte de fatores inflamatórios em doenças proliferativas e inflamatórias, como o câncer. A COX-2 também age em locais de inflamação na indução da reação inflamatória pela produção de prostaglandinas, tromboxanos e leucotrienos (DUBOIS et al., 1998; SMITH et al., 2004; VANE; BOTTING, 1996). A COX-3 foi a última a ser identificada na busca pela exato mecanismo de ação do paracetamol (BOTTING, 2000), pois este medicamento não responde pela inibição direta de COX-1 e COX-2, e acredita-se que a relativa nova descoberta desta enzima poderá ser de grande ajuda na busca por tratamentos por drogas mais seletivas e eficazes.

Dentre todas, em especial, a prostaglandina E_2 (PGE_2) possui capacidade pró-inflamatória e está relacionada com a dor e, por isso medicamentos que diminuam a formação desta prostaglandina são bastante usados na clínica (MENDIAS; TATSUMI; ALLEN, 2004). Dessa forma, as enzimas COX-1 e COX-2 são importantes alvos dos anti-inflamatórios não esteroidais (AINEs), drogas inibidoras competitivas.

Os anti-inflamatórios são classificados baseando-se na seletividade frente a inibição causada na COX-1 e COX-2 (tradicionais, AINEs não específicos) ou inibição preferencial à COX-2 (inibidores seletivos de COX-2). No entanto, outras interações de AINEs independentes de COX também são possíveis, tais como aumento da expressão dos genes NAG-1 (non-steroidal anti-inflammatory drug-activated gene activator), STAT1 (ativador do sinal de tradução e ativador de transcrição) e HSPs (*heat shock proteins*) (IGUCHI et al., 2009; TEGEDER; PFEILSCHIFTER; GEISSLINGER, 2001).

Os AINEs não são medicamentos relativamente atuais e alguns de seus efeitos colaterais estão bem definidos na literatura, sendo que há ações adversas em diferentes sistemas (WANNMACHER; BREDEMEIER, 2004). Vários estudos sugerem que o uso de inibidores seletivos de COX-2 está associado com sérios riscos cardiovasculares (BRESALIER et al., 2005; SOLOMON et al., 2005). Anti-inflamatórios tradicionais, não-esteroidais como o diclofenaco, ibuprofeno e naproxeno, inibem as enzimas COX de maneira menos seletiva. Se estes agentes possuem efeitos cardiovasculares semelhantes aos seletivos de COX-2 ainda não está bem definido, pois estudos anteriores relacionando AINEs e os riscos cardiovasculares são inconsistentes: alguns observaram aumento de riscos (GRAHAM et al., 2005; HIPPISLEY-COX; COUPLAND, 2005; JOHNSEN et al., 2005; KURTH et al., 2003; RAY et al., 2002); alguns não mostram nenhum efeito (BAK et al., 2003; CURTIS et al., 2003; GARCIA RODRIGUEZ et al., 2004; HIPPISLEY-COX; COUPLAND, 2005; JOHNSEN et al., 2003; SCHLIENGER; JICK; MEIER, 2002); enquanto outros sugerem um efeito cardioprotetor (FISCHER et al., 2004; KIMMEL et al., 2004; KO et al., 2002; RAHME; PILOTE; LELORIER, 2002; SOLOMON et al., 2002; WATSON et al., 2002).

A inibição das enzimas COX-1 e COX-2 induz redução da inflamação (se existente) e da dor em resposta à alguma lesão muscular. Porém ainda há muitos estudos controversos quanto ao aumento da expressão de COX-2 após uma lesão muscular. Algumas pesquisas mostram aumento (BONDESEN et al., 2004; BURD et al., 2010; WEINHEIMER et al., 2007) e outras nenhuma alteração na expressão após o dano muscular (MIKKELSEN et al., 2009; TRAPPE et al., 2011).

Estima-se que 70 milhões de prescrições para o uso de AINEs são anualmente feitas e que 30 bilhões de compras são feitas sem prescrição (ELNACHEF et al., 2008). No âmbito esportivo, os AINEs tem sido relatado frequentemente nas últimas décadas (HUANG; JOHNSON; PIPE, 2006; ROSE et al., 2006; THUYNE; DELBEKE, 2008; WARNER et al., 2002). O excessivo consumo de AINEs por atletas profissionais tornou esta classe de medicamentos uma das mais utilizadas no meio esportivo (CIOCCA, 2005) e entre esportistas profissionais de diferentes modalidades (DA SILVA et al., 2011). A rotina diária de treinamento dos atletas e as competições que estes participam podem causar danos musculares, inflamação e, também, diminuição da performance (MCHUGH et al., 1999). Consequentemente, o uso de AINEs tornou-se uma prática comum para diminuir a inflamação e a dor muscular (LIPPI et al., 2006), fatores parcialmente responsáveis pela perda do rendimento esportivo (ALARANTA; ALARANTA; HELENIUS, 2008; ALARANTA et al., 2006; CIOCCA, 2005).

Os AINEs são administrados antes, durante e após períodos competitivos para prevenção ou tratamento de lesão ou ainda supressão de sintomas menores de incômodo muscular e articular associados ao treinamento intenso. Uma possível razão para esse aumento no consumo de AINEs em atletas deve-se ao fato destas drogas permitirem a continuidade dos treinamentos ou, inclusive, participação dos mesmos em competições sem a necessidade de respeitar uma janela de recuperação quando pequenas lesões são detectadas (CORRIGAN; KAZLAUSKAS, 2003).. Alguns autores afirmam que muitos atletas não deixam de treinar e competir quando se lesionam, consumindo AINEs por longos períodos de tempo (ALARANTA et al., 2006; CORRIGAN; KAZLAUSKAS, 2003); outros apontam a prevenção de dor e a percepção de melhora na performance como possíveis razões para o consumo desses medicamentos por atletas (PAGE et al., 2007; WARNER et al., 2002). Contudo, os motivos pelos quais os atletas utilizam esses medicamentos ainda não estão bem esclarecidos na literatura.

Recentemente, estudos sobre o uso de AINEs em competições de nível internacional têm sido conduzidos, e os dados obtidos sobre o alto consumo desta classe de medicamentos entre esportistas profissionais de diferentes modalidades são alarmantes. Uma pesquisa recente mostrou que durante uma competição esportiva de alto nível, dos 1261 atletas participantes, 62,8 % se declararam usuários de uma ou mais drogas não banidas pela WADA, sendo os AINEs os mais frequentemente usados (mais de 65 %) (DA SILVA et al., 2011). Outros estudos relacionados, ainda indicam que até 36% dos atletas fazem o uso de AINEs (HUANG; JOHNSON; PIPE, 2006; THUYNE; DELBEKE, 2008; TSCHOLL; JUNGE; DVORAK, 2008; TSCHOLL et al., 2009). Nas Copas do Mundo de Futebol da FIFA entre 2002 e 2006, o uso de cerca de 10384 substâncias foram documentadas; sendo constatado que apenas 19,7% dos atletas não consumiam nenhum tipo de medicação. Na média de duas Copas do Mundo (2002 e 2006) os AINEs foram as substâncias mais prescritas (46,5% e 47,7%, respectivamente), onde evidenciou-se que mais de 30% dos atletas usaram AINEs previamente à partida. Dentre os diferentes princípios ativos dos AINEs, o diclofenaco foi o mais relatado (2002= 48,2%; 2006= 55,2) (TSCHOLL; JUNGE; DVORAK, 2008).

No geral, os estudos concluem que, considerando o efeito dos mesmos na diminuição da dor e a negativa associação entre dor e desempenho físico, estas drogas poderiam estar sendo usadas para o aumento do desempenho em cenários de competição esportiva. Importante ressaltar que, considerando a reação inflamatória como parte do processo necessário para a regeneração do tecido lesado, um retardo na cura de lesões associado ao consumo de AINEs têm sido sugerido por alguns autores (PETERSON et al., 2003). De acordo, alguns estudos

apontam para uma possível associação entre o uso de AINEs e a ocorrência de hiponatremia em eventos esportivos de longa duração (DAVIS et al., 2001; WHARAM et al., 2006). Embora seja incontestável o alívio da dor pelos AINEs, sua influência no processo de cura é controverso e seus efeitos no esporte ainda não são totalmente conhecidos (HOUGLUM, 1998; URSO, 2013).

1.3.1. Diclofenaco

Assim como muitos AINEs, o diclofenaco possui ação analgésica, anti-inflamatória e antipirética. O diclofenaco é o AINE mais prescrito mundialmente e desde o início de sua comercialização, vem sendo usado por mais de 1 bilhão de pessoas sendo uma das drogas mais vendidas no mundo. Estima-se que o consumo deste medicamento, humano e veterinário, seja de mais de uma tonelada por ano (GAN, 2010). Desde sua introdução no mercado americano nos anos 90, várias formulações de diclofenaco têm sido disponibilizadas ou estão sob investigação clínica. Comercialmente pode ser encontrado nas formulações oral, intravenosa, supositório, adesivo ou gel.

O diclofenaco é um derivado do ácido fenilacético (ácido 2-[2,6-dicloranilino]fenilacético), e está disponível em formulação oral na forma de sais de sódio, potássio, ou sódio/misoprostol. O diclofenaco sódico (Voltaren*) é um medicamento com revestimento entérico, de liberação lenta (GAN, 2010).

O AINE diclofenaco é amplamente prescrito para o tratamento de doenças reumáticas e também como analgésico em condições de dor muscular aguda. Contudo, quando utilizado de forma indiscriminada, pode induzir toxicidade hepática rara, mas potencialmente severa (RAMM; MALLY, 2013), devido a formação de metabólitos ativos através do metabolismo de fase I mediado pelo citocromo P450: (1) 4'-hidroxidiclofenaco e (2) 5'-hidroxidiclofenaco (KENNY et al., 2004; SCULLY; CLARKE; BARR, 1993; TANG et al., 1999). A partir da ação do citocromo P450C29 o metabolito 5'-hidroxidiclofenaco forma a p-benzoquinona imina, que nesta forma possui a capacidade de reduzir os níveis de glutationa reduzida (GSH), nicotinamida adenina dinucleótido (NADH) e nicotinamida adenina dinucleótido fosfato (NADPH), se caracterizando assim, como a principal responsável frente à formação de dano celular (KENNY et al., 2004; SHEN et al., 1997, 1999).

Todos os AINEs parecem ter propriedades anti-inflamatórias, antipiréticas e analgésicas e embora essas características possam ser largamente explicadas através da inibição da produção de prostaglandinas (RICCIOTTI; FITZGERALD, 2011), diferenças na potência,

eficácia e janela terapêutica dos AINEs podem indicar que os mecanismos de ação podem estar envolvidos nesta classe de drogas. Assim como para outros AINEs, o mecanismo de ação exato do diclofenaco ainda não está totalmente elucidado. No entanto, o mecanismo mais aceito seja a inibição da COX (VAN HECKEN et al., 2000; VANE; BOTTING, 1996). O diclofenaco está entre os mais efetivos inibidores da síntese de PGE2 e é reportado ser de 3 a 1000 vezes mais potente quando comparado a outros AINEs em sua capacidade de inibir a atividade da COX (KU et al., 1985, 1986). Importante ressaltar que há uma diferença entre os AINEs quanto à inibição relativa das duas isoformas das COXs. Warner e cols (1999) observaram que apesar do diclofenaco possuir uma seletividade 4 vezes maior para a COX-2, em níveis terapêuticos (IC80), 70% da COX-1 também é inibida. Em comparação com o ibuprofeno, cuja a porcentagem de inibição entre COX-2/COX-1 é de 80/90%, está relação para o diclofenaco é de 80/70% (MITCHELL; WARNER, 1999). Por ser um inibidor equipotente das duas COX (BURIAN; GEISSLINGER, 2005), o diclofenaco possui uma vantagem em relação a outros AINES devido a relativa baixa toxicidade para o trato gastrointestinal, e quando comparado aos inibidores seletivos de COX-2 mostra menor toxicidade cardiovascular e efeitos mínimos na atividade hepática e renal (GAN, 2010).

Além desse mecanismo de ação, investiga-se outros como: inibição da síntese de leucotrienos; inibição da fosfolipase A2; modulação dos níveis de ácido araquidônico; mecanismos neuropáticos e mediados pelo SNC; inibição do receptor-gama proliferador peroxissomal (PPAR-ɣ); redução de substâncias inflamatórias; e inibição do receptor de tromboxanos, entre outros. No entanto, mais estudos são necessários para esses mecanismos serem comprovados (GAN, 2010).

Contudo, sabe-se que atualmente os índices de intoxicação hepática medicamentosa vêm aumentando pela ampla utilização de medicamentos tais como os AINEs (LEE, 1993; WATKINS; SEEFF, 2006). Particularmente, os AINEs apresentam fácil acesso à população geral, sendo em muitos países adquiridos em supermercados e lojas (classificados como "*over-the-counter*" em inglês – ou seja, sem a necessidade de prescrição médica) o que aumenta drasticamente seu consumo não controlado. Como mencionado anteriormente, o diclofenaco é um dos fármacos mais utilizados por atletas, tendo uma frequência de quase 50% com relação aos demais AINEs utilizados nas Copas do Mundo entre 2002 e 2006 (TSCHOLL; JUNGE; DVORAK, 2008). Assim, são necessários estudos que investiguem os efeitos associados ao uso deste medicamento frente ao exercício físico.

Com base nas considerações pontuadas até aqui, observa-se que os dados da literatura muitas vezes divergem a respeito dos efeitos antioxidantes e inflamatórios do exercício, bem como, são escassos com relação aos impactos destes no tecido hepático (HOENE; WEIGERT, 2010). Estudos mostram respostas específicas do tecido muscular ao exercício agudo e ao treinamento com relação ao balanço redox e inflamatório, mas as relações estabelecidas com o tecido hepático ainda são muito escassas e variáveis (Sacheck et al., 2003). Da mesma forma, as adaptações oxidativas dos tecidos em resposta ao exercício agudo, treinamento físico e ao uso concomitante de substâncias ergogênicas necessitam de maiores esclarecimentos e comprovações científicas.

2. OBJETIVOS

2.1. Objetivo Geral

Investigar os efeitos da cafeína e diclofenaco sobre o status antioxidante, estresse oxidativo e perfil inflamatório em ratos submetidos a diferentes tipos de exercício físico.

2.2. Objetivos específicos do artigo 1

- Analisar os efeitos do treinamento físico e da cafeína sobre marcadores de dano tecidual hepático, através dos níveis plasmáticos das enzimas AST e ALT;
- Verificar o status antioxidante e estresse oxidativo em fígado de ratos treinados e suplementados com cafeína;

2.3. Objetivos específicos do artigo 2

- Analisar os efeitos do treinamento físico e da cafeína sobre a função mitocondrial hepática através dos parâmetros de potencial de membrana ($\Delta\Psi m$), edema mitocondrial, atividade do complexo I e formação de ROS,
- Verificar o status inflamatório de ratos treinados e tratados cafeína, através da atividade das enzimas acetilcolinesterase e mieloperoxidase plasmáticas.

2.4. Objetivos específicos do manuscrito 1

- Verificar os efeitos do tratamento com diclofenaco e/ou do exercício excêntrico sobre a expressão hepática de mRNA e níveis de proteínas envolvidas na cascata inflamatória via TLR4, incluindo TLR4, MyD88 e TRIF,
- Verificar os efeitos do tratamento com diclofenaco e/ou do exercício excêntrico agudo sobre a expressão e níveis da subunidade proteica de p65 do NFκB em fígado de ratos;
- Investigar os efeitos do diclofenaco e/ou do exercício excêntrico sobre a expressão de mRNA e níveis de IL-6, iNOS, TNF-α em fígado de ratos

3. USO DE ANIMAIS

Os projetos para o desenvolvimento destes estudos foram previamente aprovados pelos correspondentes comitês de ética. Os estudos que envolveram cafeína e exercício físico em ratos (Artigo 1 e Artigo 2) têm o projeto aprovado pelo Comitê de Ética e Pesquisa da Universidade Federal de Santa Maria com o número de protocolo 115/2010. O estudo que envolveu diclofenaco e exercício físico em ratos (Manuscrito 1) tem o projeto aprovado pelo Comitê de Proteção Animal da Universidade de León – Junta de Castilla y León,España – com o número do protocolo 00116/001.

4. ARTIGO 1: SUPLEMENTAÇÃO DE CAFEÍNA MODULA MARCADORES DE ESTRESSE OXIDATIVO NO FIGADO DE RATOS TREINADOS

Artigo científico publicado na revista Life Sciences, 2014

Copyright:
Licensee: Romulo P Barcelos
License Date: Jul 2, 2015
License Number: 3660900168919
Publication: Life Sciences
Title: Caffeine supplementation modulates oxidative stress markers in the liver of trained rats
Type Of Use: reuse in a thesis/dissertation

Título original:

CAFFEINE SUPPLEMENTATION MODULATES OXIDATIVE STRESS MARKERS IN THE LIVER OF TRAINED RATS

Autores:

Rômulo Pillon Barcelos, Mauren Assis Souza, Guilherme Pires Amaral, Silvio Terra Stefanello, Guilherme Bresciani, Michele Rechia Fighera, Félix Alexandre Antunes Soares, Nilda Vargas Barbosa.

Life Sciences 96 (2014) 40–45

Doi: 10.1016/j.lfs.2013.12.002

Life Sciences 96 (2014) 40–45

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Caffeine supplementation modulates oxidative stress markers in the liver of trained rats

Rômulo Pillon Barcelos [a], Mauren Assis Souza [a,b], Guilherme Pires Amaral [a], Silvio Terra Stefanello [a], Guilherme Bresciani [a,b], Michele Rechia Fighera [b,c], Félix Alexandre Antunes Soares [a,b,*], Nilda Vargas Barbosa [a]

[a] Departamento de Química, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[b] Laboratório de Bioquímica do Exercício (BioEx), Centro de Educação Física e Desportos, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[c] Departamento de Neuropsiquiatria, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 1 October 2013
Accepted 3 December 2013

Keywords:
Caffeine
Exercise training
Liver
Oxidative stress
Redox status modulation

ABSTRACT

Aims: Caffeine has been widely used in sports competitions due to its ergogenic effects. Most of the studies regarding caffeine and exercise have focused on muscle and plasma adaptations, while the impact on the liver is scarcely described. The aim is to analyze the effects of caffeine and exercise training on oxidative stress markers and injury-related parameters in the liver.
Main methods: Rats were divided into sedentary/saline, sedentary/caffeine, exercise/saline, and exercise/caffeine groups. Exercise groups underwent 4 weeks of swimming training, and caffeine (6 mg/kg, p.o.) was supplemented throughout the training protocol. Injury-related liver parameters were assessed in plasma, while redox status and oxidative stress markers were measured on liver homogenates.
Key findings: Exercise training increased muscle citrate synthase activity in the muscle, while in caffeine decreased its activity in both sedentary and trained rats. Aspartate transaminase levels were increased after training, and caffeine intake suppressed this elevation ($p < 0.05$). Caffeine also diminished alanine transaminase levels in both sedentary and exercised rats ($p < 0.05$). Exercise training induced a significant increase on the activity of the enzymes superoxide dismutase and glutathione peroxidase, as an increase on thiobarbituric acid-reactive substances levels was also reached ($p < 0.05$); caffeine intake blunted these alterations. Caffeine intake also suppressed liver catalase activity in both sedentary and exercise groups ($p < 0.05$).
Significance: Our data suggest that caffeine modified the hepatic responses associated to exercise-induced oxidative stress without affecting the performance, exerting different actions according to the tissue. However, further studies are needed to better understand caffeine's role on liver under exercise training.
© 2013 Elsevier Inc. All rights reserved.

Introduction

Caffeine (1,3,7-trimethylxanthine) is a xanthine alkaloid compound present in many commercial beverages and medicines that acts as a potent stimulant of the central nervous system (CNS) (Tunnicliffe et al., 2008). In addition, caffeine incites skeletal muscle metabolism and therefore it has been widely used in athletic competitions due to its ergogenic effects (Kalmar and Cafarelli, 2004; Tarnopolsky, 2008; Goldstein et al., 2010). Its widespread use is allowed by the World Anti-Doping Agency's despite evidence-based data that documents its physiological and performance-enhancing effects (Tarnopolsky, 2010). In this line, it is well known that caffeine may affect substrate utilization during exercise (Tunnicliffe et al., 2008; Goldstein et al., 2010; Yang et al., 2009; Davis et al., 2003). Caffeine increases fatty acid mobilization during exercise thus decreasing glycogen reliance during performance (Ivy et al., 1979; Erickson et al., 1987; Spriet et al., 1992). These effects have been broadly linked to improvements on aerobic exercise performance due to enhanced twitch strength of both skeletal and cardiac muscles, which result in delay fatigue onset (Goldstein et al., 2010; Tarnopolsky and Cupido, 2000; Simmonds et al., 2010).

On the same line, it is well known that regular exercise training plays a protective role against lifestyle-related diseases across health status and quality of life improvements (Radak et al., 2004, 2005a,b). Accordingly, it has been stated that regular exercise training may increase the resistance to various stressors via hormesis (Radak et al., 2005a). The molecular events involved in this regulation may be linked to redox status homeostasis, an oxidative stress-related adaptive response (Radak et al., 2008; Jackson, 2008; Gomez-Cabrera et al., 2008). In fact, exercise training incites regular adaptations to the continuous presence of small stimuli such as mild amounts of reactive oxygen species (ROS). In this case, the regular stimuli can trigger the expression of antioxidant enzymes and modulates other oxidative stress markers (Radak et al., 2005a; Jackson, 2008; Gomez-Cabrera et al., 2008; Ji, 1993).

* Corresponding author at: Departamento de Química, CCNE, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. Tel.: +55 55 3220 9558.
E-mail address: felix@ufsm.br (F.A.A. Soares).

0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.12.002

Although growing evidence shows the beneficial effects of caffeine intake on skeletal muscle performance during exercise training, the role of caffeine in other tissues is scarcely described. Specifically, the influence of caffeine on the oxidative responses elicited by exercise training has been mostly limited to the skeletal muscle, brain and plasma samples. Therefore, considering the remarkable metabolic role of the liver during exercise, this study aimed to analyze the isolated and/or combined effects of caffeine supplementation and exercise training on oxidative stress and tissue injury-related markers.

Materials and methods

Ethical approval

The experimental assays were conducted in accordance to national and international legislations (Brazilian College of Animal Experimentation (COBEA) and the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals-PHS Policy). The study protocol was also approved by the Ethics Committee for Animal Research of the Universidade Federal de Santa Maria (UFSM, permit number 115/2010) before experimental set beginning.

Animals and reagents

Male Wistar rats (180–250 g) were obtained from our own breeding colony and kept in plastic boxes containing a maximum of five animals per cage. After, cages were placed in controlled environment conditions (12:12 h light–dark cycle, with onset of light phase at 7:00, 25 ± 1 °C, 55% relative humidity) with food (Guabi, Santa Maria, Brazil) and water ad libitum. Assay reagents were purchased from Sigma (St. Louis, MO, USA), and the other chemicals used in this study were of analytical grade and obtained from standard commercial suppliers.

Study design

The animals were randomly divided into four groups ($n = 8$): sedentary-saline (SED-SAL), sedentary-caffeine (SED-CAF), exercise-saline (EXE-SAL) and exercise-caffeine (EXE-CAF). The exercise and sedentary groups received caffeine (6 mg/kg in saline) or its vehicle by intragastric gavage (p.o.) through the experimental period.

Training protocol

For exercise training, animals were weighed and randomly assigned to the aforementioned groups. The tank used in this study was 80 cm in length, 50 cm in width, and 90 cm in depth, and the swimming training was performed in water temperature of 31 ± 1 °C (70 cm depth) between 10 and 12 h am. The training session consisted of 50 min per day and 5 days per week (Song et al., 1998). The EXE groups performed swimming training with a 5% body weight overload attached to the back to improve endurance (Lima et al., 2013; Gobatto et al., 2001). The SED groups were placed in a separate but similar tank with shallow water (5 cm) at the same temperature for 30 min, 5 days a week without the back overload. Caffeine supplementation (6 mg/kg) was administered daily throughout the training protocol (Fredholm et al., 1999).

Tissue sampling, and organs weighting

At the end of the exercise training protocol, the rats were euthanized and, liver, adrenal gland, soleus and gastrocnemius muscles were removed and weighted. A single ratio between organ and total body weights was calculated to express this data. Samples of liver and gastrocnemius muscle were quickly removed, placed on ice, and homogenized within 10 min in 10 volumes of cold Tris 10 mM (pH 7.4). Liver and muscle homogenates were centrifuged at 4000 ×g at 4 °C for 10 min to yield the low-speed supernatant fraction that was used for different biochemical assays in all trials. Blood samples were collected and centrifuged 1500 ×g for 10 min for plasma isolation in order to perform biochemical analysis.

Plasma assays

Biochemical parameters

Plasma levels of creatinine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), triglycerides (TG), total cholesterol (TC), uric acid (UA), high density lipoprotein (HDL) and urea (UR) were estimated by standard commercially biological kits (Labtest, Lagoa Santa, Brazil).

Estimation of DNA damage

The cell death indicated by the presence of double strand DNA in the plasma (dsDNA) was measured using the PicoGreen® fluorescent assay (Ahn et al., 1996). The assay was performed according to protocol supplied by the manufacturer (Quant ItTM, Invitrogen, Brazil). The fluorescence measurements were recorded on a fluorimeter, and fluorescence emission of PicoGreen® alone (blank) and PicoGreen® with DNA were recorded at 520 nm using an excitation wavelength of 480 nm at room temperature (25 °C). The results were expressed as % of control.

Citrate synthase (CS) activity

Citrate synthase activity was determined spectrophotometrically in mixed gastrocnemius muscle and liver according to the method previously described (Srere, 1968). The enzyme activity was measured in homogenates and the amount of the complex resulting from acetyl-CoA and oxaloacetate was determined at 412 nm and 25 °C. The CS activity was expressed as percentage of control.

Liver homogenate assays

Catalase (CAT) activity

The CAT enzyme activity was determined according to the method proposed by Aebi (1984). The kinetic analysis of CAT was started after H_2O_2 addition and the color reaction was measured at 240 nm. Data were corrected by the protein content and expressed as percentage of control.

Superoxide dismutase (SOD) activity

The SOD enzyme activity was determined according to the method proposed by Misra and Fridovich (1972). The kinetic analysis of SOD was started after adrenaline addition, and the color reaction was measured at 480 nm. Data were corrected by the protein content and expressed as percentage of control.

Glutathione peroxidase (GPx) activity

The GPx activity was determined spectrophotometrically at 340 nm by NADPH consumption for 2 min at 30 °C (Flohé and Günzler, 1984). The reaction was initiated by adding the H_2O_2 to a final concentration of 0.4 mM. The GPx activity was determined using the molar extinction coefficient 6220 M^{-1} cm^{-1} and expressed as percentage of control.

Glutathione reductase (GR) activity

For GR activity determination, the measurements were made at 340 nm and initiated with addition of 20 mM GSSG, at 30 °C for 2 min (Carlberg and Mannervik, 1985). The GR activity was determined using the molar extinction coefficient 6220 M^{-1} cm^{-1}, corrected by the protein content, and expressed as percentage of control.

Fluorimetric assay of reduced (GSH) and oxidized glutathione (GSSG)

For measurement of GSH and GSSG levels, the method previously described by Hissin and Hilf (1976) was used (Hissin and Hilf, 1976).

The GSH and GSSG levels were determined from comparisons with a linear GSH or GSSG standard curve, respectively. The results were expressed as GSH/GSSG ratio.

Thiobarbituric acid reactive substance (TBAR) levels determination

Lipid peroxidation was estimated by measuring TBARS and expressed in terms of malondialdehyde (MDA) content according to the method of Ohkawa et al. (1979). The TBAR levels were measured at 532 nm, and the absorbance was compared with the standard curve using MDA and corrected by the protein content.

Protein determination

The protein content was determined as described previously (Lowry et al., 1951) using bovine serum albumin (BSA) as standard.

Statistical analysis

Statistical analysis was performed using two-way analysis of (ANOVA), followed by Student–Newman–Keuls test when appropriate to determine possible interactions. Data are expressed as means ± SEM. Values of $p < 0.05$ were considered significant.

Results

Total body weight and organ-to-body weight ratio

The EXE groups had a reduction in the body weight gain when compared to the SED groups as seen in Fig. 1 ($p < 0.05$). This exercise training effect was not modified by caffeine intake. Similarly, caffeine did not change the body weight of SED groups. No changes in the organ-to-body weight ratio were observed for liver, adrenal gland, soleus, or gastrocnemius muscles among the groups (Table 1).

Plasma biochemical levels

No significant differences in plasma lipid content (TC, TG, and HDL-cholesterol), urea, or uric acid were detected among the groups as presented in Table 2.

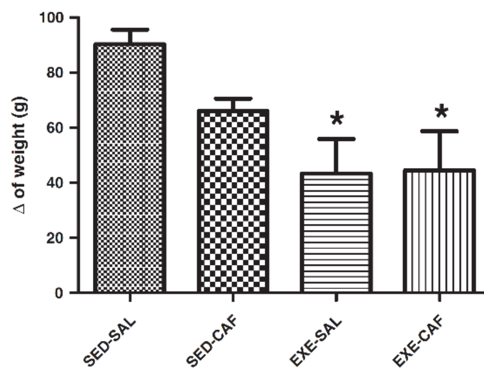


Fig. 1. Effects of exercise training and caffeine supplementation on body weight gain. Data are presented as total body weight gain after 4 weeks of exercise and expressed as means ± SEM (n = 5–8). *Denotes $p < 0.05$ when compared with the control group (SED-SAL). SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

Table 1
Effects of swimming training and caffeine on relative organ/body weight. The data are expressed as means ± SEM (n = 5–8) and presented as the ratio between tissue and total rat weight.

	SED-SAL	SED-CAF	EXE-SAL	EXE-CAF
Liver (10^{-2})	3.50 ± 0.34	3.03 ± 0.14	3.278 ± 0.11	3.275 ± 0.13
Adrenal (10^{-4})	2.58 ± 0.26	2.09 ± 0.10	1.99 ± 0.14	2.19 ± 0.16
Soleus (10^{-4})	8.60 ± 0.91	9.63 ± 1.11	11.13 ± 1.14	11.079 ± 1.06
Gastrocnemius (10^{-3})	4.79 ± 0.36	5.10 ± 0.31	5.48 ± 0.25	5.34 ± 0.27

SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

Muscle and liver citrate synthase (CS) activity

Statistical analysis showed that CS activity was higher among EXE groups in the gastrocnemius muscle (Fig. 2a). On the opposite, liver CS activity decreased with caffeine supplementation on both SED and EXE groups (Fig. 2b, $p < 0.05$).

Injury-related markers

Table 3 depicts the results of tissue injury-related markers in plasma. No differences were found for DNA PicoGreen® among the groups. Conversely, exercise training induced an increase in plasma AST and CK levels when compared to SED groups ($p < 0.05$). Caffeine intake restored the AST levels modified by exercise and diminished ALT levels in both SED/CAF and EXE/CAF rats. Caffeine intake did not alter plasma CK levels in EXE or SED rats.

Hepatic markers

Antioxidant enzyme activities

The liver antioxidant enzyme activities are summarized in Fig. 3. Caffeine intake reduced CAT activity in both SED/CAF and EXE/CAF groups (Fig. 3a, $p < 0.05$). Exercise training caused an increase in SOD and GPx activities when compared with SED/SAL rats ($p < 0.05$). These effects induced by exercise on antioxidant enzymes were blunted by caffeine intake (Fig. 3b and c, $p < 0.05$). No differences among the groups were observed regarding GR activity (data not shown).

Oxidative stress parameters

Fig. 4 presents the results of the oxidative stress markers in liver homogenates. No significant differences in the GSH/GSSG ratio were found among the groups (Fig. 4a). The levels of TBARS were significantly augmented in EXE/SAL group compared to SED/SAL rats (Fig. 4b, $p < 0.05$). This effect was suppressed by caffeine intake in EXE/CAF rats.

Discussion

In the present study, swimming training increased muscle CS activity, plasma CK and AST levels and decreased the weight gain of rats. Exercise training also induced changes on the antioxidant status and oxidative markers in the liver, specifically represented by lipid peroxidation and SOD and GPx activities increased. Most effects linked to

Table 2
Effects of swimming training and caffeine treatment on biochemical serum parameters. The data are expressed as means ± SEM (n = 5–8).

	SED-SAL	SED-CAF	EXE-SAL	EXE-CAF
TC (UI)	1.55 ± 0.07	1.41 ± 0.11	1.51 ± 0.09	1.55 ± 0.07
TG (UI)	0.61 ± 0.06	0.64 ± 0.08	0.61 ± 0.03	0.69 ± 0.09
HDL (UI)	2.96 ± 0.48	2.95 ± 0.25	2.85 ± 0.15	2.76 ± 0.24
UR (nmol/L)	8.11 ± 0.90	8.34 ± 1.14	7.64 ± 0.84	8.09 ± 0.48
UA (μmol/L)	79.90 ± 17.90	53.98 ± 5.00	55.22 ± 5.73	65.70 ± 6.25

Total cholesterol (TC); triglycerides (TG); high-density lipoprotein (HDL); urea (UR); uric acid (UA). SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

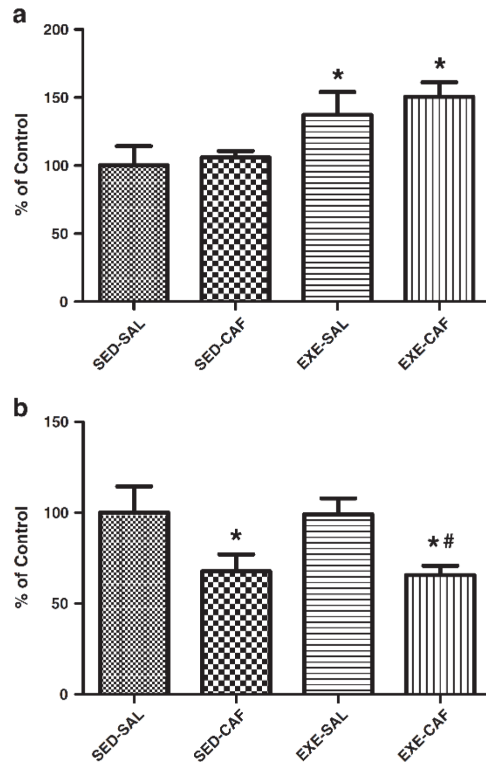


Fig. 2. Effects of exercise training and caffeine supplementation on muscle (a) and liver (b) citrate synthase activity. The data are presented as percentage of control and expressed as means ± SEM (n = 5–8). *Denotes $p < 0.05$ when compared with the control group (SED-SAL). #Denotes $p < 0.05$ when compared with the EXE-SAL group. SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

exercise training were modified by caffeine intake. Caffeine decreased CS activity in both SED/CAF and EXE/CAF groups in the liver.

Exercise trained rats exhibited a plateau in body weight gain during the 4-week training period. This finding corroborates previous data indicating that swimming training stabilizes the body weights of rats (Lima et al., 2013; Ravi Kiran et al., 2006; Clavel et al., 2002). This plateau in body weight may be related to the intensity of the swimming protocol applied in this study considering it has been long stated that intense training influences body weight in rats (Lima et al., 2013). Despite the well-described caffeine effects on the metabolism, the CAF groups did not show differences compared to SAL groups.

Table 3
Effects of swimming training and caffeine treatment on tissue injury-related marker levels. The data are expressed as means ± SEM (n = 5–8) comparing to control group (%).

	SED-SAL	SED-CAF	EXE-SAL	EXE-CAF
AST	100.00 ± 5.83	91.49 ± 7.84	132.66 ± 10.76*	104.19 ± 5.46#
ALT	100.00 ± 10.62	63.29 ± 3.55*	84.51 ± 9.13	66.22 ± 6.20*#
DNA	100.00 ± 4.54	102.60 ± 4.26	102.34 ± 3.91	95.02 ± 3.45
CK	100.0 ± 10.84	140.6 ± 23.86	225.41 ± 46.11*	233.3 ± 40.49*

Aspartate transaminase (AST); and alanine transaminase (ALT); creatine kinase (CK). SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

* Denotes $p < 0.05$ when compared with the control group (SED-SAL).

\# Denotes $p < 0.05$ when compared with the EXE-SAL group.

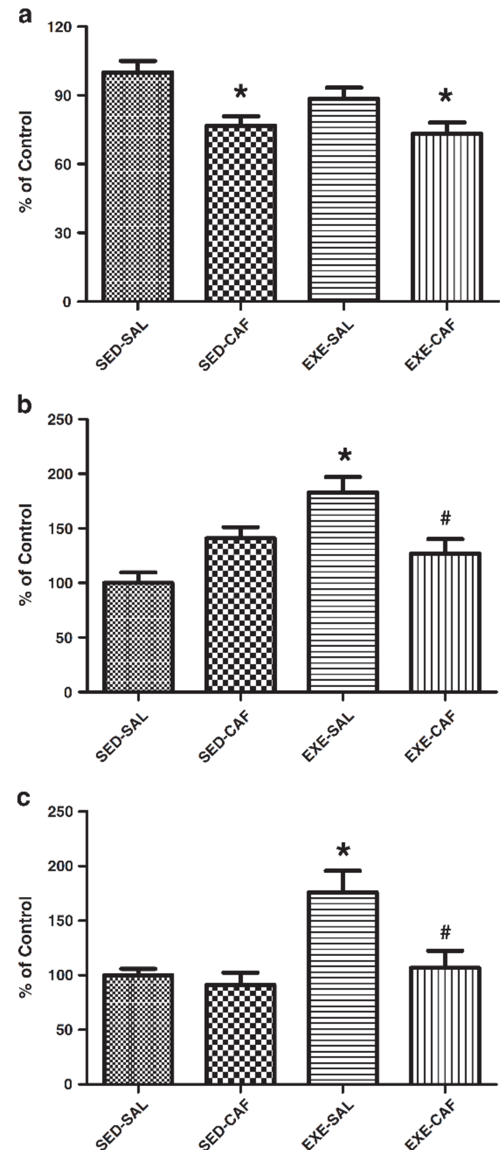


Fig. 3. Effects of exercise training and caffeine supplementation on liver catalase (a), superoxide dismutase (b), and glutathione peroxidase (c) activities. The data are presented as means ± SEM (n = 5–8) and expressed as percentage of control. *Denotes $p < 0.05$ when compared with the control group (SED-SAL). #Denotes $p < 0.05$ when compared with EXE-SAL group. SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

The EXE groups exhibited higher CK levels, potentially indicating exercise-related damage caused by intense swimming training. In this regard, there is evidence that high CK levels lead to oxidative damage and increased lipid peroxidation, which was also seen in this study through increased TBAR levels on the EXE/SAL group (Itoh et al., 2000; Jiménez et al., 2001; Márquez et al., 2001; Zajac et al., 2001). On the same line, muscle CS activity was higher in the trained groups, supporting previous reports on favorable adaptations of the aerobic

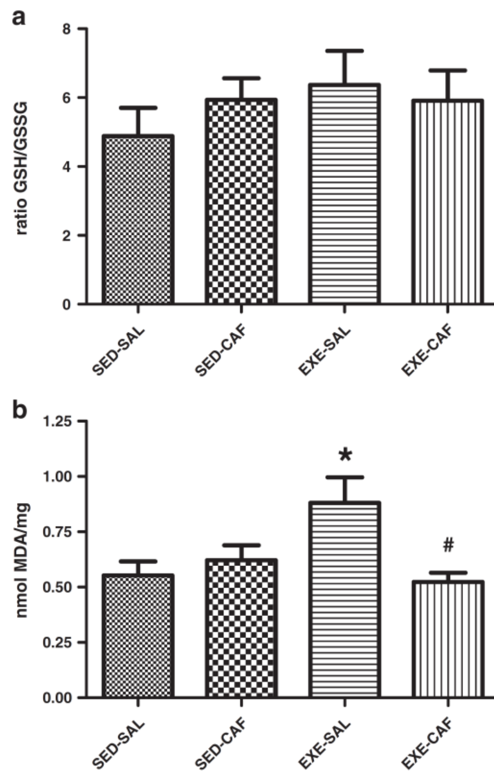


Fig. 4. Effects of exercise and caffeine on liver GSH/GSSG ratio (a) and TBAR levels (b). The data are represented as means ± SEM (n = 5–8). *Denotes $p < 0.05$ when compared with the control group (SED-SAL). #Denotes $p < 0.05$ when compared with EXE-SAL group. SED-SAL: sedentary-saline; SED-CAF: sedentary-caffeine; EXE-SAL: exercise-saline; EXE-CAF: exercise-caffeine.

metabolism to regular training (Jackson, 2008; Gomez-Cabrera et al., 2008; Ji, 1993; Ristow and Schmeisser, 2011; Ji et al., 2006). However, our results showed that the liver CS activity was decreased in CAF groups, which may indicate a differential caffeine modulation according to the analyzed tissue. The decreased liver CS activity was accompanied by a similar tissue injury-related marker modulation. AST and ALT are useful plasmatic parameters of hepatic injury that are associated with liver toxicity (Qin et al., 2007; Ozer et al., 2008; de David et al., 2011). In this study, increases in AST levels among EXE/SAL rats were observed. This is in agreement with previous studies that suggest exhaustive exercise may increase AST and ALT activities (Bowers et al., 1978). Interestingly, caffeine supplementation protected the liver from increased AST levels induced by swimming training, and reduced ALT levels in SED/SAL animals. In this sense, ALT levels showed a similar trend as CS activity in the liver, indicating a decrease on liver metabolism through caffeine supplementation. These are remarkable findings and corroborate previous studies showing that caffeine may prevent increases in plasma AST and ALT and thus decrease the risk of chronic liver damage (Honjo et al., 2001; Ruhl and Everhart, 2005; Tanaka et al., 1998; Cadden et al., 2007). These data suggest that exercise-induced liver adaptations may be modified by caffeine supplementation on a tissue-specific trend.

To the best of our knowledge, this is the first study that investigates the isolated and combined effects of caffeine on the liver redox status homeostasis of sedentary and trained rats. Regular exercise imposes a mild beneficial ROS production, which increases organic resistance to various diseases (Radak et al., 2004, 2005a,b). This recent hypothesis has been extended to the ROS-generating effects of exercise (Radak et al., 2005a; Ji et al., 2006). Regular exercise appears to exert a dual effect: (1) the generation of oxidative stress and, consequently, (2) increased antioxidant enzyme activities that minimize the deleterious effects of these oxidants (Liu et al., 2000; Ji, 1996). Unlike acute and exhaustive exercises which allow insufficient recovery for biochemical adaptations, these antioxidant modulations have been observed during regular exercise training such as the swimming training herein described (Fukai et al., 2000; Rush et al., 2003). In our experimental protocol, swimming training induced compensatory responses regarding oxidative stress. Specifically, SOD and GPx activities were enhanced probably due to increased TBAR content in the EXE/SAL group. Similarly, the increase on GPx activity following vigorous exercise has been suggested as an adaptation to efficiently eliminate exercise-related ROS production and to minimize ROS-mediated damage (Oh-ishi et al., 1997). Therefore, it seems that TBAR increases were compensated by equal increases on SOD and GPx activities in order to maintain redox status. Nevertheless, EXE/CAF presented similar redox status when comparing to SED groups, indicating the antioxidant role of caffeine. Interestingly, this antioxidant activity of caffeine in the liver did not influence the muscle aerobic capacity herein assayed across the CS activity.

Accumulating evidence has suggested a potential antioxidant role for caffeine (Aoyama et al., 2011; Rossowska and Nakamoto, 1994; Zeidán-Chuliá et al., 2013). Chemical studies have proposed a ROS scavenging role for caffeine, particularly the hydroxyl radical (OH·) (Shi et al., 1991; Devasagayam et al., 1996). The beneficial effects of caffeine are usually attributed to its major metabolites 1-methylxanthine and methyluric acid, which are highly effective antioxidants (Lee, 2000). Herein, we verify that caffeine intake blunted exercise-induced increases in TBARS, SOD, and GPx in the liver. These effects were accompanied by decreases on CS activity in the liver of CAF groups, which indicates a decreased aerobic metabolism. In fact, epidemiologic studies have demonstrated that caffeine intake is associated with reduced levels of oxidative stress biomarkers (Ofluoglu et al., 2009); however, a growing number of evidence has indicated the deleterious effects of the antioxidant treatment to performance. In exercise training it has been described that an antioxidant supplementation may prevent the expected adaptations associated with regular training in the muscle (Jackson, 2008; Gomez-Cabrera et al., 2008). In this study, we observed a similar association with caffeine supplementation during regular exercise training which blunted antioxidant adaptations in the liver. Although caffeine have exhibited antioxidant properties in liver redox status, the aerobic training performance markers (muscle CS activity) did not changed by caffeine administration in our experimental protocol. Moreover, caffeine supplementation significantly reduced the liver CS activity, showing that in this specific tissue caffeine may influence cell bioenergetics and reduce its metabolism. Interestingly, this decrease in liver metabolism did not affect exercise performance, considering both EXE groups presented similar CS activity in the muscle. Moreover, EXE/CAF group showed the same aerobic capacity as EXE/SAL in spite of redox status and injury-related markers decreases, indicating that the liver was protected by caffeine supplementation without performance impairment.

Conclusion

In addition to previous findings in skeletal muscle, brain and plasma, our results suggest that exercise training induced liver adaptations associated to oxidative stress. Caffeine, which has antioxidant properties and is widely used in athletic competitions, blunted these exercise-related responses and decreased the liver CS activity. Thus, this study points out the differential antioxidant role of caffeine in the liver without performance deterioration. However, further investigations to undercover the role of caffeine under exercise training on the liver are still needed.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The work was supported by the PRONEM # 11/2029-1 research grant of FINEP. F.A.A.S. and N.B.V.B. received a fellowship from CNPq. R.P.B., S.T.S., G.P.A. and G.B. received a fellowship from CAPES.

References

Aebi H. Catalase in vitro. Methods Enzymol 1984:105121–6.

Ahn SJ, Costa J, Emanuel JR. PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. Nucleic Acids Res 1996;24(13):2623–5.

Aoyama K, Matsumura N, Watabe M, Wang F, Kikuchi-Utsumi K, Nakaki T. Caffeine and uric acid mediate glutathione synthesis for neuroprotection. Neuroscience 2011:181206–15.

Bowers WD, Hubbard RW, Leav I, Daum R, Conlon M, Hamlet MP, et al. Alterations of rat liver subsequent to heat overload. Arch Pathol Lab Med 1978;102(3):154–7.

Cadden IS, Partovi N, Yoshida EM. Review article: possible beneficial effects of coffee on liver disease and function. Aliment Pharmacol Ther 2007;26(1):1–8.

Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985:113484–90.

Clavel S, Farout L, Briand M, Briand Y, Jouanel P. Effect of endurance training and/or fish oil supplemented diet on cytoplasmic fatty acid binding protein in rat skeletal muscles and heart. Eur J Appl Physiol 2002;87(3):193–201.

Davis JM, Zhao Z, Stock HS, Mehl KA, Buggy J, Hand GA. Central nervous system effects of caffeine and adenosine on fatigue. Am J Physiol Regul Integr Comp Physiol 2003;284(2):R399–404.

de David C, Rodrigues G, Bona S, Meurer L, González-Gallego J, Tuñón MJ, et al. Role of quercetin in preventing thioacetamide-induced liver injury in rats. Toxicol Pathol 2011;39(6):949–57.

Devasagayam TP, Kamat JP, Mohan H, Kesavan PC. Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. Biochim Biophys Acta 1996;1282(1):63–70.

Erickson MA, Schwarzkopf RJ, McKenzie RD. Effects of caffeine, fructose, and glucose ingestion on muscle glycogen utilization during exercise. Med Sci Sports Exerc 1987;19(6):579–83.

Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984:105114–21.

Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999;51(1):83–133.

Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000;105(11):1631–9.

Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol A Mol Integr Physiol 2001;130(1):21–7.

Goldstein ER, Ziegenfuss T, Kalman D, Kreider R, Campbell B, Wilborn C, et al. International society of sports nutrition position stand: caffeine and performance. J Int Soc Sports Nutr 2010;7(1):5.

Gomez-Cabrera MC, Domenech E, Viña J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 2008;44(2):126–31.

Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 1976;74(1):214–26.

Honjo S, Kono S, Coleman MP, Shinchi K, Sakurai Y, Todoroki I, et al. Coffee consumption and serum aminotransferases in middle-aged Japanese men. J Clin Epidemiol 2001;54(8):823–9.

Itoh H, Ohkuwa T, Yamazaki Y, Shimoda T, Wakayama A, Tamura S, et al. Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. Int J Sports Med 2000;21(5):369–74.

Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. Med Sci Sports 1979;11(1):6–11.

Jackson MJ. Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? Free Radic Biol Med 2008;44(2):132–41.

Ji LL. Antioxidant enzyme response to exercise and aging. Med Sci Sports Exerc 1993;25(2):225–31.

Ji LL. Exercise, oxidative stress, and antioxidants. Am J Sports Med 1996;24(6 Suppl.):S20–4.

Ji LL, Gomez-Cabrera MC, Vina J. Exercise and hormesis: activation of cellular antioxidant signaling pathway. Ann N Y Acad Sci 2006:1067425–35.

Jiménez L, Lefèvre G, Richard R, Couderc R, Saint George M, Duvallet A, et al. Oxidative stress in hemodialyzed patients during exhausting exercise. J Sports Med Phys Fitness 2001;41(4):513–20.

Kalmar JM, Cafarelli E. Caffeine: a valuable tool to study central fatigue in humans? Exerc Sport Sci Rev 2004;32(4):143–7.

Lee C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. Clin Chim Acta 2000;295(1–2):141–54.

Lima FD, Stamm DN, Della-Pace ID, Dobrachinski F, de Carvalho NR, Royes LF, et al. Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. PLoS One 2013;8(2):e55668.

Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 2000;89(1):21–8.

Lowry O, Roserbrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265–75.

Márquez R, Santángelo G, Sastre J, Goldschmidt P, Luyckx J, Pallardó FV, et al. Cyanoside chloride and chromocarbe diethylamine are more effective than vitamin C against exercise-induced oxidative stress. Pharmacol Toxicol 2001;89(5):255–8.

Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247(10):3170–5.

Ofluoglu E, Pasaoglu H, Pasaoglu A. The effects of caffeine on L-arginine metabolism in the brain of rats. Neurochem Res 2009;34(3):395–9.

Oh-ishi S, Kizaki T, Ookawara T, Sakurai T, Izawa T, Nagata N, et al. Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. Am J Respir Crit Care Med 1997;156(5):1579–85.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95(2):351–8.

Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology 2008;245(3):194–205.

Qin LQ, Wang Y, Xu JY, Kaneko T, Sato A, Wang PY. One-day dietary restriction changes hepatic metabolism and potentiates the hepatotoxicity of carbon tetrachloride and chloroform in rats. Tohoku J Exp Med 2007;212(4):379–87.

Radák Z, Chung HY, Naito H, Takahashi R, Jung KJ, Kim HJ, et al. Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. FASEB J 2004;18(6):749–50.

Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. Biogerontology 2005a;6(1):71–5.

Radak Z, Goto S, Nakamoto H, Udud K, Papai Z, Horvath I. Lung cancer in smoking patients inversely alters the activity of hOGG1 and hNTH1. Cancer Lett 2005b;219(2):191–5.

Radak Z, Chung HY, Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med 2008;44(2):153–9.

Ravi Kiran T, Subramanyam MV, Prathima S, Asha Devi S. Blood lipid profile and myocardial superoxide dismutase in swim-trained young and middle-aged rats: comparison between left and right ventricular adaptations to oxidative stress. J Comp Physiol B 2006;176(8):749–62.

Ristow M, Schmeisser S. Extending life span by increasing oxidative stress. Free Radic Biol Med 2011;51(2):327–36.

Rossowska MJ, Nakamoto T. Effects of chronic caffeine feeding on the activities of oxygen free radical defense enzymes in the growing rat heart and liver. Experientia 1994;50(5):465–8.

Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. Gastroenterology 2005;128(1):24–32.

Rush JW, Turk JR, Laughlin MH. Exercise training regulates SOD-1 and oxidative stress in porcine aortic endothelium. Am J Physiol Heart Circ Physiol 2003;284(4):H1378–87.

Shi X, Dalal NS, Jain AC. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. Food Chem Toxicol 1991;29(1):1–6.

Simmonds MJ, Minahan CL, Sabapathy S. Caffeine improves supramaximal cycling but not the rate of anaerobic energy release. Eur J Appl Physiol 2010;109(2):287–95.

Song YJ, Sawamura M, Ikeda K, Igawa S, Nara Y, Yamori Y. Training in swimming reduces blood pressure and increases muscle glucose transport activity as well as GLUT4 contents in stroke-prone spontaneously hypertensive rats. Appl Human Sci 1998;17(6):275–80.

Spriet LL, MacLean DA, Dyck DJ, Hultman E, Cederblad G, Graham TE. Caffeine ingestion and muscle metabolism during prolonged exercise in humans. Am J Physiol 1992;262(6 Pt 1):E891–8.

Srere PA. Studies on purified citrate-enzymes: metabolic interpretations. Biochem Soc Symp 1968:2711–21.

Tanaka K, Tokunaga S, Kono S, Tokudome S, Akamatsu T, Moriyama T, et al. Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. Int J Epidemiol 1998;27(3):438–43.

Tarnopolsky MA. Effect of caffeine on the neuromuscular system—potential as an ergogenic aid. Appl Physiol Nutr Metab 2008;33(6):1284–9.

Tarnopolsky MA. Caffeine and creatine use in sport. Ann Nutr Metab 2010;57:21–8. [Suppl.].

Tarnopolsky M, Cupido C. Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers. J Appl Physiol 2000;89(5):1719–24.

Tunnicliffe JM, Erdman KA, Reimer RA, Lun V, Shearer J. Consumption of dietary caffeine and coffee in physically active populations: physiological interactions. Appl Physiol Nutr Metab 2008;33(6):1301–10.

Yang JN, Chen JF, Fredholm BB. Physiological roles of A1 and A2A adenosine receptors in regulating heart rate, body temperature, and locomotion as revealed using knockout mice and caffeine. Am J Physiol Heart Circ Physiol 2009;296(4):H1141–9.

Zajac A, Waśkiewicz Z, Pilis W. Anaerobic power, creatine kinase activity, lactate concentration, and acid–base equilibrium changes following bouts of exhaustive strength exercises. J Strength Cond Res 2001;15(3):357–61.

Zeidán-Chuliá FF, Gelain DP, Kolling EA, Rybarczyk-Filho JL, Ambrosi P, Resende Terra S, et al. Major components of energy drinks (caffeine, taurine, and guarana) exert cytotoxic effects on human neuronal SH-SY5Y cells by decreasing reactive oxygen species production. Oxid Med Cell Longev 2013;2013:1–22. [2013791795].

5. ARTIGO 2: CONSUMO DE CAFEINA PODE MODULAR OS MARCADORES DE INFLAMAÇÃO EM RATOS TREINADOS

Artigo científico publicado na revista Nutrients, 2014

Título original:

CAFFEINE INTAKE MAY MODULATE INFLAMMATION MARKERS IN TRAINED RATS

Autores:

Rômulo Pillon Barcelos, Mauren Assis Souza, Guilherme Pires Amaral, Silvio Terra Stefanello, Guilherme Bresciani, Michele Rechia Fighera, Félix Alexandre Antunes Soares, Nilda Vargas Barbosa.

Nutrients 2014, 6, 1678-1690

Doi:10.3390/nu6041678

Nutrients **2014**, *6*, 1678-1690; doi:10.3390/nu6041678

OPEN ACCESS

nutrients

ISSN 2072-6643

www.mdpi.com/journal/nutrients

Article

Caffeine Intake May Modulate Inflammation Markers in Trained Rats

Rômulo Pillon Barcelos [1], Mauren Assis Souza [1,2], Guilherme Pires Amaral [1], Silvio Terra Stefanello [1], Guilherme Bresciani [1,2,3], Michele Rechia Fighera [2,4], Félix Alexandre Antunes Soares [1,2,]* and Nilda de Vargas Barbosa [1]

[1] Departamento de Química, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), 97105-900 Santa Maria, Brazil;
E-Mails: romulo1604@hotmail.com (R.P.B.); maurensouza@gmail.com (M.A.S.); guipiresa@yahoo.com.br (G.P.A.); silviotstefanello@hotmail.com (S.T.S.); guilhermebresciani@gmail.com (G.B.); nvbarbosa@yahoo.com.br (N.V.B.)

[2] Laboratório de Bioquímica do Exercício (BioEx), Centro de Educação Física e Desportos, Universidade Federal de Santa Maria (UFSM), 97105-900 Santa Maria, Brazil;
E-Mail: mrfighera@yahoo.com.br (M.R.F.)

[3] Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, 4810101, Temuco, Chile

[4] Departamento de Neuropsiquiatria, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), 97105-900 Santa Maria, Brazil

* Author to whom correspondence should be addressed; E-Mail: felix@ufsm.br;
Tel.: +55-55-3220-9558.

Received: 28 February 2014; in revised form: 19 March 2014 / Accepted: 25 March 2014 / Published: 21 April 2014

Abstract: Caffeine is presented in many commercial products and has been proven to induce ergogenic effects in exercise, mainly related to redox status homeostasis, inflammation and oxidative stress-related adaptation mechanisms. However, most studies have mainly focused on muscle adaptations, and the role of caffeine in different tissues during exercise training has not been fully described. The aim of this study was therefore, to analyze the effects of chronic caffeine intake and exercise training on liver mitochondria functioning and plasma inflammation markers. Rats were divided into control, control/caffeine, exercise, and exercise/caffeine groups. Exercise groups underwent four weeks of swimming training and caffeine groups were supplemented with 6 mg/kg/day. Liver mitochondrial swelling and complex I activity, and plasma myeloperoxidase (MPO)

and acetylcholinesterase (AChE) activities were measured. An anti-inflammatory effect of exercise was evidenced by reduced plasma MPO activity. Additionally, caffeine intake alone and combined with exercise decreased the plasma AChE and MPO activities. The *per se* anti-inflammatory effect of caffeine intake should be highlighted considering its widespread use as an ergogenic aid. Therefore, caffeine seems to interfere on exercise-induced adaptations and could also be used in different exercise-related health treatments.

Keywords: caffeine; exercise training; mitochondria; inflammation; myeloperoxidase

---

1. Introduction

Aerobic physical training demands great amounts of energy turnover, which is mainly promoted by increased oxygen consumption. In this sense, it is well known that exercise induces several metabolic changes, which can disrupt the mitochondrial functioning in different ways [1]. Among them the oxygen uptake rate during exercise training is considered an important factor to the mitochondrial excessive reactive oxygen species production (ROS) [2]. In this context, mitochondrial dysfunction seems to be closely related to oxidative damage caused by exercise in different tissues [3].

Considering the complexity of exercise-induced cell damage, more comprehensive strategies to understand the associated mechanisms are of interest. In this line, mitochondria are the major site of cellular ROS production while at the same time are also ROS targets [4,5], indicating that mitochondrial dysfunction play a key role in exercise performance [6]. Of note, we have recently described a positive antioxidant modulation of liver mitochondria to exercise training [6]. As such, mitochondria could also bring to light relevant information on exercise mediated-cell antioxidant adaptation.

In animal models, chronic exercise has been attributed a key role in tissue homeostasis, associated with both increased antioxidant defenses and aerobic metabolism [7] and decreased liver inflammation [8], as well as the stimulation of tissue turnover [9,10]. Additionally, epidemiological data and human intervention studies have confirmed the potential benefits of low-to-moderate intensity chronic exercise on muscle health [11,12]. However, studies considering exercise-related adaptations on the liver are still scarce [13].

Caffeine is a xanthine alkaloid compound presented in many commercial beverages and medicines, and its concomitant use with regular exercise may influence the physiological response to effort [14]. Ergogenic effects of caffeine are mainly on central and peripheral mechanisms [15–17], but there is a lack of information concerning its chronic effects. A few studies have suggested that chronic caffeine intake decreases inflammatory injury and chronic inflammation in the liver and brain [8,18,19]. These studies have attributed this protective effect to the antioxidant effects of chronic caffeine intake and decreased activation of resident macrophages (Kupfer cells) and microglia. Moreover, chronic caffeine intake decreases the expression of inflammatory cytokines in blood monocytes and resident macrophages, indicating it may chronically decrease local inflammation [19,20]. Besides, in a mouse model of liver injury, chronic caffeine intake decreased the expression of the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β [19].

However, it is still unknown whether caffeine acts as an energetic buffer and/or trigger of peripheral mechanisms of antioxidant and inflammation modulation. Although there is evidence suggesting beneficial effects of chronic caffeine supplementation on oxidative stress markers, the mechanisms by which these adaptations occur are still to be clarified. In addition, the interaction between exercise and caffeine in the liver is poorly described in the literature, despite the remarkable role of this organ on energy turnover during exercise. Therefore, the aim of this study was to investigate the effect of chronic caffeine intake in liver mitochondria and plasma markers of oxidative metabolism and inflammation in trained rats.

2. Materials and Methods

2.1. Animals and Reagents

Male Wistar rats (180–250 g) were obtained from our own breeding colony and kept in plastic boxes containing a maximum of five animals per cage under controlled environment conditions (12:12 h light-dark cycle, with onset of light phase at 7:00, 25 ± 1 °C, 55% relative humidity) with food (Guabi, Santa Maria, Brazil) and water *ad libitum*. All experiments were conducted in accordance with national and international legislation (Brazilian College of Animal Experimentation (COBEA) and the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals-PHS Policy) and with the approval of the Ethics Committee for Animal Research of the Universidade Federal de Santa Maria (UFSM). Assay reagents were purchased from Sigma (St. Louis, MO, USA). The other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. Training Protocol

For exercise training, animals were weighed (270–340 g) and randomly assigned to the following groups: control, control-caffeine, exercise, and exercise-caffeine. The training consisted of four weeks of swimming, 50 min per day and five sessions per week. The tank used in this study was 80 cm in length, 50 cm in width, and 90 cm in depth, and the swimming training was performed in water temperature of 31 ± 1 °C (70 cm depth) between 10:00 and 12:00 a.m. The exercise groups performed the swimming training with a 5% body weight overload attached to the back to improve endurance [21]. The control groups were placed in a separate but similar tank with shallow water (5 cm) at the same temperature for 30 min, five days a week without the back overload. Caffeine administration was performed daily by intragastric gavage at a dose of 6 mg/kg (in saline) throughout the training protocol [22]. Control groups received saline. Animals were sacrificed 24 h after the last training bout to avoid possible exercise bias.

2.3. Liver Mitochondrial Parameters

2.3.1. Mitochondria Isolation

The liver mitochondria were isolated at 4 °C by differential centrifugation [23] with some modifications. The sample of the liver was rapidly removed and immersed in ice-cold "isolation buffer I" at 4 °C (100 mM sucrose, 10 mM EDTA, 100 mM Tris-HCl, 46 mM KCl, at pH 7.4). The tissue was

then homogenized and the resulting suspension was centrifuged for 5 min at 2000× *g* in a Hitachi CR21E centrifuge (Koki, Tokyo, Japan). After centrifugation, the supernatant was recentrifuged for 20 min at 12,000× *g*. The pellet was gently resuspended in "isolation buffer II" (100 mM sucrose, 10 mM EDTA, 100 mM Tris-HCl, 46 mM KCl, and 0.5% fatty-acid free bovine serum albumin (BSA) free of fatty acids, at pH 7.4) and recentrifuged at 12,000× *g* for 10 min. The supernatant was decanted, and the final pellet was gently washed and resuspended in "isolation buffer III" (270 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl at pH 7.4).

2.3.2. Measurement of Mitochondrial Transmembrane Electrical Potential ($\Delta\Psi m$)

The mitochondrial $\Delta\Psi m$ determination was estimated by fluorescence changes in safranine-O assayed according to Akerman and Wikstron (1976) [24]. The fluorescence analysis was performed at 495 nm for excitation and 586 nm for emission, with slit widths of 5 nm. The $\Delta\Psi m$ was presented as arbitrary fluorescence units per second (AFU/s).

2.3.3. Estimation of Mitochondrial ROS Production

The mitochondrial generation of ROS was determined spectrofluorimetrically using the membrane permeable fluorescent dye H2-DCFDA [25]. The fluorescence analysis was performed at 488 nm for excitation and 525 nm for emission, with slit widths of 5 nm.

2.3.4. Mitochondrial Swelling

Measurement of mitochondrial swelling was performed using a RF-5301 Shimadzu espectrofluorometer at 600 nm and slit 1.5 nm for excitation and emission. The mitochondria (0.1 mg protein/mL) were incubated in the presence of 100 μM Ca^{2+} [26]. Data for mitochondrial swelling was expressed as arbitrary absorbance units per second (AAU/s).

2.3.5. Mitochondrial Complex I Assay

The activity of complex I (NADH dehydrogenase) was measured by following the oxidation of NADH [27,28]. Absorbance at 600 nm was monitored for 2 min to follow the rate of oxidation of NADH, and the activity was determined using an extinction coefficient of 6.22 $mM^{-1}\cdot cm^{-1}$. After thawing, the mitochondria were found to be completely permeable to NADH. Results are expressed as % of control.

2.4. Myeloperoxidase (MPO) Activity

The plasma activity of the pro-inflammatory MPO enzyme was measured spectrophotometrically by a modified peroxidase-coupled assay system involving phenol, 4-aminoantipyrine (AAP) and H_2O_2 as previously described [29]. The results were expressed in micromol of quinoneimine produced at 30 min.

2.5. Acetylcholinesterase (AChE) Activity

The AChE activity was estimated in plasma by the Ellman method [30], using acetylthiocholine iodide (ATC) as substrate and etopropazine as butyrylcholinesterase (BChE) inhibitor [31]. Data were expressed in μmol of hydrolyzed ATC/min/mL.

2.6. Protein Determination

The protein content was determined as described previously [32] using bovine serum albumin (BSA) as standard.

2.7. Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was performed using two-way analysis of (ANOVA), followed by Student-Newman-Keuls test when appropriate or two-way analysis of variance to determine possible interactions. Values of $p < 0.05$ were considered significant.

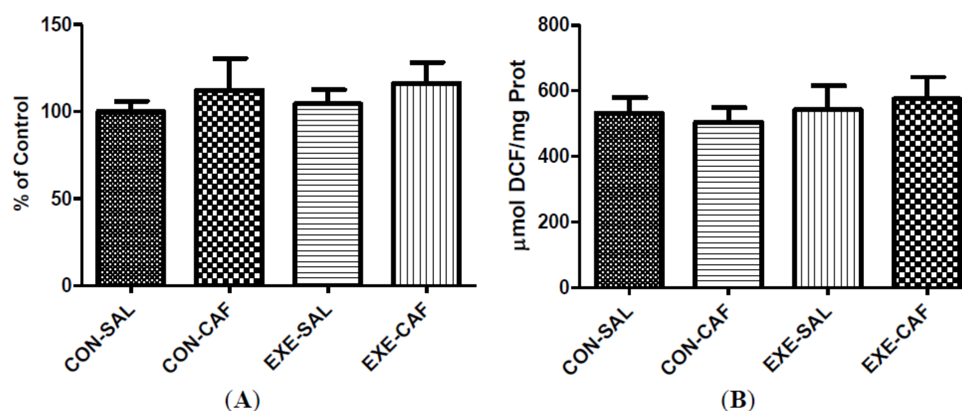
3. Results

3.1. Liver Mitochondrial Parameters

3.1.1. Liver Mitochondria Oxygen Metabolism

Figure 1 depicts the data regarding oxygen metabolism on liver mitochondria. In this sense, no effect of exercise training or caffeine, nor the combination of both, have affected the activity of the complex I (1A). In same way, no significant differences were found between the groups on mitochondrial ROS production (1B).

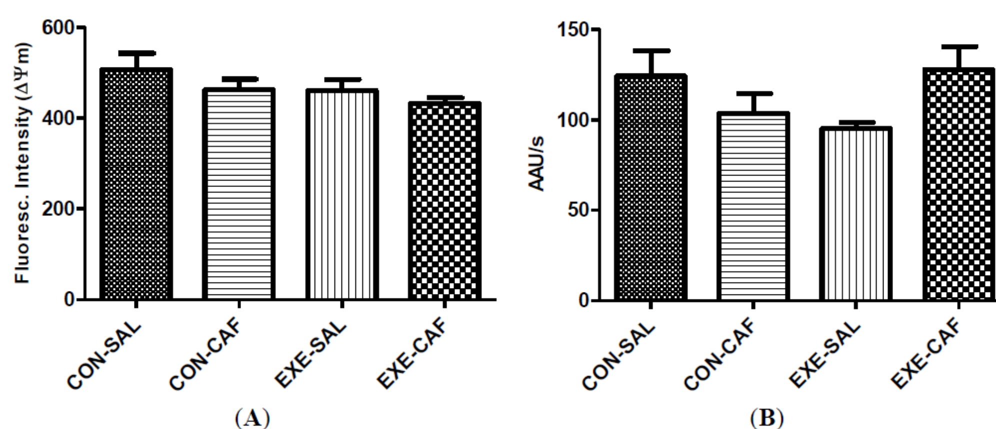
Figure 1. the effects of chronic caffeine intake and exercise training on (**A**) mitochondrial complex I activity; and (**B**) ROS production. Means without a common letter differ significantly ($p < 0.05$). CON: control; CON-CAF: control-caffeine; EXE: exercise; EXE-CAF: exercise-caffeine.



3.1.2. Liver Mitochondrial Function

No effect of the exercise training, caffeine and the combination of both were found on mitochondria functioning parameters. The Figure 2 depicts the data obtained for mitochondria swelling and membrane potential. Exercise, caffeine, and/or control conditions did not affect mitochondrial $\Delta\Psi m$ (2A) and mitochondrial swelling (2B) in the liver.

Figure 2. the effects of chronic caffeine intake and exercise training on (**A**) mitochondrial membrane potential; and (**B**) swelling. Means without a common letter differ significantly ($p < 0.05$). CON-SAL: control; CON-CAF: control-caffeine; EXE-SAL: exercise; EXE-CAF: exercise-caffeine.



3.2. Inflammation Markers

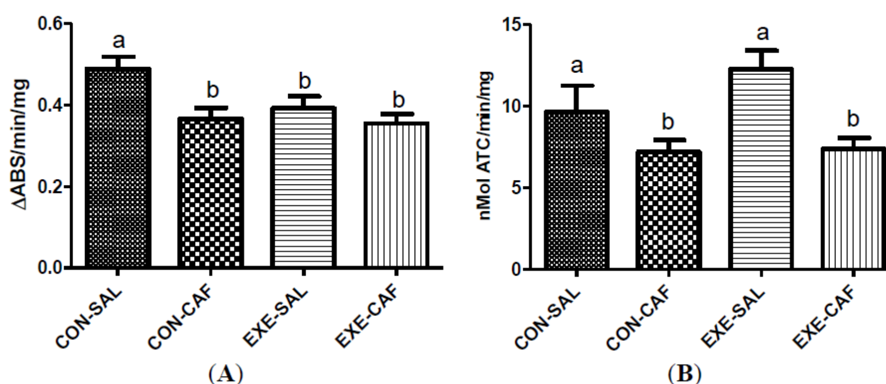
3.2.1. Myeloperoxidase Activity

Trained rats exhibited decreased MPO activities when compared with control animals, and caffeine intake did not modify this response when compared to the trained rats (Figure 3A, $p < 0.05$). However, caffeine alone decreased MPO activity when compared to the control rats. The two-way ANOVA did not detect interactions between exercise training and caffeine intake.

3.2.2. Acetylcholinesterase Activity

No differences in AChE activities were observed between control and trained rats. However, caffeine intake decreased AChE activities in both control and exercised rats (Figure 3B, $p < 0.05$). Again, no interaction between exercise and caffeine intake was found by two-way ANOVA.

Figure 3. the effects of chronic caffeine intake and exercise training on plasma (**A**) myeloperoxidase; and (**B**) acetylcholinesterase activities. Means without a common letter differ significantly ($p < 0.05$). CON-SAL: control; CON-CAF: control-caffeine; EXE-SAL: exercise; EXE-CAF: exercise-caffeine.



4. Discussion

In this study, we demonstrate a systemic anti-inflammatory role of exercise training evidenced by the reduced MPO activity in plasma. Additionally, we also showed that chronic caffeine alone might modulate systemic inflammatory status herein measured through both MPO and AChE activities. Moreover, chronic caffeine intake has also decreased the AChE activity of trained rats. Despite no changes on mitochondrial function and metabolism, caffeine chronic intake may modulate systemic inflammatory markers combined or not with exercise training.

In a previous study, we have demonstrated the training adaptations of this swimming protocol on specific exercise-related markers [7]. Additionally, this training protocol has also induced liver antioxidant modulation, whereas chronic caffeine intake blunted these adaptations [7]. These were relevant findings due to the massive intake of supplementation commercial products with caffeine used by athletes during training or competitions [14]. Our previous study demonstrated that caffeine acted like an “energy spear” mechanism in the liver, with supplemented rats presenting the same exercise capacity with less metabolic demand in the liver. However, if this mechanism was related to mitochondria energy buffering or to a differential inflammatory modulation was still to be elucidated. Therefore, in this study we investigated whether caffeine could modulate the exercise-related mitochondrial oxidative metabolism and function, and systemic inflammatory markers.

It is hypothesized that aerobic exercise increases ROS production causing oxidative stress and mitochondrial dysfunction [33]. However, in our study, no increase was detected on liver mitochondrial ROS production or any of the other mitochondrial parameters measured among exercised animals. This lack of changes in the liver might also be a consequence of increases observed on the antioxidant defense system, including GPx and SOD activities [7]. These results are in accordance with Sun *et al.* [33] who found no alterations in liver mitochondrial ROS production following exercise training. Apparently, mitochondrial energy turnover and ROS production are directly related to the most active organs during exercise, such as the muscle [34]. We have also

previously seen liver mitochondria adaptations on membrane potential and swelling [6], which were not found in this study. A possible explanation for this discrepancy relies on the training intensity and duration, which were both lower in this study. It has been long stated that training duration and intensity are highly related to exercise oxidative metabolism modulation [35].

On the other side, clinical studies and other experimental sets have demonstrated that MPO activity, a marker of neutrophil infiltration, is associated with exercise-induced tissue damage, including muscle, liver, and heart [36,37]. We observed a reduction in plasma MPO activity among trained rats, likely due to the chronic stimulus and mild tissue damage elicited by this swimming protocol. In agreement with our findings, previous studies have shown that exercise training may increase the efficiency of immune functioning and decrease serum levels of inflammation markers [10,38,39]. Interestingly, caffeine alone reduced the MPO activity in control rats, demonstrating a *per se* anti-inflammatory role. In this sense, the anti-inflammatory role of caffeine in different tissues of rats has been previously described [8,40].

The hydrolytic enzyme AChE, which is anchored to the membranes of erythrocytes, platelets, leukocytes, and endothelial cells, continuously regulates acetylcholine (ACh) levels [41,42]. ACh has anti-inflammatory functions and suppresses the production of pro-inflammatory cytokines [43–46]. Thus, ACh levels are reduced when AChE activity is increased, leading to a reduction on the anti-inflammatory actions exerted by ACh [47]. Reduced plasma AChE activity indirectly reduces local and systemic inflammatory events due to the absence of negative feedback control exerted by ACh [47]. In our study, caffeine was able to reduce plasma AChE activity in both control and trained rats, indicating a possible anti-inflammatory role. Accordingly, recent studies have demonstrated that the use of AChE inhibitors suppress systemic inflammation and enhance the survival of animals exposed to lipopolysaccharides [48,49] or infection [50]. Regarding exercise, it seems that inflammatory responses rely mainly on duration and intensity [51], which is important considering inflammation blunts exercise performance as seen in studies with different chronic diseases [52–54]. Additionally, similarly to the MPO data, the AChE *per se* suppression on control rats is also a remarkable finding to be highlighted. Finally, these data suggest that caffeine could be used in combination with training protocols as a firstline health promotion nutrient.

5. Conclusions

In this study, we demonstrated that exercise training presents anti-inflammatory effects herein evidenced by decreased and MPO activity. Moreover, we have also found a *per se* anti-inflammatory effect of caffeine intake through reduction on both MPO and AChE activities on control animals. These are interesting findings since caffeine has long been used as an antioxidant molecule, in spite of the anti-inflammatory role it may exert. These adaptations are linked to an increased exercise performance as seen in our previous study, which corroborates previous data. In this sense, in this exercise training protocol caffeine is not acting directly on the hepatic oxygen metabolism to induce higher exercise capacity as we have not found liver mitochondria to be affected by caffeine intake or training. Apparently, in this swimming protocol the exercise capacity is related to increased antioxidant (as seen in our previous study) and inflammatory modulation. Future studies are needed to

clarify the metabolic pathways related to both antioxidant and anti-inflammatory adaptations elicited by caffeine intake and exercise training.

Acknowledgments

Work supported by the PRONEM # 11/2029-1 research grant by FINEP, "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00. INCT—National Institute of Science and Technology for Excitotoxicity and Neuroprotection/CNPq also supported this work. F.A.A.S. and N.B.V.B. received a fellowship from CNPq. R.P.B., S.T.S., G.P.A and G.B. received a fellowship from CAPES.

Author Contributions

Conception and design of the experiments: R.P.B., M.A.S., G.P.A. and S.T.S. Collection, analysis and interpretation of data: R.P.B., G.B., F.A.A.S. and N.V.B. Drafting the article and revising it critically for important intellectual content: R.P.B., G.B., M.R.F., F.A.A.S., N.V.B. All authors approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rasmussen, U.F.; Krustrup, P.; Bangsbo, J.; Rasmussen, H.N. The effect of high-intensity exhaustive exercise studied in isolated mitochondria from human skeletal muscle. *Pflugers Arch.* **2001**, *443*, 180–187.
2. Radák, Z.; Sasvári, M.; Nyakas, C.; Taylor, A.W.; Ohno, H.; Nakamoto, H.; Goto, S. Regular training modulates the accumulation of reactive carbonyl derivatives in mitochondrial and cytosolic fractions of rat skeletal muscle. *Arch. Biochem. Biophys.* **2000**, *383*, 114–118.
3. Willis, W.T.; Jackman, M.R. Mitochondrial function during heavy exercise. *Med. Sci. Sports Exerc.* **1994**, *26*, 1347–1353.
4. Beckman, K.B.; Ames, B.N. The free radical theory of aging matures. *Physiol. Rev.* **1998**, *78*, 547–581.
5. Echtay, K.S. Mitochondrial uncoupling proteins—What is their physiological role? *Free Radic. Biol. Med.* **2007**, *43*, 1351–1371.
6. Lima, F.D.; Stamm, D.N.; Della-Pace, I.D.; Dobrachinski, F.; de Carvalho, N.R.; Royes, L.F.; Soares, F.A.; Rocha, J.B.; González-Gallego, J.; Bresciani, G. Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. *PLoS One* **2013**, *8*, e55668.
7. Barcelos, R.P.; Souza, M.A.; Amaral, G.P.; Stefanello, S.T.; Bresciani, G.; Fighera, M.R.; Soares, F.A.; Barbosa, N.V. Caffeine supplementation modulates oxidative stress markers in the liver of trained rats. *Life Sci.* **2014**, *96*, 40–45.
8. Cechella, J.L.; Leite, M.R.; Dobrachinski, F.; da Rocha, J.T.; Carvalho, N.R.; Duarte, M.M.; Soares, F.A.; Bresciani, G.; Royes, L.F.; Zeni, G. Moderate swimming exercise and caffeine

supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats. *Amino Acids* **2014**, doi:10.1007/s00726-014-1679-1.
9. Ravi Kiran, T.; Subramanyam, M.V.; Asha Devi, S. Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: Relationship to swim intensity and duration. *Comp. Biochem. Physiol. Part. B Biochem. Mol. Biol.* **2004**, *137*, 187–196.
10. Lira, F.S.; Koyama, C.H.; Yamashita, A.S.; Rosa, J.C.; Zanchi, N.E.; Batista, M.L.; Seelaender, M.C. Chronic exercise decreases cytokine production in healthy rat skeletal muscle. *Cell Biochem. Funct.* **2009**, *27*, 458–461.
11. Campbell, P.T.; Gross, M.D.; Potter, J.D.; Schmitz, K.H.; Duggan, C.; McTiernan, A.; Ulrich, C.M. Effect of exercise on oxidative stress: A 12-month randomized, controlled trial. *Med. Sci. Sports Exerc.* **2010**, *42*, 1448–1453.
12. Waters, D.L.; Baumgartner, R.N.; Garry, P.J.; Vellas, B. Advantages of dietary, exercise-related, and therapeutic interventions to prevent and treat sarcopenia in adult patients: An update. *Clin. Interv. Aging* **2010**, *5*, 259–270.
13. Hoene, M.; Weigert, C. The stress response of the liver to physical exercise. *Exerc. Immunol. Rev.* **2010**, *16*, 163–183.
14. Tunnicliffe, J.M.; Erdman, K.A.; Reimer, R.A.; Lun, V.; Shearer, J. Consumption of dietary caffeine and coffee in physically active populations: Physiological interactions. *Appl. Physiol. Nutr. Metab.* **2008**, *33*, 1301–1310.
15. Goldstein, E.R.; Ziegenfuss, T.; Kalman, D.; Kreider, R.; Campbell, B.; Wilborn, C.; Taylor, L.; Willoughby, D.; Stout, J.; Graves, B.S.; *et al.* International society of sports nutrition position stand: Caffeine and performance. *J. Int. Soc. Sports Nutr.* **2010**, *7*, 5.
16. Kalmar, J.M.; Cafarelli, E. Caffeine: A valuable tool to study central fatigue in humans? *Exerc. Sport Sci. Rev.* **2004**, *32*, 143–147.
17. Tarnopolsky, M.A. Effect of caffeine on the neuromuscular system—Potential as an ergogenic aid. *Appl. Physiol. Nutr. Metab.* **2008**, *33*, 1284–1289.
18. Chen, G.Q.; Chen, Y.Y.; Wang, X.S.; Wu, S.Z.; Yang, H.M.; Xu, H.Q.; He, J.C.; Wang, X.T.; Chen, J.F.; Zheng, R.Y. Chronic caffeine treatment attenuates experimental autoimmune encephalomyelitis induced by guinea pig spinal cord homogenates in Wistar rats. *Brain Res.* **2010**, *1309*, 116–125.
19. Lv, X.; Chen, Z.; Li, J.; Zhang, L.; Liu, H.; Huang, C.; Zhu, P. Caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress. *Inflamm. Res.* **2010**, *59*, 635–645.
20. Chavez-Valdez, R.; Wills-Karp, M.; Ahlawat, R.; Cristofalo, E.A.; Nathan, A.; Gauda, E.B. Caffeine modulates TNF-alpha production by cord blood monocytes: The role of adenosine receptors. *Pediatr. Res.* **2009**, *65*, 203–208.
21. Gobatto, C.A.; de Mello, M.A.; Sibuya, C.Y.; de Azevedo, J.R.; dos Santos, L.A.; Kokubun, E. Maximal lactate steady state in rats submitted to swimming exercise. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2001**, *130*, 21–27.
22. Fredholm, B.B.; Bättig, K.; Holmén, J.; Nehlig, A.; Zvartau, E.E. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev.* **1999**, *51*, 83–133.

23. Bhattacharya, S.K.; Thakar, J.H.; Johnson, P.L.; Shanklin, D.R. Isolation of skeletal muscle mitochondria from hamsters using an ionic medium containing ethylenediaminetetraacetic acid and nagarse. *Anal. Biochem.* **1991**, *192*, 344–349.
24. Akerman, K.E.; Wikström, M.K. Safranine as a probe of the mitochondrial membrane potential. *FEBS Lett.* **1976**, *68*, 191–197.
25. García-Ruiz, C.; Colell, A.; Marí, M.; Morales, A.; Fernández-Checa, J.C. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *J. Biol. Chem.* **1997**, *272*, 11369–11377.
26. Votyakova, T.V.; Reynolds, I.J. Ca^{2+}-induced permeabilization promotes free radical release from rat brain mitochondria with partially inhibited complex I. *J. Neurochem.* **2005**, *93*, 526–537.
27. Bottje, W.; Iqbal, M.; Tang, Z.X.; Cawthon, D.; Okimoto, R.; Wing, T.; Cooper, M. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. *Poult. Sci.* **2002**, *81*, 546–555.
28. Galante, Y.M.; Hatefi, Y. Resolution of complex I and isolation of NADH dehydrogenase and an iron—Sulfur protein. *Methods Enzymol.* **1978**, *53*, 15–21.
29. Pimentel, V.C.; Pinheiro, F.V.; Kaefer, M.; Moresco, R.N.; Moretto, M.B. Assessment of uric acid and lipid peroxidation in serum and urine after hypoxia-ischemia neonatal in rats. *Neurol. Sci.* **2011**, *32*, 59–65.
30. Ellman, G.L.; Courtney, K.D.; Andres, V., Jr.; Feather-Stone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95.
31. Worek, F.; Mast, U.; Kiderlen, D.; Diepold, C.; Eyer, P. Improved determination of acetylcholinesterase activity in human whole blood. *Clin. Chim. Acta* **1999**, *288*, 73–90.
32. Lowry, O.; Roserbrough, N.; Farr, A.; Randall, R. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
33. Sun, L.; Shen, W.; Liu, Z.; Guan, S.; Liu, J.; Ding, S. Endurance exercise causes mitochondrial and oxidative stress in rat liver: Effects of a combination of mitochondrial targeting nutrients. *Life Sci.* **2010**, *86*, 39–44.
34. Bo, H.; Jiang, N.; Ji, L.L.; Zhang, Y. Mitochondrial redox metabolism in aging: Effect of exercise interventions. *J. Sport Health Sci.* **2013**, *2*, 67–74.
35. Radak, Z.; Chung, H.Y.; Koltai, E.; Taylor, A.W.; Goto, S. Exercise, oxidative stress and hormesis. *Ageing Res. Rev.* **2008**, *7*, 34–42.
36. Belcastro, A.N.; Arthur, G.D.; Albisser, T.A.; Raj, D.A. Heart, liver, and skeletal muscle myeloperoxidase activity during exercise. *J. Appl. Physiol.* **1996**, *80*, 1331–1335.
37. Fielding, R.A.; Manfredi, T.J.; Ding, W.; Fiatarone, M.A.; Evans, W.J.; Cannon, J.G. Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *Am. J. Physiol.* **1993**, *265*, R166–R172.
38. Petersen, A.M.; Pedersen, B.K. The anti-inflammatory effect of exercise. *J. Appl. Physiol.* **2005**, *98*, 1154–1162.
39. Belotto, M.F.; Magdalon, J.; Rodrigues, H.G.; Vinolo, M.A.; Curi, R.; Pithon-Curi, T.C.; Hatanaka, E. Moderate exercise improves leucocyte function and decreases inflammation in diabetes. *Clin. Exp. Immunol.* **2010**, *162*, 237–243.

40. Lou, Y.; Peng, Q.; Li, T.; Nolan, B.; Bernard, J.J.; Wagner, G.C.; Lin, Y.; Shih, W.J.; Conney, A.H.; Lu, Y. Oral caffeine during voluntary exercise markedly inhibits skin carcinogenesis and decreases inflammatory cytokines in UVB-treated mice. *Nutr. Cancer* **2013**, *65*, 1002–1013.
41. Cicco, G.; Vetrugno, M.; Rotelli, M.T.; Sborgia, G.; Pennetta, M.; Vico, P.P.; Memeo, V.; Nitti, L.; Sborgia, C. Red blood cell (RBC) surface acetylcholinesterase showing a hemorheological pattern during glaucoma treatment. *Clin. Hemorheol. Microcirc.* **2006**, *35*, 149–154.
42. Cousin, X.; Strähle, U.; Chatonnet, A. Are there non-catalytic functions of acetylcholinesterases? Lessons from mutant animal models. *Bioessays* **2005**, *27*, 189–200.
43. Borovikova, L.V.; Ivanova, S.; Zhang, M.; Yang, H.; Botchkina, G.I.; Watkins, L.R.; Wang, H.; Abumrad, N.; Eaton, J.W.; Tracey, K.J. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* **2000**, *405*, 458–462.
44. Pavlov, V.A.; Tracey, K.J. Neural regulators of innate immune responses and inflammation. *Cell. Mol. Life Sci.* **2004**, *61*, 2322–2331.
45. Pavlov, V.A.; Tracey, K.J. Controlling inflammation: The cholinergic anti-inflammatory pathway. *Biochem. Soc. Trans.* **2006**, *34*, 1037–1040.
46. Wang, H.; Liao, H.; Ochani, M.; Justiniani, M.; Lin, X.; Yang, L.; Al-Abed, Y.; Metz, C.; Miller, E.J.; Tracey, K.J.; Ulloa, L. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat. Med.* **2004**, *10*, 1216–1221.
47. Das, U.N. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. *Med. Sci. Monit.* **2007**, *13*, RA214–RA221.
48. Pavlov, V.A.; Parrish, W.R.; Rosas-Ballina, M.; Ochani, M.; Puerta, M.; Ochani, K.; Chavan, S.; Al-Abed, Y.; Tracey, K.J. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain Behav. Immun.* **2009**, *23*, 41–45.
49. Liu, Z.H.; Ma, Y.F.; Wu, J.S.; Gan, J.X.; Xu, S.W.; Jiang, G.Y. Effect of cholinesterase inhibitor galanthamine on circulating tumor necrosis factor alpha in rats with lipopolysaccharide-induced peritonitis. *Chin. Med. J.* **2010**, *123*, 1727–1730.
50. Fernandez-Cabezudo, M.J.; Lorke, D.E.; Azimullah, S.; Mechkarska, M.; Hasan, M.Y.; Petroianu, G.A.; al-Ramadi, B.K. Cholinergic stimulation of the immune system protects against lethal infection by Salmonella enterica serovar Typhimurium. *Immunology* **2010**, *130*, 388–398.
51. Handschin, C.; Spiegelman, B.M. The role of exercise and PGC1alpha in inflammation and chronic disease. *Nature* **2008**, *454*, 463–469.
52. Kadoglou, N.P.; Perrea, D.; Iliadis, F.; Angelopoulou, N.; Liapis, C.; Alevizos, M. Exercise reduces resistin and inflammatory cytokines in patients with type 2 diabetes. *Diabetes Care* **2007**, *30*, 719–721.
53. Tang, K.; Murano, G.; Wagner, H.; Nogueira, L.; Wagner, P.D.; Tang, A.; Dalton, N.D.; Gu, Y.; Peterson, K.L.; Breen, E.C. Impaired exercise capacity and skeletal muscle function in a mouse model of pulmonary inflammation. *J. Appl. Physiol.* **2013**, *114*, 1340–1350.

54. Van de Weert-van Leeuwen, P.B.; Arets, H.G.; van der Ent, C.K.; Beekman, J.M. Infection, inflammation and exercise in cystic fibrosis. *Respir. Res.* **2013**, *14*, 32.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).

6. MANUSCRITO 1: EFEITOS DO DICLOFENACO NA RESPOSTA INFLAMATÓRIA AO EXERCÍCIO EXCÊNTRICO MEDIADA POR TOLL-LIKE RECEPTOR 4/NUCLEAR FACTOR KAPPA B EM FIGADO DE RATOS

Artigo científico submetido na revista Mediators of Inflammation, 2015; encontra-se na fase de Revisão R1.

Título original:

EFFECTS OF DICLOFENAC ON THE TOLL-LIKE RECEPTOR 4/NUCLEAR FACTOR KAPPA B-MEDIATED INFLAMMATORY RESPONSE TO ECCENTRIC EXERCISE IN RAT LIVER

Autores:

Rômulo Pillon Barcelos, Guilherme Bresciani, Paula Rodriguez-Miguelez, Maria José Cuevas, Félix Alexandre Antunes Soares, Nilda Vargas Barbosa,Javier González-Gallego

Effects of diclofenac pretreatment on the toll-like receptor 4/nuclear factor kappa B-mediated inflammatory response to eccentric exercise in rat liver

Rômulo Pillon Barcelos[a*], Guilherme Bresciani[b], Paula Rodriguez-Miguelez[d], Maria José Cuevas[c], Félix Alexandre Antunes Soares[a], Nilda Vargas Barbosa[a],Javier González-Gallego[c]

[a]*Departamento de Química, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil*

[b]*Universidad Autónoma de Chile, Chile*

[c]*Institute of Biomedicine (IBIOMED) and CIBERehd, University of León, Campus Universitario, 24071 León, Spain*

[d]*Division of Clinical Translational Science, Georgia Prevention Institute, Department of Pediatrics, Georgia Regents University, USA*

*Corresponding author: Rômulo Pillon Barcelos

Departamento de Química, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. Tel: +55 55 32209522; e-mail: romulo1604@hotmail.com

Abstract

Acute exercise is a stress stimulus that may cause cell damage through the activation of the toll-like receptor (TLR)4 pathway, resulting in the translocation of nuclear factor kappa B (NF-κB) into the cell nucleus and the upregulation of inflammatory genes. Nonsteroidal anti-inflammatory drugs, such as diclofenac, are often prescribed to counteract exercise-induced inflammation. This study analyzed effects of diclofenac pretreatment on the TLR4/NF-κB pathway in rat liver after an acute eccentric exercise. Twenty male Wistar rats were divided in four groups: control-saline, control-diclofenac, exercise-saline and exercise-diclofenac. The rats received saline or diclofenac (10 mg/kg) for 7 days prior to an eccentric exercise bout. After exercise there was an increase in TLR4, myeloid differentiation primary response gene 88 (MyD88), TIR domain-containing adaptor inducing interferon (TRIF) and p65 NF-κB subunit protein levels. Exercise also resulted in increased mRNA and protein expression of interleukin (IL)-6, inducible nitric oxide synthase (iNOS) and tumour necrosis factor (TNF)-α. Proinflammatory effects of exercise were prevented by the administration of diclofenac, which blunted the activation of the TLR4/NF-κB pathway and the inflammatory response in the liver of exercised rats. Results from the present study highlight the role of TLR4 as a target for anti-inflammatory interventions.

Key words: Acute exercise; Inflammation; TLR4; NF-κB; Diclofenac; Liver

1 Introduction

Inflammation and exercise are correlated as massively indicated in the literature. Currently, it is well known that acute exercise represents a stress stimulus that transiently disrupts cell homeostasis [1, 2]. Unaccustomed exercise, especially involving rigorous eccentric muscle contractions, causes significant damage to skeletal muscle fibers [3], leading to increased levels of inflammatory markers, such as adhesion molecules and proinflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-6 [4]. This cascade triggers an inflammatory response characterized by the increase in the number of inflammatory circulating cells and cytokines [5-8].

Nuclear factor-κB (NF-κB) is one of the most important signaling pathway activated during eccentric exercise [9]. This transcription factor is inactive within the cytoplasm due to its binding to inhibitory IκB proteins, while stimuli such as a reactive oxygen species (ROS) and cytokines release during acute exercise, may induce its activation [10]. IκB is then phosphorylated and proteolytically degraded, resulting in the translocation of NF-κB into the nucleus. This directly alters the expression of different proinflammatory cytokines [9].

The toll-like receptors (TLRs) are type I trans-membrane glycoproteins which play an important regulatory role in the NF-κB signaling pathway [11] and act as key factors in the inflammatory responses to exercise [12, 13]. One of these TLRs, TLR4, has been shown to highly respond to different exercise protocols [11, 14]. TLR4 mediates several processes in the inflammatory cascade, triggering inflammation through myeloid differentiation primary response gene 88 (MyD88)-dependent and MyD88-independent [15] pathways. The cell surface or the endosomal localization of TLR4 leads to the recruitment of MyD88 or the toll/interleukin-1 receptor (TIR) domain containing adapter inducing interferon-β (TRIF) proteins[16] proteins, which results in the activation of NF-κB [17]. Different studies have shown that training programs reduce TLR4-mediated activation of the proinflammatory

response through MyD88-dependent and -independent pathways [18]. Nevertheless, the studies about the association of TLR4 and acute exercise are still contradictory; while some authors have reported a decrease in TLR4 expression after a single bout of exercise [19, 20], others did not find differences [21] or even an enhanced expression [22]. In peripheral blood mononuclear cells, eccentric training is known to partially prevent the increased expression of proteins involved in the TLR4 pathway following an acute bout of eccentric exercise [11, 18]. Different studies have shown the existence of an inflammatory liver response following acute exercise [23-26]. However, as far as we know, there are no data supporting the relationship between liver and TLR-4/NF-κB activation after an eccentric exercise.

In sports, the use of medications to counteract the inflammatory effects of exercise, such as the nonsteroidal anti-inflammatory drugs (NSAIDs), has become a common practice [27]. NSAIDs are pharmacologic agents which provide analgesic effects [28] blocking the synthesis of prostaglandins and thromboxanes [29]. The daily routine of training and highly demanding competitions cause muscle damage, inflammation and loss of performance and, in many cases, NSAIDs are commonly prescribed to reduce muscle pain and inflammation derived from training and competition [30]. Among the NSAIDs, diclofenac is widely prescribed as an analgesic drug against acute pain [31]. It is among the most commonly used NSAIDs by either athletes or non-athletes [32, 33] who are unaware of its potential toxicities, especially on the liver. Indeed, its indiscriminate consumption can produce rare but sometimes serious hepatotoxicity due to its metabolization into reactive metabolites [34].

Considering the major metabolic role of the liver during exercise [25] and the lack of information concerning the combined effects of NSAIDS and acute exercise on the hepatic inflammatory pathways, this study was aimed to investigate the effects of an acute bout of eccentric exercise on the TLR4/NF-κB pathway in the liver of diclofenac-treated rats.

2 Materials and methods

2.1 Animals

All experiments were approved by the Institutional Animal Care Committee of the University of León and according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Male Wistar rats weighing 270 ± 25 g were used in the experiments. The animals were fed standard laboratory chow and allowed free access to water in an air-conditioned room with a 12:12-h light-dark cycle.

2.2 Experimental design and diclofenac administration

Twenty animals were randomly divided into four groups: control-saline (CS), control-diclofenac (CD), exercise-saline (ES) and exercise-diclofenac (ED). The animals from the C and E groups received saline, while the groups CD and ED received diclofenac treatment during seven days previous to the eccentric exercise bout. 24hr after the last administration, exercised groups ES an ED performed an acute bout of eccentric exercise, which consisted on intermittent downhill running (-16° incline) at 16 m/min for a total of 90 min (5 min/bout (18 bouts) separated by 2 min of rest) [3]. Diclofenac was administered by intragastric gavage at a dose of 10 mg/kg body wt (in saline) during 7 days. The selected dose is that prescribed in clinical practice and does not cause adverse effects [35, 36]. Animals were killed 2 h after cessation of the exercise [3] and, after exsanguination, the liver was immediately excised, freeze-clamped between aluminum tongs, precooled with liquid nitrogen, and stored at -80°C for biochemical assays.

2.3 Western blot analysis

For western blot analysis, liver tissue was physical disrupted after suspended the samples on 150 μl of 0.25mM sucrose, 1mM EDTA, 10 mM Tris and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Samples containing 40 μg of protein were fractionated by SDS–polyacrilamide gel electrophoresis with polyacrilamide gels (9% for TRL-4 and iNOS; 11% for

MyD88, p65 and TRIF; 14% for TNF-α and IL-6) and then, transferred to a PVDF membrane by a Trans-Blot® TurboTM Transfer System (Bio-Rad®, Hercules, CA). Non-specific binding was blocked by pre-incubation of the PVDF membranes in PBS containing 5% non-fat milk for 1 h. Then, membranes were incubated overnight at 4°C with the corresponding antibodies. Antibodies against TLR4 (95 KDa), MyD88 (33 KDa), iNOS (130 KDa), TNF-α (26 KDa), IL-6 (25 KDa) and p65 (65 KDa) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); antibody against TRIF (66 KDa) was purchased from Abcam® (Cambridge, UK). After washing with TBST, the membranes were incubated for 1 hr at room temperature with secondary HRP conjugated antibody (Dako, Glostrup, Denmark, 1:5,000), and visualized using ECL detection kit (Amersham Pharmacia; Uppsala, Sweden). The blots were stripped and probed again for anti-β-actin (42 kDa) antibody (Sigma-Aldrich, St. Louis, MO, USA) to verify equal protein loading[36]. The density of the specific bands was quantified with an imaging densitometer (Scion Image J Software 1.46a; Bethesda, MD, USA)

.

2.4 Real-time quantitative RT-PCR

Total RNA was obtained by using a Trizol reagent (Life Technologies, Carlsbad, CA) and quantified by spectrophotometry (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA). Residual genomic DNA was removed by incubating RNA with RQ1 RNase-free DNase (Promega, Madison, WI). First-standard cDNA was synthesized using High-Capacity cDNA Archive Kit (Applied Biosystems, Weiterstadt, Germany). The negative control (no transcriptase control) was performed in parallel. First-standard complementary DNA (cDNA) was synthesized using High-Capacity cDNA Archive Kit (Applied Biosystems®, Paisley, UK), and then, it was amplified using TaqMan® Universal PCR Master Mix (Applied Biosystems®) on a StepOnePlus™ Real-Time PCR Systems (Applied Biosystems®)[37]. TaqMan primers and probes for iNOS (GenBank accession no. D12520.1 and Rn00561646_m1), IL-6 (GenBank accession no. M26744.1 and Rn99999011_m1), TNF-α (GenBank accession no.

AJ002278.1 and Rn99999017_m1) and GAPDH (GenBank accession no. NM_017008.13 and Rn01775763_g1) as housekeeping gene were derived from the commercially available TaqMan® Gene Expression Assays (Applied Biosystems). Relative changes in gene expression levels were determined using the $2^{-\Delta\Delta CT}$ method as described previously[38, 39]. The cycle number at which the transcripts were detectable (CT) was normalized to the cycle number of GADPH detection, referred to as ΔCT.

2.5 Statistical Analysis

Results are shown as mean ± SEM. Statistical analysis was performed using one-way analysis of (ANOVA), followed by Student-Newman-Keuls test when appropriate or two-way ANOVA to determine possible interactions (exercise x diclofenac). Values of $p<0.05$ were considered significant.

3 Results

3.1 Effect of acute eccentric exercise and diclofenac pretreatment on the TRL4/NF-κB pathway

As shown in Figure 1A the acute bout of eccentric exercise induced a significant increase in the level of protein TLR4 (+187, $p<0.001$). The expression of both MyD88 and TRIF proteins also increased significantly (+65%, $p<0.001$ and +73%, $p<0.001$, respectively) (Fig. 1B and 1C), indicating that the acute bout of exercise induced the activation of the Myd88-dependent and MyD88-independent pathways. As expected, these changes were accompanied by an enhanced protein concentration of the p65 NF-kB subunit (+136%, $p< 0.001$) (Fig. 1D). The pretreatment with diclofenac blunted the effects of the acute eccentric exercise on the expression of proteins TLR4 (-48%, $p<0.001$), Myd88 (-68%, $p<0.01$), TRIF (-71%, $p<0.05$) and p65 (-62%, $p<0.05$) (Fig. 1).

3.2 Effect of acute eccentric exercise and diclofenac pretreatment on the expression of inflammation-related genes

We then tested effects of exercise and diclofenac on the mRNA and protein expression of different inflammation-related genes. When mRNA levels were measured by quantitative RT-PCR results indicated a transcriptional upregulation of IL-6 (+28%, $p<0.001$) (Fig. 2A), TNF-α (+86%, $p<0.001$) (Fig. 2B) and INOS (+427%, $p<0.001$) (Fig. 2C). These changes were accompanied by a parallel increase in protein levels (IL-6: +35%, $p<0.001$; TNF-α: +68%, $p<0.001$; iNOS: +70%, $p<0.001$) (Fig 3A, 3B and 3C). Pretreatment with diclofenac prevented the increase in mRNA expression and protein levels for both IL-6 (-67%, $p<0.001$; -63%, $p<0.001$, respectively) and TNF-α (−59%, $p<0.001$; -36%, $p<0.001$, respectively) (Fig 2A, 3A, 2B and 3B). However, no significant change was observed in iNOS expression when compared to ES group (Fig. 2C and 3C).

4 Discussion

The present investigation was designed to deepen into the liver molecular inflammatory signaling associated with acute eccentric exercise and the potential of diclofenac to reduce the inflammatory response. Results indicated that an acute 90 min bout of eccentric exercise provoked a proinflammatory response, which was associated with an activation of TLR4 signaling pathway. Diclofenac pretreatment attenuated the proinflammatory response associated with acute eccentric exercise through a down-regulation of both the MyD88-dependent and TRIF-dependent pathways.

The elevated TLR4 protein concentration in the liver of exercised animals suggested that acute eccentric exercise results in a proinflammatory response in this organ. Previous research has shown that eccentric exercise induces an increased expression of TLR4 in skeletal muscle [14] or peripheral blood mononuclear cells [11, 18]. This finding contrasts with several

investigations which have described a down-regulated response of TLR4 after a bout of aerobic exercise [40, 41]. However, while eccentric exercise is generally considered as a proinflammatory stimulus, aerobic exercise often results in downregulation of immune and inflammatory responses [11]. Therefore, activation of the TLR4 pathway appears to be dependent, among other factors, on exercise modality.

The complex TLR4 downstream signaling is far from being completely understood. The cell surface or endosomal localization of TLR4 drives recruitment of MyD88 or TRIF, which mediate the early- and late-phase activation of NF-κB, respectively [16]. Results from the present investigation showed that TLR4 activation through the MyD88-dependent pathway plays an important role in the exercise-induced liver inflammatory response, since the acute bout of eccentric exercise elevated MyD88 protein concentration. The reported increase agrees with results from previous research in rat adipose tissue [22], human and rat skeletal muscle [19, 20] or human peripheral blood mononuclear cells [18]. Activation of MyD88 results in up-regulation of tumor necrosis factor receptor-associated factor (TRAF6), which plays a critical role in the TLR-induced NF-κB pathway [42], triggering the rapid phosphorylation of IκB proteins by a multiprotein complex termed the IKK complex, and leading to the translocation of p65 NF-κB subunits to the nucleus [43]. Additionally to the MyD88-dependent pathway, NF-κB can also be activated in TLR signaling by a MyD88-independent pathway [44], in which TRIF directly binds TRAF6 via TRAF-6-binding motifs in the N-terminal region, leading to NF-κB activation [45]. Previous reports have also shown that acute eccentric exercise may result in activation of this pathway in human peripheral blood mononuclear cells [11, 18]. In the present study, the acute exercise protocol induced a significant increase of p65 protein content, asserting the liver inflammatory effect of acute exercise. As an expected consequence to the up-regulation of the two inflammatory pathways, NF-κB p65 levels also increased in the liver of exercised rats. It is noteworthy to highlight that there is almost no information in the

literature related to the effects of exercise on the NF-κB pathway in the liver. Only one study indicates that NF-kB activation is attenuated by regular exercise in aged rats [46], but this is the first report of changes induced by acute exercise. As expected,activation of the NF-κB pathway resulted in transcriptional upregulation of proinflammatory genes, with increased mRNA and protein expression of iNOS, IL-6 and TNF-α These results corroborate existing data on the proinflammatory effects of acute exercise in skeletal muscle [10, 47, 48], heart [49], blood [50, 51], or peripheral blood mononuclear cells [9].

Data from the present study also demonstrate that pretreatment with diclofenac partially abrogates the exercise-induced inflammation in the liver. Our results confirm previous research in liver HepG2 cells, in which it has been reported that diclofenac exerts anti-inflammatory effects by directly affecting the TNF-α-induced nuclear translocation of NF-κB through inhibition of IκBα phosphorylation and degradation [34]. Keeping in mind that the TLR4 signaling cascade is one of the principal pathways activating NF-κB, it is tempting to propose that the inhibition of the TLR4 pathway (via both MyD88-dependent and TRIF-dependent mechanisms) leads to a reduction in NF-κB activation, resulting in a downregulation of the proinflammatory cytokines TNF-α and IL-6 at both, transcriptional and post-transcriptional levels. However, diclofenac pretreatment did not decrease the mRNA expression and protein levels of iNOS. Although further research would be necessary to identify the mechanisms responsible for this lack of effect, it is known that iNOS may be overexpressed in different tissues through an activation of MAP kinases not mediated via the NF-κB pathway [52]. Moreover, iNOS expression may be induced in liver cells through NF-κB-independent pathways related to upregulation of IL1-RI through the inhibition of Akt phosphorylation [53]. Therefore, it is possible to speculate that these pathways nondependent on NF-κB activation are not inhibited by diclofenac. A previous report indicated that in RAW264.7 macrophages and in human epithelial Caco-2 and HT-29 cells, agonists of peroxisome proliferator activated

receptors (PPAR) amplify NOS expression while inhibiting NF-κB activity [54]. Diclofenac is known to activate PPAR-γ in different tissues [55, 56].

In summary, this study examined the molecular inflammatory signaling associated with acute exercise and diclofenac administration in the liver of rats. Acute eccentric exercise increased TLR4-mediated NF-κB activation, and induced an increased expression of different proinflammatory genes. In addition, we found that pretreatment with diclofenac reduced TLR4-mediated activation of the proinflammatory response through MyD88-dependent and TRIF-dependent pathways. Although further research is needed to fully evaluate the potential effects of NSAIDs on the inflammatory response induced by acute exercise in different organs, results from the present study highlight the role of the TLR4/NF-κB pathway as a target for anti-inflammatory interventions.

Conflict of interests

The authors declare that there are no conflicts of interest.

Financial support

The work was supported by the PRONEM # 11/2029-1 research grant of CNPQ/FAPERGS. F.A.A.S. and N.B.V.B. received a fellowship from CNPq. R.P.B. received a fellowship from CAPES. CIBERehd is funded by Instituto de la Salud Carlos III, Spain.

References

1. Cristi-Montero, C., et al., *[Effect of an acute exercise bout on Toll-like receptor 4 and inflammatory mechanisms in rat heart].* Rev Med Chil, 2012. **140**(10): p. 1282-8.
2. Lima-Cabello, E., et al., *Eccentric exercise induces nitric oxide synthase expression through nuclear factor-kappaB modulation in rat skeletal muscle.* J Appl Physiol (1985), 2010. **108**(3): p. 575-83.
3. Armstrong, R.B., R.W. Ogilvie, and J.A. Schwane, *Eccentric exercise-induced injury to rat skeletal muscle.* J Appl Physiol Respir Environ Exerc Physiol, 1983. **54**(1): p. 80-93.

4. Niebauer, J., *Effects of exercise training on inflammatory markers in patients with heart failure.* Heart Fail Rev, 2008. **13**(1): p. 39-49.
5. Pradeu, T. and E.L. Cooper, *The danger theory: 20 years later.* Front Immunol, 2012. **3**: p. 287.
6. Evans, W.J., et al., *Metabolic changes following eccentric exercise in trained and untrained men.* J Appl Physiol (1985), 1986. **61**(5): p. 1864-8.
7. Fielding, R.A., et al., *Enhanced protein breakdown after eccentric exercise in young and older men.* J Appl Physiol (1985), 1991. **71**(2): p. 674-9.
8. Peake, J., K. Nosaka, and K. Suzuki, *Characterization of inflammatory responses to eccentric exercise in humans.* Exerc Immunol Rev, 2005. **11**: p. 64-85.
9. Jiménez-Jiménez, R., et al., *Eccentric training impairs NF-kappaB activation and over-expression of inflammation-related genes induced by acute eccentric exercise in the elderly.* Mech Ageing Dev, 2008. **129**(6): p. 313-21.
10. Alonso, M., P.S. Collado, and J. González-Gallego, *Melatonin inhibits the expression of the inducible isoform of nitric oxide synthase and nuclear factor kappa B activation in rat skeletal muscle.* J Pineal Res, 2006. **41**(1): p. 8-14.
11. Fernandez-Gonzalo, R., et al., *Effects of eccentric exercise on toll-like receptor 4 signaling pathway in peripheral blood mononuclear cells.* J Appl Physiol (1985), 2012. **112**(12): p. 2011-8.
12. Gleeson, M., B. McFarlin, and M. Flynn, *Exercise and Toll-like receptors.* Exerc Immunol Rev, 2006. **12**: p. 34-53.
13. Flynn, M.G. and B.K. McFarlin, *Toll-like receptor 4: link to the anti-inflammatory effects of exercise?* Exerc Sport Sci Rev, 2006. **34**(4): p. 176-81.
14. Zbinden-Foncea, H., et al., *TLR2 and TLR4 activate p38 MAPK and JNK during endurance exercise in skeletal muscle.* Med Sci Sports Exerc, 2012. **44**(8): p. 1463-72.
15. Akira, S., *TLR signaling.* Curr Top Microbiol Immunol, 2006. **311**: p. 1-16.
16. Kenny, E.F. and L.A. O'Neill, *Signalling adaptors used by Toll-like receptors: an update.* Cytokine, 2008. **43**(3): p. 342-9.
17. Kawai, T. and S. Akira, *Signaling to NF-kappaB by Toll-like receptors.* Trends Mol Med, 2007. **13**(11): p. 460-9.
18. Rodriguez-Miguelez, P., et al., *Role of Toll-like receptor 2 and 4 signaling pathways on the inflammatory response to resistance training in elderly subjects.* Age (Dordr), 2014. **36**(6): p. 9734.
19. Lancaster, G.I., et al., *The physiological regulation of toll-like receptor expression and function in humans.* J Physiol, 2005. **563**(Pt 3): p. 945-55.
20. Liao, P., et al., *Eccentric contraction induces inflammatory responses in rat skeletal muscle: role of tumor necrosis factor-alpha.* Am J Physiol Regul Integr Comp Physiol, 2010. **298**(3): p. R599-607.
21. McFarlin, B.K., et al., *TLR4 is lower in resistance-trained older women and related to inflammatory cytokines.* Med Sci Sports Exerc, 2004. **36**(11): p. 1876-83.
22. Rosa, J.C., et al., *Exhaustive exercise increases inflammatory response via Toll like receptor-4 and NF-κBp65 pathway in rat adipose tissue.* J Cell Physiol, 2011. **226**(6): p. 1604-7.
23. Barcelos, R.P., et al., *Caffeine intake may modulate inflammation markers in trained rats.* Nutrients, 2014. **6**(4): p. 1678-90.
24. Hoene, M. and C. Weigert, *The stress response of the liver to physical exercise.* Exerc Immunol Rev, 2010. **16**: p. 163-83.
25. Shephard, R.J. and N. Johnson, *Effects of physical activity upon the liver.* Eur J Appl Physiol, 2015. **115**(1): p. 1-46.
26. Lima, F.D., et al., *Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts.* PLoS One, 2013. **8**(2): p. e55668.
27. Gorski, T., et al., *Use of NSAIDs in triathletes: prevalence, level of awareness and reasons for use.* Br J Sports Med, 2011. **45**(2): p. 85-90.

28. Feucht, C.L. and D.R. Patel, *Analgesics and anti-inflammatory medications in sports: use and abuse.* Pediatr Clin North Am, 2010. **57**(3): p. 751-74.
29. Amer, M., et al., *Use of nonsteroidal anti-inflammatory drugs in patients with cardiovascular disease: a cautionary tale.* Cardiol Rev, 2010. **18**(4): p. 204-12.
30. Lippi, G., et al., *Non-steroidal anti-inflammatory drugs in athletes.* Br J Sports Med, 2006. **40**(8): p. 661-2; discussion 662-3.
31. Gómez-Lechón, M.J., et al., *Diclofenac induces apoptosis in hepatocytes by alteration of mitochondrial function and generation of ROS.* Biochem Pharmacol, 2003. **66**(11): p. 2155-67.
32. Inotai, A., B. Hankó, and A. Mészáros, *Trends in the non-steroidal anti-inflammatory drug market in six Central-Eastern European countries based on retail information.* Pharmacoepidemiol Drug Saf, 2010. **19**(2): p. 183-90.
33. Ziltener, J.L., S. Leal, and P.E. Fournier, *Non-steroidal anti-inflammatory drugs for athletes: an update.* Ann Phys Rehabil Med, 2010. **53**(4): p. 278-82, 282-8.
34. Fredriksson, L., et al., *Diclofenac inhibits tumor necrosis factor-α-induced nuclear factor-κB activation causing synergistic hepatocyte apoptosis.* Hepatology, 2011. **53**(6): p. 2027-41.
35. Liles, J.H. and P.A. Flecknell, *The use of non-steroidal anti-inflammatory drugs for the relief of pain in laboratory rodents and rabbits.* Lab Anim, 1992. **26**(4): p. 241-55.
36. Ramm, S. and A. Mally, *Role of drug-independent stress factors in liver injury associated with diclofenac intake.* Toxicology, 2013. **312**: p. 83-96.
37. Crespo, I., et al., *Melatonin prevents the decreased activity of antioxidant enzymes and activates nuclear erythroid 2-related factor 2 signaling in an animal model of fulminant hepatic failure of viral origin.* J Pineal Res, 2010. **49**(2): p. 193-200.
38. Filippin, L.I., et al., *The role of nitric oxide during healing of trauma to the skeletal muscle.* Inflamm Res, 2011. **60**(4): p. 347-56.
39. Tuñón, M.J., et al., *Melatonin attenuates apoptotic liver damage in fulminant hepatic failure induced by the rabbit hemorrhagic disease virus.* J Pineal Res, 2011. **50**(1): p. 38-45.
40. García-Mediavilla, M.V., et al., *Differential contribution of hepatitis C virus NS5A and core proteins to the induction of oxidative and nitrosative stress in human hepatocyte-derived cells.* J Hepatol, 2005. **43**(4): p. 606-13.
41. Oliveira, M. and M. Gleeson, *The influence of prolonged cycling on monocyte Toll-like receptor 2 and 4 expression in healthy men.* Eur J Appl Physiol, 2010. **109**(2): p. 251-7.
42. Simpson, R.J., et al., *Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans.* Brain Behav Immun, 2009. **23**(2): p. 232-9.
43. Lambert, C.P., et al., *Exercise but not diet-induced weight loss decreases skeletal muscle inflammatory gene expression in frail obese elderly persons.* J Appl Physiol (1985), 2008. **105**(2): p. 473-8.
44. Coll, R.C. and L.A. O'Neill, *New insights into the regulation of signalling by toll-like receptors and nod-like receptors.* J Innate Immun, 2010. **2**(5): p. 406-21.
45. Kawai, T. and S. Akira, *TLR signaling.* Cell Death Differ, 2006. **13**(5): p. 816-25.
46. Sasai, M., et al., *Direct binding of TRAF2 and TRAF6 to TICAM-1/TRIF adaptor participates in activation of the Toll-like receptor 3/4 pathway.* Mol Immunol, 2010. **47**(6): p. 1283-91.
47. Radák, Z., et al., *Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise.* FASEB J, 2004. **18**(6): p. 749-50.
48. Hollander, J., et al., *Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle.* Pflugers Arch, 2001. **442**(3): p. 426-34.
49. Ji, L.L., et al., *Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle.* FASEB J, 2004. **18**(13): p. 1499-506.
50. Veneroso, C., et al., *Melatonin reduces cardiac inflammatory injury induced by acute exercise.* J Pineal Res, 2009. **47**(2): p. 184-91.
51. Vider, J., et al., *Physical exercise induces activation of NF-kappaB in human peripheral blood lymphocytes.* Antioxid Redox Signal, 2001. **3**(6): p. 1131-7.

52. Richlin, V.A., et al., *Stress-induced enhancement of NF-kappaB DNA-binding in the peripheral blood leukocyte pool: effects of lymphocyte redistribution.* Brain Behav Immun, 2004. **18**(3): p. 231-7.
53. Doi, M., et al., *Cytokine-activated Jak-2 is involved in inducible nitric oxide synthase expression independent from NF-kappaB activation in vascular smooth muscle cells.* Atherosclerosis, 2002. **160**(1): p. 123-32.
54. Matsui, K., et al., *Active hexose correlated compound inhibits the expression of proinflammatory biomarker iNOS in hepatocytes.* Eur Surg Res, 2011. **47**(4): p. 274-83.
55. Cernuda-Morollón, E., et al., *PPAR agonists amplify iNOS expression while inhibiting NF-kappaB: implications for mesangial cell activation by cytokines.* J Am Soc Nephrol, 2002. **13**(9): p. 2223-31.
56. Ayoub, S.S., et al., *Activation of macrophage peroxisome proliferator-activated receptor-gamma by diclofenac results in the induction of cyclooxygenase-2 protein and the synthesis of anti-inflammatory cytokines.* Mol Cell Biochem, 2009. **327**(1-2): p. 101-10.
57. Kaur, J. and S.N. Sanyal, *Modulation of inflammatory changes in early stages of colon cancer through activation of PPARgamma by diclofenac.* Eur J Cancer Prev, 2010. **19**(5): p. 319-27.

Figure legends

Fig. 1. Effects of exercise and diclofenac administration on the protein expression of TLR4 (A), MyD88 (B), TRIF (C) and p65 (D) in rat liver. Densitometric quantification and representative Western blots are shown. Equal loading of proteins is illustrated by b-actin bands. CS: control; CD: control+diclofenac pretreatment; ES: eccentric exercise; ED: eccentric exercise+diclofenac pretreatment. Exercise consisted of 90 min of intermittent downhill running. Diclofenac was given at 10 mg/kg body wt i.g. for 7 days. Data are mean ± SEM for n=4-6 in each group. Means for a variable with superscripts without a common letter differ. $P < 0.05$.

Fig. 2. Effects of exercise and diclofenac administration on the mRNA expression of IL-6 (A), TNF-α (B) and iNOS (C) in rat liver. Levels of mRNA were analyzed by quantitative RT- PCR assays and were normalized to GADPH. CS: control; CD: control+diclofenac pretreatment; ES: eccentric exercise; ED: eccentric exercise+diclofenac pretreatment. Exercise consisted of 90 min of intermittent downhill running. Diclofenac was given at 10 mg/kg body wt i.g. for 7 days. Data are mean ± SEM for n=4-6 in each group. Means for a variable with superscripts without a common letter differ. $P < 0.05$.

Fig. 3. Effects of exercise and diclofenac administration on protein expression of, IL-6 (A), TNF-α (B) and iNOS (C) in rat liver. Densitometric quantification and representative Western blots are shown. Equal loading of proteins is illustrated by β-actin bands. CS: control; CD: control+diclofenac pretreatment; ES: eccentric exercise; ED: eccentric exercise+diclofenac pretreatment. Exercise consisted of 90 min of intermittent downhill running. Diclofenac was

given at 10 mg/kg body wt i.g. for 7 days. Data are mean ± SEM for n=4-6 in each group. Means for a variable with superscripts without a common letter differ. *P*<0.05.

FIGURES

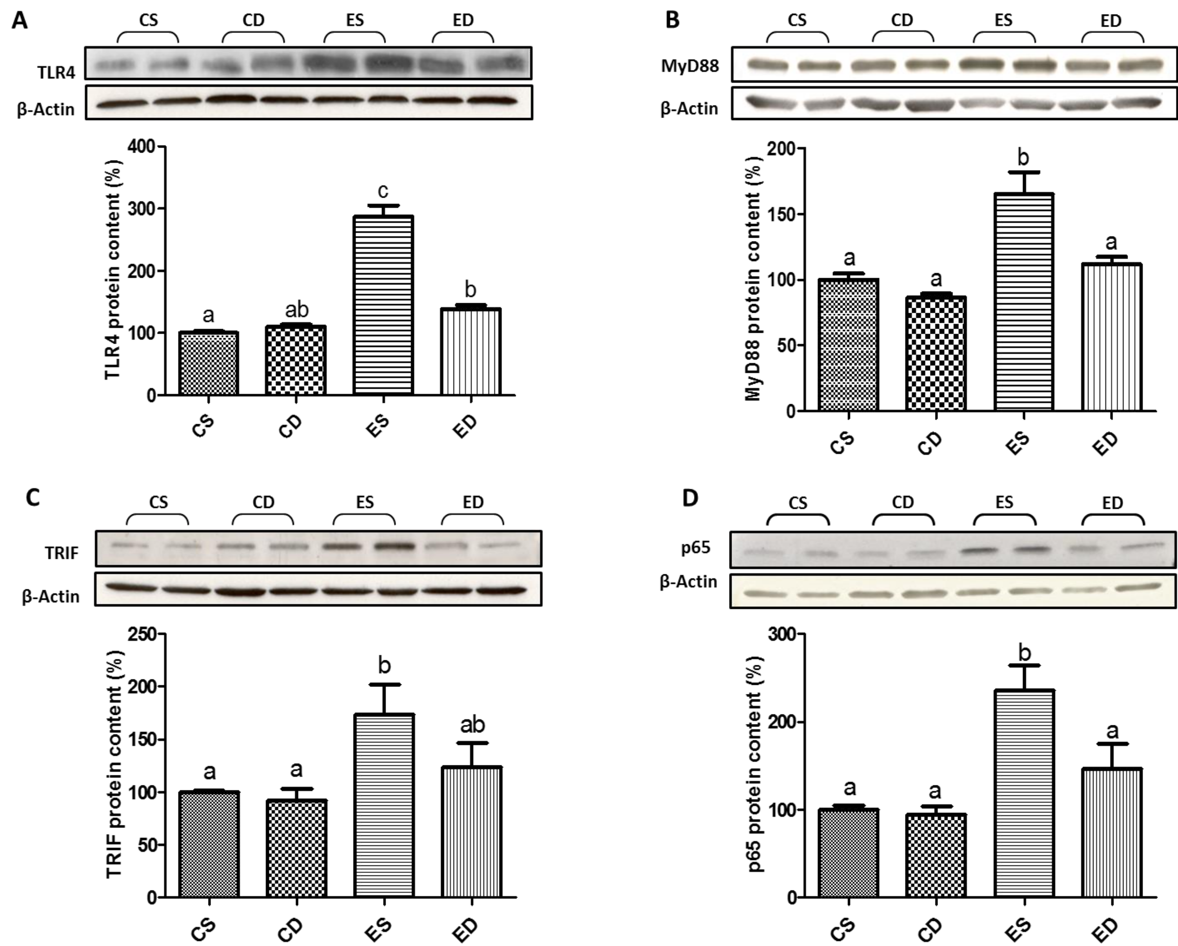


FIGURE 1

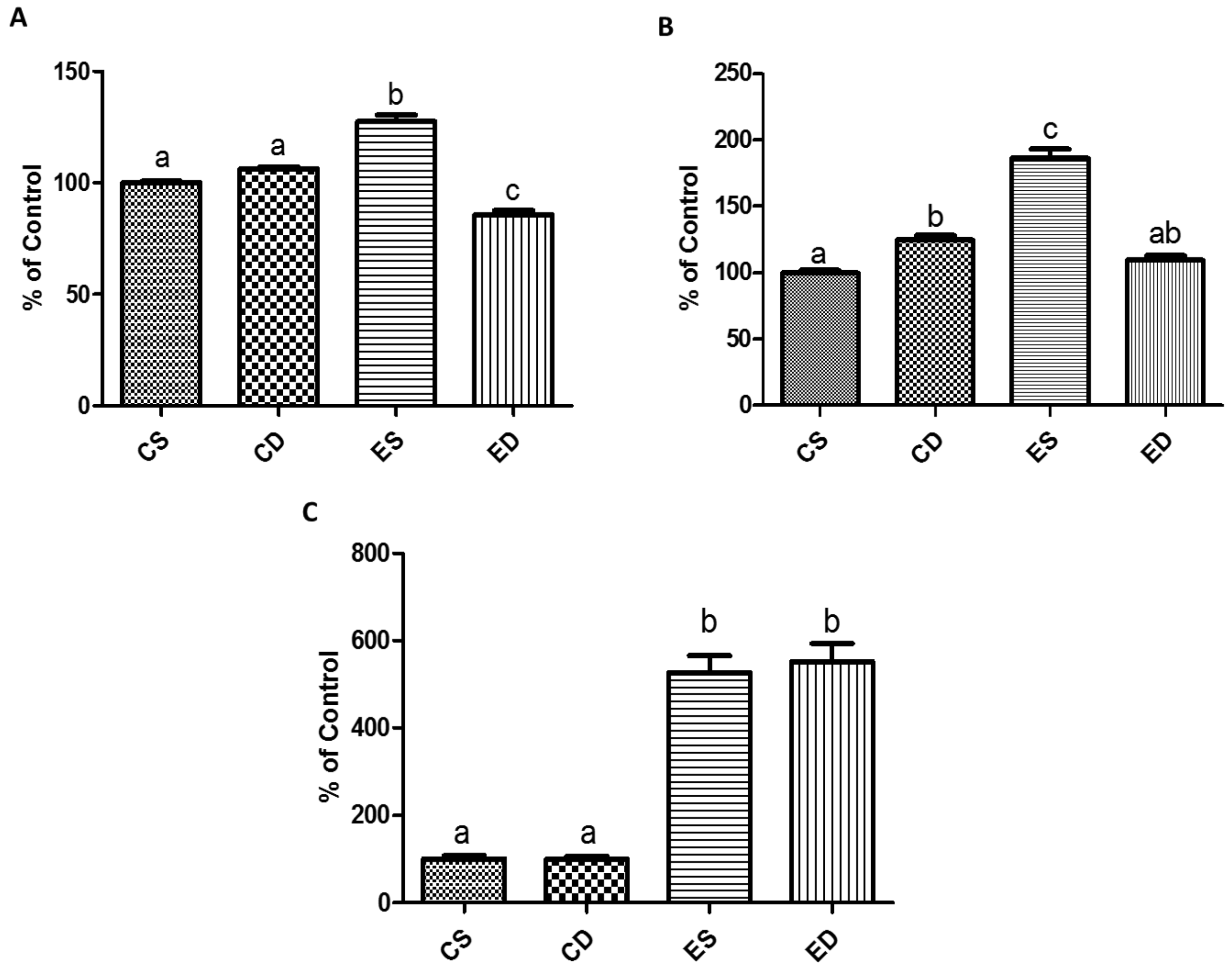


FIGURE 2

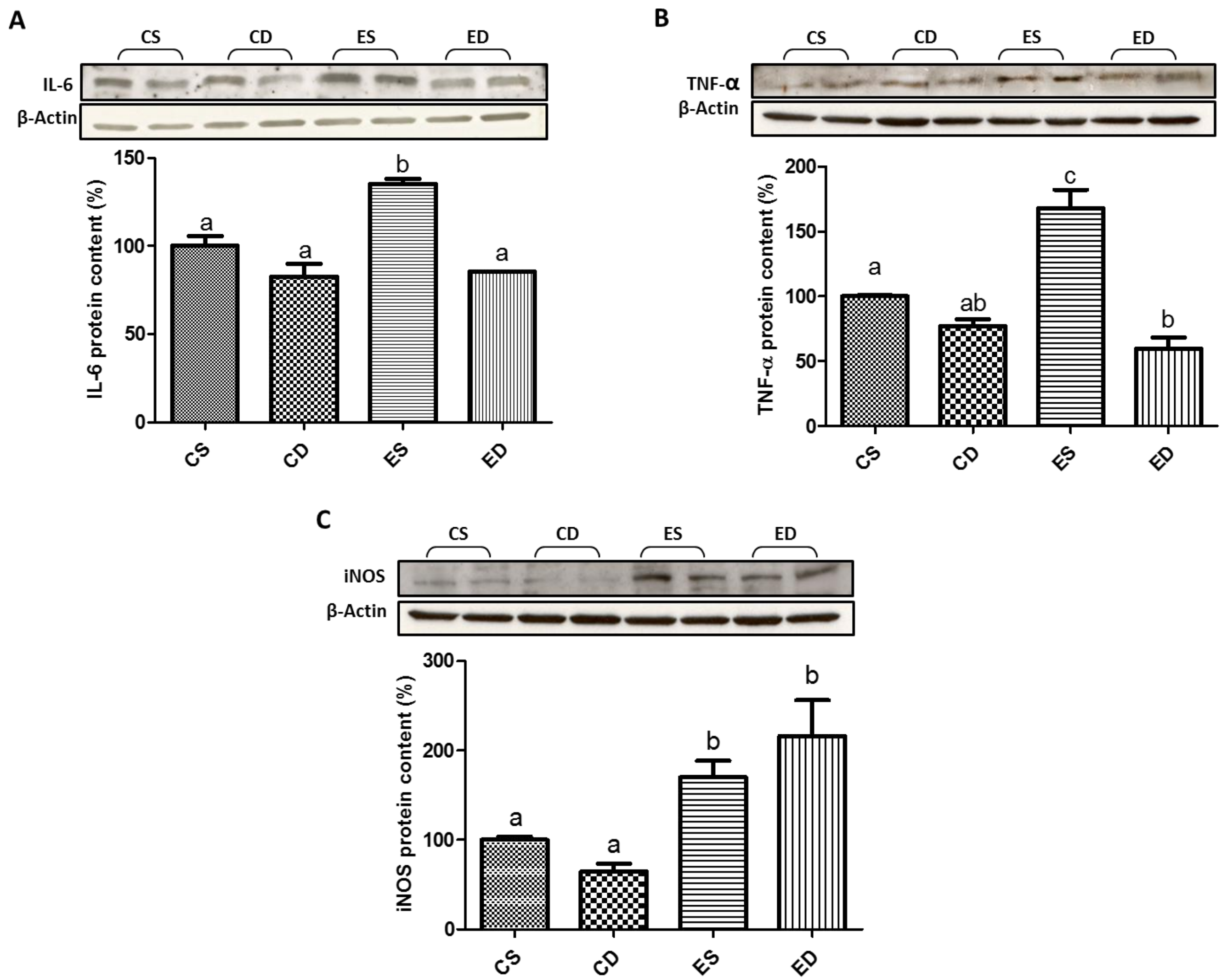


FIGURE 3

7. DISCUSSÃO

Atualmente, a prática regular de exercícios físicos moderados está bem consolidada na sociedade devido ao grande leque de benefícios à saúde (NIEMAN, 2003; GLEESON, 2007), como: melhora do humor (TALL, 2002), ação sobre o sistema cardiovascular e o metabolismo (BOOTH; CHAKRAVARTHY; SPANGENBURG, 2002), redução da pressão sanguínea, e também no combate e prevenção de doenças como diabetes melittus, obesidade, doenças cardiovasculares e pulmonares, problemas musculares e articulares, depressão (PEDERSEN; SALTIN, 2006;BLAIR; JACKSON, 2001), entre outros.

Durante cada sessão de treino, variáveis como carga, duração, pausa entre os estímulos, ação muscular, velocidade de execução do movimento, frequência dos exercícios, número de exercícios por sessão de treino, amplitude dos movimentos e combinação dos exercícios devem ser manipuladas para um melhor aproveitamento do planejamento e para o alcance das metas desejadas (TOIGO; BOUTELLIER, 2006).

Por outro lado, da mesma forma que o exercício físico causa benefícios, o mesmo também pode culminar com a produção de EROs, principalmente por aumentar a taxa de consumo de oxigênio (BOVERIS; NAVARRO, 2008). Um aumento excessivo na produção de EROs pode levar a um desbalanço do sistema antioxidante e assim, causar estresse oxidativo. Este quadro ocorre principalmente em exercícios exaustivos. Sabe-se que o exercício físico extenuante induz diversas alterações metabólicas que podem desregular a função mitocondrial (RASMUSSEN et al., 2001). A formação de EROs mitocondrial, devido à alta taxa de consumo de oxigênio, está entre os principais eventos celulares associados com o exercício intenso e os danos oxidativos (RADÁK et al., 2000; WILLIS; JACKMAN, 1994).

Pouco ainda se sabe sobre a relação entre exercício físico agudo e crônico e as respostas desenvolvidas pelo fígado. Assim, no presente estudo destacamos os efeitos tanto do exercício crônico quanto do agudo referente aos processos oxidativos e inflamatórios, principalmente no tecido hepático. Os dois tipos de exercício causaram mudanças significativas no status antioxidante e inflamatório do respectivo tecido.

A partir dos resultados dos artigos envolvendo cafeína e exercício, constatou-se que o treinamento físico crônico aumentou atividade da CS no músculo. Esse é um marcador importante para comprovar a eficácia do protocolo de exercício físico, bem como um indicador de treinamento aeróbio e de adaptação (GOMEZ-CABRERA; DOMENECH; VIÑA, 2008; JACKSON, 2008; JI; GOMEZ-CABRERA; VINA, 2006; RISTOW; SCHMEISSER, 2011)

Concomitantemente, níveis elevados de CK, AST e TBARS foram encontrados nos animais treinados, sugerindo indícios de dano oxidativo no fígado após o exercício (OZER et

al., 2008). O aumento da lipoperoxidação neste tecido pode ter sido uma consequência da contínua geração de EROs causada pelo exercício. Por outro lado, o treinamento também induziu um aumento na atividade de enzimas antioxidantes no fígado, fato verificado também em outros trabalhos (GOMEZ-CABRERA; DOMENECH; VIÑA, 2008); e que leva a inferir que há uma adaptação redox do tecido frente ao estresse causado pelo exercício (OH-ISHI et al., 1997).

Similarmente, o treinamento induziu respostas celulares condizentes com redução da inflamação sistêmica, como diminuição da atividade da mieloperoxidase (MPO) plasmática. Esta enzima, sintetizada originalmente em granulócitos (BORISH et al., 1989), é um marcador da ativação neutrofílica após o exercício (NIESS et al., 2000). Sua atividade está associada ao desenvolvimento de estresse oxidativo e inflamação tecidual, onde os neutrófilos podem permanecer por até 48h na circulação após o dano (AOI et al., 2004; MAHONEY et al., 2008). Tanto os neutrófilos quanto os macrófagos podem produzir EROs através da ação da MPO (BELCASTRO et al., 1996; REID, 2008) e induzir um dano oxidativo em proteínas, lipídios e DNA (MOROZOV et al., 2006). Sabe-se também que sua atividade aumenta proporcionalmente com a intensidade do exercício físico (NIEMAN et al., 1998; PEAKE et al., 2005). No nosso estudo, detectamos uma menor atividade plasmática desta enzima após o treinamento, fato que pode estar indicando que o exercício crônico exerce uma atividade anti-inflamatória, como já foi comprovado em outros estudos (BELOTTO et al., 2010; LIRA et al., 2009; PETERSEN; PEDERSEN, 2005).

Concomitantemente ao treinamento físico, a suplementação com cafeína, uma substância mundialmente conhecida e muito utilizada no meio esportivo, principalmente por sua característica ergogênica (TARNOPOLSKY, 2010; TUNNICLIFFE et al., 2008), bloqueou os efeitos do exercício. Mostramos primeiramente que o tratamento com cafeína fez com que a atividade das enzimas SOD e GPx dos animais treinados ficasse nos mesmos níveis dos animais sedentários. O tratamento com cafeína também reduziu os níveis de TBARS no fígado dos animais treinados. Estes resultados podem estar relacionados com a atividade antioxidante da cafeína, fato mostrado em outros estudos pelo aumento na formação de glutationa reduzida e diminuindo a formação e EROS (AOYAMA et al., 2011; ZEIDÁN-CHULIÁ et al., 2013). Além disso, vários estudos têm defendido que a suplementação com antioxidantes pode impedir a adaptação esperada do organismo ao exercício físico (GOMEZ-CABRERA; DOMENECH; VIÑA, 2008; JACKSON, 2008). De fato, nossos resultados estão de acordo com essa teoria.

O efeito da cafeína em parâmetros inflamatórios também foi investigado. Observamos que o tratamento com cafeína diminuiu a atividade da MPO e da AChE. Esses achados indicam

um efeito anti-inflamatório da cafeína, o que está de acordo com outros estudos, porém, realizados em tecidos e condições diferentes (CECHELLA et al., 2014; LOU et al., 2013). Uma redução na atividade da MPO poderia reduzir a formação de EROS e de inflamação (PETERSEN; PEDERSEN, 2005). Da mesma forma, como já foi abordado anteriormente, a inibição da AChE, diminui a taxa de degradação da ACh, aumentando assim a presença dessa molécula no meio. Sabe-se que a ACh pode agir como uma substância anti-inflamatória, uma vez que inibe a produção de TNF-α, IL-1β em macrófagos (BOROVIKOVA et al., 2000; DAS, 2012).

A maioria dos mecanismos antioxidantes e anti-inflamatórios em protocolos de exercício tem sido discutidos sob o contexto de adaptações crônicas do exercício regular. No entanto, existem evidências que o exercício em seções isoladas também gera de forma transitória, mas benéfico, diversos tipos de mudanças teciduais (THOMPSON et al., 2001). Há adaptações metabólicas derivadas de uma sessão única de exercício que geram alterações celulares ao nível genético, levando a efeitos cumulativos do treinamento. Já foi demonstrado que o exercício agudo causa passageiras reduções nos níveis de triglicerídeos, aumento dos níveis de HDL, diminuição da pressão sanguínea, redução na resistência insulínica e melhoras no controle da glicemia (THOMPSON et al., 2001). Estes efeitos fortalecem o importante papel das seções individuais de exercício no status de saúde. Assim, sessão única de exercício também tem impacto relevante para a saúde.

Já está bem documentado que o exercício agudo, particularmente o exercício excêntrico, induz alterações em muitos componentes do sistema imune (AKIMOTO et al., 2002), incluindo elevados níveis de IL-1β, IL-6 e TNF-α (OSTROWSKI et al., 1999; VASSILAKOPOULOS et al., 2003). Estas citocinas são conhecidas por estarem envolvidas na regulação da resposta imune e inflamação e são consideradas críticas para o sistema de defesa do organismo (MEKSAWAN et al., 2004). Neste contexto, trabalhos mostram que o exercício físico intenso tem um papel importante na indução da ativação do NFκB pelo TLR4 através das vias dependentes e independentes de MyD88 nas células mononucleares do sangue periférico (PBMC) de homens (FERNANDEZ-GONZALO et al., 2012) e idosos (JIMÉNEZ-JIMÉNEZ et al., 2008). Corroborando com esses dados, no nosso estudo envolvendo exercício agudo excêntrico (manuscrito 1) vimos um aumento da rota inflamatória através do receptor TLR4, passando por um aumento na expressão gênica e síntese proteica desde o próprio TLR4, MyD88, TRIF, translocação de NFκB ao núcleo, culminando no aumento da síntese de proteínas e enzimas pró-inflamatórias (IL-6, TNF-α e iNOS) em fígado. Apesar de muitos estudos terem demonstrado a ativação dessa rota em células sanguíneas e músculo, pouco se

sabe sobre essa rota no fígado. Pode-se relacionar esses dados ao fato de que apesar de o exercício excêntrico gerar uma resposta pró-inflamatória local, há também uma resposta sistêmica firmemente atribuída às citocinas anti-inflamatórias e caracterizada pela alteração nos receptores de leucócitos circulantes e sua atividade funcional (PEAKE; NOSAKA; SUZUKI, 2005), levando a inflamação e alterações moleculares no fígado. Constatamos que o pré-tratamento com o AINE diclofenaco bloqueou quase a totalidade dos efeitos pró-inflamatórios induzidos pelo exercício excêntrico. De fato, o pré-tratamento com diclofenaco diminui a expressão de TLR4, MyD88, TRIF, NFκB, IL-6 e TNF-α aumentada pelo exercício físico, ou seja, diminuindo a inflamação via rota do TLR4.

No nosso estudo, observamos que o exercício agudo induz uma resposta inflamatória culminando com o aumento da formação de citocinas, como por exemplo a IL-6. Esta interleucina exerce vários efeitos metabólicos e compartilha mecanismos na adaptação ao treinamento e a melhora imunológica (STARKIE et al., 2003). Assim, a diminuição da expressão dessa proteína no fígado poderia estar afetando a lipólise (STARKIE et al., 2003), reduzindo os efeitos anti-inflamatórios do exercício e atenuando a expressão de inúmeros genes metabólicos ativados pelo exercício (PILEGAARD et al., 2002).

A inflamação, uma resposta imune inata, é fundamentalmente uma resposta protetora que tem o objetivo de eliminar o agente indutor do dano (que pode ser um microrganismo, estímulo físico, agente químico, etc.), bem como, crítica para a regeneração de novas fibras musculares após o dano (TIDBALL; VILLALTA, 2010). A ausência de uma inflamação adequada em uma célula/tecido lesado poderá fazer com que o dano às células/tecidos não seja cessado. Por isso, a inflamação pode ser tanto benéfica quanto potencialmente danosa (DAS, 2007). A nível molecular, a ativação do fator de transcrição NFκB nas células danificadas induz uma resposta inflamatória, marcada pelo aumento de IL-1β, TNF-α IL-6, iNOS e COX-2 (JI, 2008).

Há vários exemplos da importância da inflamação exercendo um papel significativo na modelação e adaptação do músculo esquelético em resposta ao exercício físico, bem como a interferência de tratamentos com anti-inflamatórios. Por exemplo, o tratamento local com anti-inflamatórios atrasa a regeneração muscular após a sessão de exercício em animais jovens (MONDA et al., 2009), bloqueia a proliferação de células-tronco em humanos após um exercício excêntrico (MIKKELSEN et al., 2009), diminui a hipertrofia em ratos (NOVAK et al., 2009; SOLTOW et al., 2006) e retarda a recuperação da força muscular (MISHRA et al., 1995). Estes dados sugerem que a modulação inflamatória tem um papel crucial na adaptação muscular ao exercício físico e que o tratamento com anti-inflamatórios interfere de maneira

negativa no processo de adaptação e de regeneração tecidual. Neste contexto, nossos resultados utilizando o AINE diclofenaco corroboram essa influência negativa do tratamento com anti-inflamatórios concomitante à atividade física, destacando os efeitos no fígado.

8. CONCLUSÃO

A partir dos resultados obtidos nesta tese foi possível verificar que as substâncias cafeína e diclofenaco interferem na modulação das respostas antioxidantes e inflamatórias induzidas pelo exercício físico.

Constatou-se que a cafeína, conhecida por ser um psico-estimulante do SNC e usada comumente no meio esportivo como uma substância ergogênica para aumentar a performance atlética, modulou as respostas de dano e estresse oxidativo hepático. Verificou-se também a alteração de respostas inflamatórias induzidas pelo treinamento físico em plasma e fígado, enquanto que nenhuma alteração foi detectada nos parâmetros mitocondriais hepáticos.

Da mesma forma, o uso de diclofenaco antes de uma sessão de exercício excêntrico exaustivo diminui a inflamação hepática gerada por este tipo de exercício físico. Em termos de mecanismos, identificamos a participação de fatores inflamatórios ativados pela rota TLR4-NFκB-interleucinas.

Baseado no conjunto de resultados obtidos, pode-se afirmar que a suplementação com antioxidantes, como cafeína, e anti-inflamatórios, como o diclofenaco, concomitantemente ao exercício físico podem limitar a modulação natural do organismo ao estresse de cunho oxidativo/inflamatório gerado por diferentes tipos de atividades físicas.

O presente estudo torna-se relevante considerando a quantidade e frequência do consumo de cafeína e diclofenaco mundialmente. Nossos resultados apontam para que treinadores, atletas ou qualquer indivíduo que deseja melhorar sua performance esportiva, devem avaliar os riscos e benefícios antes do uso de qualquer tipo de substância, mesmo que permitidas por organizações esportivas e de uso comum, pois estas podem resultar em efeitos não desejados a curto ou longo prazo.

REFERÊNCIAS

AKIMOTO, T. et al. Increased plasma concentrations of intercellular adhesion molecule-1 after strenuous exercise associated with muscle damage. **Eur J Appl Physiol**, v. 86, n. 3, p. 185–90, 2002.

AKIRA, S.; SATO, S. Toll-like receptors and their signaling mechanisms. **Scand J Infect Dis**, v. 35, p. 555–562, 2003.

AKIRA, S.; UEMATSU, S.; TAKEUCHI, O. Pathogen recognition and innate immunity. **Cell**, v. 124, n. 4, p. 783–801, 2006.

ALARANTA, A. et al. Ample use of physician-prescribed medications in Finnish elite athletes. **Int J Sports Med**, v. 27, p. 919–925, 2006.

ALARANTA, A.; ALARANTA, H.; HELENIUS, I. Use of prescription drugs in athletes. **Sports medicine (Auckland, N.Z.)**, v. 38, n. 6, p. 449–463, 2008.

ALEXOPOULOU, L. et al. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. **Nature**, v. 413, n. 6857, p. 732–8, 2001.

AOI, W. et al. Oxidative stress and delayed-onset muscle damage after exercise. **Free Radical Biology and Medicine**, v. 37, n. 4, p. 480–487, 2004.

AOYAMA, K. et al. Caffeine and uric acid mediate glutathione synthesis for neuroprotection. **Neuroscience**, v. 181, p. 206–215, 2011.

ARCIERO, P. J. et al. **Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men.The American journal of physiology**. [s.l: s.n.].

ASTORINO, T. A.; ROBERSON, D. W. Efficacy of acute caffeine ingestion for short-term high-intensity exercise performance: a systematic review. **Journal of strength and conditioning research / National Strength & Conditioning Association**, v. 24, n. 1, p. 257–265, 2010.

BACKHOUSE, S. H. et al. Caffeine ingestion, affect and perceived exertion during prolonged cycling. **Appetite**, v. 57, n. 1, p. 247–252, 2011.

BAK, S. et al. Risk of stroke associated with nonsteroidal anti-inflammatory drugs: a nested case-control study. **Stroke; a journal of cerebral circulation**, v. 34, n. 2, p. 379–386, 2003.

BANCHEREAU, J.; STEINMAN, R. M. Dendritic cells and the control of immunity. **Nature**, v. 392, n. 6673, p. 245–252, 1998.

BELCASTRO, A. N. et al. Heart, liver, and skeletal muscle myeloperoxidase activity during exercise. **J Appl Physiol**, v. 80, p. 1331–1335, 1996.

BELOTTO, M. F. et al. Moderate exercise improves leucocyte function and decreases inflammation in diabetes. **Clin Exp Immunol**, v. 162, p. 237–243, 2010.

BENATTI, F. B.; PEDERSEN, B. K. Exercise as an anti-inflammatory therapy for rheumatic diseases-myokine regulation. **Nature reviews. Rheumatology**, v. 11, n. 2, p. 86–97, Feb. 2015.

BLAIR, S. N. et al. Physical fitness and all-cause mortality. A prospective study of healthy men and women. **JAMA : the journal of the American Medical Association**, v. 262, n. 17, p. 2395–2401, 1989.

BLAISE, G. A.; STEWART, D. J.; GUÉRARD, M. J. Acetylcholine stimulates release of endothelium-derived relaxing factor in coronary arteries of human organ donors. **The Canadian journal of cardiology**, v. 9, n. 9, p. 813–820, 1993.

BLOOMER, R. J. Effect of exercise on oxidative stress biomarkers. **Advances in clinical chemistry**, v. 46, p. 1–50, 2008.

BONDESEN, B. A. et al. The COX-2 pathway is essential during early stages of skeletal muscle regeneration. **American journal of physiology. Cell physiology**, v. 287, n. 2, p. C475–C483, 2004.

BORISH, L. et al. Activation of neutrophils by recombinant interleukin 6. **Cellular immunology**, v. 121, n. 2, p. 280–289, 1989.

BOROVIKOVA, L. V et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. **Nature**, v. 405, p. 458–462, 2000.

BORRÁS, C.; GÓMEZ-CABRERA, M. C.; VIÑA, J. The dual role of p53: DNA protection and antioxidant. **Free radical research**, v. 45, n. 6, p. 643–652, 2011.

BOTEZELLI, J. D. et al. Different exercise protocols improve metabolic syndrome markers, tissue triglycerides content and antioxidant status in rats. **Diabetol Metab Syndr**, v. 3, p. 35, 2011.

BOTTING, R. M. Mechanism of action of acetaminophen: is there a cyclooxygenase 3? **Clinical infectious diseases : an official publication of the Infectious Diseases Society of America**, v. 31 Suppl 5, p. S202–S210, 2000.

BOVERIS, A.; CHANCE, B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. **The Biochemical journal**, v. 134, n. 3, p. 707–716, 1973.

BOVERIS, A.; NAVARRO, A. Systemic and mitochondrial adaptive responses to moderate exercise in rodents. **Free Radical Biology and Medicine**, v. 44, n. 2, p. 224–229, 2008.

BRESALIER, R. S. et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. **The New England journal of medicine**, v. 352, n. 11, p. 1092–102, 17 Mar. 2005.

BURD, N. A. et al. Effect of a cyclooxygenase-2 inhibitor on postexercise muscle protein synthesis in humans. **American journal of physiology. Endocrinology and metabolism**, v. 298, n. 2, p. E354–61, Feb. 2010.

BURIAN, M.; GEISSLINGER, G. **COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sitesPharmacology and Therapeutics**, 2005.

BYRKJELAND, R. et al. Inflammatory markers as related to disease severity in patients with chronic heart failure: limited effects of exercise training. **Scandinavian Journal of Clinical and Laboratory Investigation**, v. 71, p. 598–605, 2011.

ÇAKIR, B. et al. Stress-induced multiple organ damage in rats is ameliorated by the antioxidant and anxiolytic effects of regular exercise. **Cell Biochemistry and Function**, v. 28, p. 469–479, 2010.

CARMICHAEL, M. D. et al. Role of brain macrophages on IL-1beta and fatigue following eccentric exercise-induced muscle damage. **Brain Behav Immun**, v. 24, p. 564–568, 2010.

CECHELLA, J. L. et al. Moderate swimming exercise and caffeine supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats. **Amino Acids**, 2014.

CHANDRASEKHARAN, N. V et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. **Proceedings of the National Academy of Sciences of the United States of America**, v. 99, n. 21, p. 13926–13931, 2002.

CIOCCA, M. Medication and supplement use by athletes. **Clin Sports Med**, v. 24, p. 719–38, x–xi, 2005.

CIPOLLONE, F. et al. Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. **Circulation**, v. 104, n. 8, p. 921–7, 21 Aug. 2001.

COLBERG, S. R. et al. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. **Diabetes care**, v. 33, n. 12, p. e147–67, Dec. 2010.

COLLIER, S. R. et al. Effect of 4 weeks of aerobic or resistance exercise training on arterial stiffness, blood flow and blood pressure in pre- and stage-1 hypertensives. **Journal of human hypertension**, v. 22, p. 678–686, 2008.

CONNOLLY, D. J.; O'NEILL, L. A. New developments in Toll-like receptor targeted therapeutics. **Curr Opin Pharmacol**, v. 12, p. 510–518, 2012.

CORRIGAN, B.; KAZLAUSKAS, R. Medication use in athletes selected for doping control at the Sydney Olympics (2000). **Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine**, v. 13, n. 1, p. 33–40, 2003.

COYLE, E. F. Fluid and fuel intake during exercise. **Journal of sports sciences**, v. 22, n. 1, p. 39–55, 2004.

CRISTI-MONTERO, C. et al. [Effect of an acute exercise bout on Toll-like receptor 4 and inflammatory mechanisms in rat heart]. **Rev Med Chil**, v. 140, p. 1282–1288, 2012.

CRISTOFARO, P.; OPAL, S. M. Role of Toll-like receptors in infection and immunity: clinical implications. **Drugs**, v. 66, p. 15–29, 2006.

CURRIE, K. D.; THOMAS, S. G.; GOODMAN, J. M. Effects of short-term endurance exercise training on vascular function in young males. **Eur J Appl Physiol**, v. 107, p. 211–218, 2009.

CURTIS, J. P. et al. Aspirin, ibuprofen, and mortality after myocardial infarction: retrospective cohort study. **BMJ (Clinical research ed.)**, v. 327, n. 7427, p. 1322–3, 6 Dec. 2003.

CZEPLUCH, F. S. et al. Strenuous physical exercise adversely affects monocyte chemotaxis. **Thrombosis and Haemostasis**, v. 105, p. 122–130, 2011.

CZURA, C. J.; FRIEDMAN, S. G.; TRACEY, K. J. Neural inhibition of inflammation: the cholinergic anti-inflammatory pathway. **Journal of endotoxin research**, v. 9, n. 6, p. 409–413, 2003.

DA SILVA, E. R. et al. Non-steroidal anti-inflammatory use in the XV Pan-American Games (2007). **Br J Sports Med**, v. 45, p. 91–94, 2011.

DAS, U. N. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. **Med Sci Monit**, v. 13, p. RA214–21, 2007.

DAS, U. N. Acetylcholinesterase and butyrylcholinesterase as markers of low-grade systemic inflammation. **Annals of Hepatology**, v. 11, n. 3, p. 409–411, 2012.

DAVIES, K. J. et al. Free radicals and tissue damage produced by exercise. **Biochem Biophys Res Commun**, v. 107, p. 1198–1205, 1982.

DAVIS, D. P. et al. Exercise-associated hyponatremia in marathon runners: A two-year experience. **Journal of Emergency Medicine**, v. 21, n. 1, p. 47–57, 2001.

DAVIS, J. K.; GREEN, J. M. **Caffeine and anaerobic performance: Ergogenic value and mechanisms of actionSports Medicine**, 2009.

DAVIS, J. M. et al. Central nervous system effects of caffeine and adenosine on fatigue. **Am J Physiol Regul Integr Comp Physiol**, v. 284, p. R399–404, 2003.

DELBEKE, F. T.; DEBACKERE, M. Caffeine: use and abuse in sports. **International journal of sports medicine**, v. 5, n. 4, p. 179–182, 1984.

DELLA GATTA, P. A. et al. Effect of exercise training on skeletal muscle cytokine expression in the elderly. **Brain, Behavior, and Immunity**, v. 39, p. 80–86, 2014.

DENG, B. et al. IL-10 triggers changes in macrophage phenotype that promote muscle growth and regeneration. **Journal of immunology (Baltimore, Md. : 1950)**, v. 189, p. 3669–80, 2012.

DEVAN, A. E. et al. Regular aerobic exercise protects against impaired fasting plasma glucose-associated vascular endothelial dysfunction with aging. **Clinical science (London, England : 1979)**, v. 124, p. 325–331, 2013.

DISHMAN, R. K. et al. Neurobiology of exercise. **Obesity (Silver Spring, Md.)**, v. 14, n. 3, p. 345–356, 2006.

DOHERTY, M.; SMITH, P. M. Effects of caffeine ingestion on exercise testing: A meta-analysis. **International Journal of Sport Nutrition and Exercise Metabolism**, v. 14, n. 6, p. 626–646, 2004.

DREXEL, H. et al. Metabolic and anti-inflammatory benefits of eccentric endurance exercise - A pilot study. **European Journal of Clinical Investigation**, v. 38, p. 218–226, 2008.

DUBOIS, R. N. et al. Cyclooxygenase in biology and disease. **The FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, v. 12, n. 12, p. 1063–1073, 1998.

DURRANT, J. R. et al. Voluntary wheel running restores endothelial function in conduit arteries of old mice: direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase. **The Journal of physiology**, v. 587, n. Pt 13, p. 3271–85, 1 Jul. 2009.

ELNACHEF, N. et al. Changing perceptions and practices regarding aspirin, nonsteroidal anti-inflammatory drugs, and cyclooxygenase-2 selective nonsteroidal anti-inflammatory drugs among US primary care providers. **Alimentary pharmacology & therapeutics**, v. 28, n. 10, p. 1249–1258, 2008.

FERNANDEZ-GONZALO, R. et al. Effects of eccentric exercise on toll-like receptor 4 signaling pathway in peripheral blood mononuclear cells. **J Appl Physiol (1985)**, v. 112, p. 2011–2018, 2012.

FINAUD, J.; LAC, G.; FILAIRE, E. Oxidative stress : relationship with exercise and training. **Sports medicine (Auckland, N.Z.)**, v. 36, n. 4, p. 327–58, Jan. 2006.

FISCHER, L. M. et al. Discontinuation of nonsteroidal anti-inflammatory drug therapy and risk of acute myocardial infarction. **Arch Intern Med**, v. 164, n. 22, p. 2472–2476, 2004.

FISHER-WELLMAN, K.; BLOOMER, R. J. Acute exercise and oxidative stress: a 30 year history. **Dynamic medicine : DM**, v. 8, p. 1, 2009.

FLYNN, M. G. et al. Toll-like receptor 4 and CD14 mRNA expression are lower in resistive exercise-trained elderly women. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 95, p. 1833–1842, 2003.

FLYNN, M. G.; MCFARLIN, B. K. Toll-like receptor 4: link to the anti-inflammatory effects of exercise? **Exerc Sport Sci Rev**, v. 34, p. 176–181, 2006.

FOX, S. M.; HASKELL, W. L. Physical activity and the prevention of coronary heart disese. **Bulletin of the New York Academy of Medicine**, v. 44, n. 8, p. 950–9, 1968.

FREDHOLM, B. B. et al. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. **Pharmacol Rev**, v. 51, p. 83–133, 1999.

GAN, T. J. Diclofenac: an update on its mechanism of action and safety profile. **Current medical research and opinion**, v. 26, n. 7, p. 1715–1731, 2010.

GARCIA RODRIGUEZ, L. A. et al. Nonsteroidal antiinflammatory drugs and the risk of myocardial infarction in the general population. **Circulation**, v. 109, n. 24, p. 3000–3006, 2004.

GARCÍA-LÓPEZ, D. et al. Effects of eccentric exercise on NF-kappaB activation in blood mononuclear cells. **Med Sci Sports Exerc**, v. 39, p. 653–664, 2007.

GIBALA, M. J. et al. Physiological adaptations to low-volume, high-intensity interval training in health and disease. **The Journal of physiology**, v. 590, n. 5, p. 1077–1084, 2012.

GLEESON, M. Immune function in sport and exercise. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 103, n. 2, p. 693–699, 2007.

GLEESON, M. et al. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. **Nature reviews. Immunology**, v. 11, p. 607–615, 2011.

GLEESON, M.; MCFARLIN, B.; FLYNN, M. **Exercise and toll-like receptorsExercise Immunology Review**, 2006.

GOLDSTEIN, E. R. et al. International society of sports nutrition position stand: caffeine and performance. **Journal of the International Society of Sports Nutrition**, v. 7, n. 1, p. 5, Jan. 2010.

GOMES, A. C. **Treinamento desportivo, estruturação e periodização**. 1. ed. Porto Alegre: Artmed, 2003.

GOMES, E. C.; SILVA, A. N.; OLIVEIRA, M. R. DE. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. **Oxidative Medicine and Cellular Longevity**, v. 2012, 2012.

GOMEZ-CABRERA, M. C. et al. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. **American Journal of Clinical Nutrition**, v. 87, n. 1, p. 142–149, 2008.

GOMEZ-CABRERA, M. C.; DOMENECH, E.; VIÑA, J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. **Free Radic Biol Med**, v. 44, p. 126–131, 2008.

GOMEZ-CABRERA, M. C.; VIÑA, J.; JI, L. L. Interplay of oxidants and antioxidants during exercise: implications for muscle health. **Phys Sportsmed**, v. 37, p. 116–123, 2009.

GOMEZ-CABRERA, M.-C. et al. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. **The Journal of physiology**, v. 567, p. 113–120, 2005.

GRAHAM, D. J. et al. Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective non-steroidal anti-inflammatory drugs: nested case-control study. **Lancet**, v. 365, n. 9458, p. 475–481, 2005.

GRAHAM, T. E. Caffeine and exercise: metabolism, endurance and performance. **Sports Med**, v. 31, p. 785–807, 2001.

GRAHAM, T. E. et al. Does caffeine alter muscle carbohydrate and fat metabolism during exercise? **Applied physiology, nutrition, and metabolism = Physiologie appliquée, nutrition et métabolisme**, v. 33, n. 6, p. 1311–8, Dec. 2008.

GRAHAM, T. E.; SPRIET, L. L. Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. **J Appl Physiol**, v. 78, p. 867–874, 1995.

GUL, M. et al. Effects of endurance training on tissue glutathione homeostasis and lipid peroxidation in streptozotocin-induced diabetic rats. **Scand J Med Sci Sports**, v. 12, p. 163–170, 2002.

HEREDIA, J. E. et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. **Cell**, v. 153, n. 2, p. 376–88, 11 Apr. 2013.

HIPPISLEY-COX, J.; COUPLAND, C. Risk of myocardial infarction in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis. **BMJ (Clinical research ed.)**, v. 330, n. 7504, p. 1366, 2005.

HOENE, M.; WEIGERT, C. The stress response of the liver to physical exercise. **Exerc Immunol Rev**, v. 16, p. 163–183, 2010.

HOFFMANN, A.; BALTIMORE, D. Circuitry of nuclear factor kappaB signaling. **Immunol Rev**, v. 210, p. 171–186, 2006.

HOLLANDER, J. et al. Superoxide dismutase gene expression in skeletal muscle: fiber-specific adaptation to endurance training. **Am J Physiol**, v. 277, p. R856–62, 1999.

HOUGLUM, J. E. Pharmacologic considerations in the treatment of injured athletes with nonsteroidal anti-inflammatory drugs. **Journal of athletic training**, v. 33, n. 3, p. 259–63, Jul. 1998.

HU, J.-P. et al. Exercise improves cognitive function in aging patients. **International journal of clinical and experimental medicine**, v. 7, n. 10, p. 3144–9, Jan. 2014.

HUANG, C. C.; TSAI, S. C.; LIN, W. T. Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats. **Exp Gerontol**, v. 43, p. 571–577, 2008.

HUANG, L. E. et al. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its α subunit. **Journal of Biological Chemistry**, v. 271, n. 50, p. 32253–32259, 1996.

HUANG, S. H.; JOHNSON, K.; PIPE, A. L. The use of dietary supplements and medications by Canadian athletes at the Atlanta and Sydney Olympic Games. **Clin J Sport Med**, v. 16, p. 27–33, 2006.

IGUCHI, G. et al. A reciprocal relationship exists between non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1) and cyclooxygenase-2. **Cancer Letters**, v. 282, n. 2, p. 152–158, 2009.

INOUE, K. et al. Long-term Mild Exercise Training Enhances Hippocampus-dependent Memory in Rats. **International journal of sports medicine**, 27 Nov. 2014.

INTO, T. et al. **Autophagy in regulation of Toll-like receptor signalingCellular Signalling**, 2012.

ISCHIROPOULOS, H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. **Archives of biochemistry and biophysics**, v. 356, n. 1, p. 1–11, 1998.

JACKSON, M. J. Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? **Free Radic Biol Med**, v. 44, p. 132–141, 2008.

JEŽEK, P.; HLAVATÁ, L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. **International Journal of Biochemistry and Cell Biology**, v. 37, n. 12, p. 2478–2503, 2005.

JI, L. L. Antioxidant enzyme response to exercise and aging. **Med Sci Sports Exerc**, v. 25, p. 225–231, 1993.

JI, L. L. et al. Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. **FASEB J**, v. 18, p. 1499–1506, 2004.

JI, L. L. Modulation of skeletal muscle antioxidant defense by exercise: Role of redox signaling. **Free Radical Biology and Medicine**, v. 44, n. 2, p. 142–152, 2008.

JI, L. L.; GOMEZ-CABRERA, M. C.; VINA, J. Exercise and hormesis: activation of cellular antioxidant signaling pathway. **Ann N Y Acad Sci**, v. 1067, p. 425–435, 2006.

JIMÉNEZ-JIMÉNEZ, R. et al. Eccentric training impairs NF-kappaB activation and over-expression of inflammation-related genes induced by acute eccentric exercise in the elderly. **Mech Ageing Dev**, v. 129, p. 313–321, 2008.

JOHNSEN, S. P. et al. Nonaspirin nonsteroidal anti-inflammatory drugs and risk of hospitalization for intracerebral hemorrhage: a population-based case-control study. **Stroke (00392499)**, v. 34, n. 2, p. 387–391, 2003.

JOHNSEN, S. P. et al. Risk of hospitalization for myocardial infarction among users of rofecoxib, celecoxib, and other NSAIDs: a population-based case-control study. **Archives of internal medicine**, v. 165, n. 9, p. 978–984, 2005.

JOSEFSSON, T.; LINDWALL, M.; ARCHER, T. Physical exercise intervention in depressive disorders: meta-analysis and systematic review. **Scandinavian journal of medicine & science in sports**, v. 24, n. 2, p. 259–72, 2014.

JUHN, M. S. Ergogenic aids in aerobic activity. **Current sports medicine reports**, v. 1, n. 4, p. 233–238, 2002.

JUHN, M. S. **Popular Sports Supplements and Ergogenic AidsSports Medicine**, 2003.

KAKARLA, P.; VADLURI, G.; REDDY KESIREDDY, S. Response of hepatic antioxidant system to exercise training in aging female rat. **J Exp Zool A Comp Exp Biol**, v. 303, p. 203–208, 2005.

KALMAR, J. M.; CAFARELLI, E. Caffeine: a valuable tool to study central fatigue in humans? **Exerc Sport Sci Rev**, v. 32, p. 143–147, 2004.

KALOW, W.; TANG, B. K. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. **Clinical pharmacology and therapeutics**, v. 50, n. 5 Pt 1, p. 508–519, 1991.

KAROLKIEWICZ, J. et al. Response of oxidative stress markers and antioxidant parameters to an 8-week aerobic physical activity program in healthy, postmenopausal women. **Archives of gerontology and geriatrics**, v. 49, n. 1, p. e67–71, Jan. 2009.

KASAPIS, C.; THOMPSON, P. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. **… of the American College of …**, v. 45, n. 10, p. 1563–9, 2005.

KAWAI, T.; AKIRA, S. TLR signaling. **Cell Death Differ**, v. 13, p. 816–825, 2006.

KAWANISHI, N. et al. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. **Exercise Immunology Review**, v. 16, p. 105–118, 2010.

KAWASHIMA, K.; FUJII, T. **The lymphocytic cholinergic system and its contribution to the regulation of immune activityLife Sciences**, 2003.

KELLER, C. et al. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 99, n. 6, p. 2075–2079, 2005.

KENNY, J. R. et al. Syntheses and characterization of the acyl glucuronide and hydroxy metabolites of diclofenac. **Journal of Medicinal Chemistry**, v. 47, n. 11, p. 2816–2825, 2004.

KILMARTIN, B.; REEN, D. J. HSP60 induces self-tolerance to repeated HSP60 stimulation and cross-tolerance to other pro-inflammatory stimuli. **European Journal of Immunology**, v. 34, n. 7, p. 2041–2051, 2004.

KIMMEL, S. E. et al. The effects of nonselective non-aspirin non-steroidal anti-inflammatory medications on the risk of nonfatal myocardialinfarction and their interaction with aspirin. **Journal of the American College of Cardiology**, v. 43, n. 6, p. 985–990, 2004.

KO, D. et al. Nonsteroidal antiinflammatory drugs after acute myocardial infarction. **American Heart Journal**, v. 143, n. 3, p. 475–481, 2002.

KORIVI, M. et al. Ginsenoside-Rg1 Protects the Liver against Exhaustive Exercise-Induced Oxidative Stress in Rats. **Evid Based Complement Alternat Med**, v. 2012, p. 932165, 2012.

KU, E. C. et al. The effects of diclofenac sodium on arachidonic acid metabolism. **Seminars in arthritis and rheumatism**, v. 15, n. 2 Suppl 1, p. 36–41, 1985.

KU, E. C. et al. Effect of diclofenac sodium on the arachidonic acid cascade. **Am J Med**, v. 80, p. 18–23, 1986.

KURTH, T. et al. Inhibition of clinical benefits of aspirin on first myocardial infarction by nonsteroidal antiinflammatory drugs. **Circulation**, v. 108, n. 10, p. 1191–1195, 2003.

LAMBERT, C. P. et al. Exercise but not diet-induced weight loss decreases skeletal muscle inflammatory gene expression in frail obese elderly persons. **J Appl Physiol (1985)**, v. 105, p. 473–478, 2008.

LAMINA, S.; OKOYE, C. G.; DAGOGO, T. T. Therapeutic effect of an interval exercise training program in the management of erectile dysfunction in hypertensive patients. **Journal of clinical hypertension (Greenwich, Conn.)**, v. 11, p. 125–129, 2009.

LANCASTER, G. I. et al. The physiological regulation of toll-like receptor expression and function in humans. **J Physiol**, v. 563, p. 945–955, 2005.

LANG, C. H. et al. Endotoxin stimulates in vivo expression of inflammatory cytokines tumor necrosis factor alpha, interleukin-1beta, -6, and high-mobility-group protein-1 in skeletal muscle. **Shock (Augusta, Ga.)**, v. 19, n. 6, p. 538–546, 2003.

LAURIN, D. et al. Physical activity and risk of cognitive impairment and dementia in elderly persons. **Archives of neurology**, v. 58, n. 3, p. 498–504, 2001.

LAZAREVIC, G. et al. A physical activity programme and its effects on insulin resistance and oxidative defense in obese male patients with type 2 diabetes mellitus. **Diabetes & metabolism**, v. 32, p. 583–590, 2006.

LEE, I. M.; SKERRETT, P. J. Physical activity and all-cause mortality: what is the dose-response relation? **Medicine and science in sports and exercise**, v. 33, n. 6 Suppl, p. S459–S471; discussion S493–S494, 2001.

LEE, W. M. Acute liver failure. **The New England journal of medicine**, v. 329, n. 25, p. 1862–72, 16 Dec. 1993.

LEES, P. et al. Pharmacodynamics and pharmacokinetics of miloxicam in the horse. **The British veterinary journal**, v. 147, n. 2, p. 97–108, Jan. 1991.

LEEUWENBURGH, C.; JI, L. L. Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. **Arch Biochem Biophys**, v. 316, p. 941–949, 1995.

LEONARD, B. E. **Inflammation, depression and dementia: Are they connected?Neurochemical Research**, 2007.

LEW, H.; PYKE, S.; QUINTANILHA, A. Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rats. **FEBS Lett**, v. 185, p. 262–266, 1985.

LIAO, P. et al. Eccentric contraction induces inflammatory responses in rat skeletal muscle: role of tumor necrosis factor-alpha. **Am J Physiol Regul Integr Comp Physiol**, v. 298, p. R599–607, 2010.

LIMA, F. D. et al. Adaptation to oxidative challenge induced by chronic physical exercise prevents Na+,K+-ATPase activity inhibition after traumatic brain injury. **Brain Research**, v. 1279, p. 147–155, 7 Jul. 2009.

LIMA, F. D. et al. Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. **PLoS One**, v. 8, p. e55668, 2013.

LIN, Y. et al. The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. **The Journal of biological chemistry**, v. 275, n. 32, p. 24255–63, 11 Aug. 2000.

LIPPI, G. et al. Non-steroidal anti-inflammatory drugs in athletes. **Br J Sports Med**, v. 40, p. 661–663, 2006.

LIRA, F. S. et al. Chronic exercise decreases cytokine production in healthy rat skeletal muscle. **Cell Biochem Funct**, v. 27, p. 458–461, 2009.

LIU, J. et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. **J Appl Physiol (1985)**, v. 89, p. 21–28, 2000.

LOU, Y. et al. Oral caffeine during voluntary exercise markedly inhibits skin carcinogenesis and decreases inflammatory cytokines in UVB-treated mice. **Nutr Cancer**, v. 65, p. 1002–1013, 2013.

LUTTRELL, M. J. et al. Effect of age and exercise training on protein:protein interactions among eNOS and its regulatory proteins in rat aortas. **European journal of applied physiology**, v. 113, n. 11, p. 2761–8, Nov. 2013.

LYONS, A. S.; PETRUCELLI, R. J. **Medicine: An Illustrated History by Albert S. Lyons, R. Joseph Petrucelli: Harry N.** New York: [s.n.].

MA, Y.; HE, M.; QIANG, L. Exercise therapy downregulates the overexpression of TLR4, TLR2, MyD88 and NFkB after cerebral ischemia in rats. **International Journal of Molecular Sciences**, v. 14, n. 2, p. 3718–3733, 2013.

MACERA, C. A.; HOOTMAN, J. M.; SNIEZEK, J. E. Major public health benefits of physical activity. **Arthritis and rheumatism**, v. 49, n. 1, p. 122–8, 15 Feb. 2003.

MAHONEY, D. J. et al. Gene expression profiling in human skeletal muscle during recovery from eccentric exercise. **American journal of physiology. Regulatory, integrative and comparative physiology**, v. 294, n. 6, p. R1901–R1910, 2008.

MÃRTENSSON, J.; MEISTER, A. Glutathione deficiency decreases tissue ascorbate levels in newborn rats: ascorbate spares glutathione and protects. **Proceedings of the National Academy of Sciences of the United States of America**, v. 88, p. 4656–4660, 1991.

MASTORAKOS, G.; PAVLATOU, M. Exercise as a stress model and the interplay between the hypothalamus-pituitary-adrenal and the hypothalamus-pituitary-thyroid axes. **Horm Metab Res**, v. 37, p. 577–584, 2005.

MATHUR, N.; PEDERSEN, B. K. **Exercise as a mean to control low-grade systemic inflammationMediators of Inflammation**, 2008.

MAUGHAN, R. **The athlete's diet: nutritional goals and dietary strategies.The Proceedings of the Nutrition Society**, 2002.

MAUGHAN, R. J.; KING, D. S.; LEA, T. Dietary supplements. **Journal of sports sciences**, v. 22, n. 1, p. 95–113, 2004.

MCFARLIN, B. K. et al. TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. **Med Sci Sports Exerc**, v. 36, p. 1876–1883, 2004.

MCFARLIN, B. K. et al. Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. **J Gerontol A Biol Sci Med Sci**, v. 61, n. 4, p. 388–393, 2006.

MCHUGH, M. P. et al. Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. **Sports Med**, v. 27, p. 157–170, 1999.

MCLEAN, C.; GRAHAM, T. E. Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrheic women. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 93, n. 4, p. 1471–8, 1 Oct. 2002.

MEDLOW, P. et al. Exercise training protects the LDL I subfraction from oxidation susceptibility in an aged human population. **Atherosclerosis**, v. 239, n. 2, p. 516–522, 7 Feb. 2015.

MEDZHITOV, R. Toll-like receptors and innate immunity. **Nature reviews. Immunology**, v. 1, n. 2, p. 135–145, 2001.

MEKSAWAN, K. et al. Effect of dietary fat intake and exercise on inflammatory mediators of the immune system in sedentary men and women. **Journal of the American College of Nutrition**, v. 23, n. 4, p. 331–40, Aug. 2004.

MENDIAS, C. L.; TATSUMI, R.; ALLEN, R. E. Role of cyclooxygenase-1 and -2 in satellite cell proliferation, differentiation, and fusion. **Muscle & nerve**, v. 30, n. 4, p. 497–500, Oct. 2004.

MEYER, A. A. et al. Improvement of Early Vascular Changes and Cardiovascular Risk Factors in Obese Children After a Six-Month Exercise Program. **Journal of the American College of Cardiology**, v. 48, p. 1865–1870, 2006.

MIETTINEN, M. et al. IFNs activate toll-like receptor gene expression in viral infections. **Genes and Immunology**, v. 2, n. 6, p. 349–355, 2001.

MIKKELSEN, U. R. et al. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 107, n. 5, p. 1600–1611, 2009.

MISHRA, D. K. et al. Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. **The Journal of bone and joint surgery. American volume**, v. 77, n. 10, p. 1510–1519, 1995.

MITCHELL, J. A.; WARNER, T. D. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. **British journal of pharmacology**, v. 128, n. 6, p. 1121–1132, 1999.

MOISEY, L. L. et al. Caffeinated coffee consumption impairs blood glucose homeostasis in response to high and low glycemic index meals in healthy men. **Am J Clin Nutr**, v. 87, p. 1254–1261, 2008.

MOMAYA, A.; FAWAL, M.; ESTES, R. Performance-Enhancing Substances in Sports: A Review of the Literature. **Sports Medicine**, 2015.

MONDA, M. et al. Inhibition of prostaglandin synthesis reduces the induction of MyoD expression in rat soleus muscle. **Journal of Muscle Research and Cell Motility**, v. 30, n. 3-4, p. 139–144, 2009.

MOOPANAR, T. R.; ALLEN, D. G. The activity-induced reduction of myofibrillar Ca2+ sensitivity in mouse skeletal muscle is reversed by dithiothreitol. **The Journal of physiology**, v. 571, n. Pt 1, p. 191–200, 2006.

MOROZOV, V. I. et al. The effects of high-intensity exercise on skeletal muscle neutrophil myeloperoxidase in untrained and trained rats. **European Journal of Applied Physiology**, v. 97, n. 6, p. 716–722, 2006.

MUZIO, M. et al. Toll-like receptor family and signalling pathway. **Biochemical Society transactions**, v. 28, n. 5, p. 563–566, 2000.

MUZIO, M.; MANTOVANI, A. Toll-like receptors. **Microbes and infection / Institut Pasteur**, v. 2, n. 3, p. 251–255, 2000.

NAVARRO, A. et al. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. **Am J Physiol Regul Integr Comp Physiol**, v. 286, p. R505–11, 2004.

NIEMAN, D. C. et al. Infectious episodes in runners before and after the Los Angeles Marathon. **Journal of Sports Medicine and Physical Fitness**, v. 30, n. 3, p. 316–328, 1990.

NIEMAN, D. C. et al. Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 84, n. 4, p. 1252–9, Apr. 1998.

NIESS, A. M. et al. Expression of the inducible nitric oxide synthase (iNOS) in human leukocytes: responses to running exercise. **Medicine & Science in Sports & Exercise**, v. 32, n. 7, p. 1220–1225, 2000.

NIESS, A. M.; SIMON, P. Response and adaptation of skeletal muscle to exercise – the role of reactive oxygen species. **Frontiers in Bioscience**, v. 12, p. 4826–4838, 2007.

NIKOLAIDIS, M. G. et al. **The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: Magnitude and time-course considerationsSports Medicine**, 2008.

NOVAK, M. L. et al. COX-2 inhibitor reduces skeletal muscle hypertrophy in mice. **American journal of physiology. Regulatory, integrative and comparative physiology**, v. 296, n. 4, p. R1132–R1139, 2009.

NYBO, L. et al. Interleukin-6 release from the human brain during prolonged exercise. **J Physiol**, v. 542, n. Pt 3, p. 991–995, 2002.

O’NEILL, L. A. J.; BOWIE, A. G. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. **Nature reviews. Immunology**, v. 7, n. 5, p. 353–364, 2007.

OGAWA, K. et al. Resistance exercise training-induced muscle hypertrophy was associated with reduction of inflammatory markers in elderly women. **Mediators of Inflammation**, v. 2010, 2010.

OH-ISHI, S. et al. Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. **Am J Respir Crit Care Med**, v. 156, p. 1579–1585, 1997.

OSHIUMI, H. et al. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. **Nat Immunol**, v. 4, p. 161–167, 2003.

OSTROWSKI, K. et al. **Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans.The Journal of physiology**. [s.l: s.n.].

OUCHI, N. et al. Adipokines in inflammation and metabolic disease. **Nature reviews. Immunology**, v. 11, n. 2, p. 85–97, 2011.

OZER, J. et al. The current state of serum biomarkers of hepatotoxicity. **Toxicology**, v. 245, p. 194–205, 2008.

PACKER, L.; CADENAS, E. Oxidants and antioxidants revisited. New concepts of oxidative stress. **Free radical research**, v. 41, n. 9, p. 951–2, Sep. 2007.

PAGE, A. J. et al. Exercise-associated hyponatremia, renal function, and nonsteroidal antiinflammatory drug use in an ultraendurance mountain run. **Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine**, v. 17, n. 1, p. 43–48, 2007.

PAHL, H. L. Activators and target genes of Rel/NF-kappaB transcription factors. **Oncogene**, v. 18, p. 6853–6866, 1999.

PALOMERO, J. et al. Heat shock factor activation in human muscles following a demanding intermittent exercise protocol is attenuated with hyperthermia. **Acta Physiologica**, v. 193, n. 1, p. 79–88, 2008.

PALUSKA, S. A. Caffeine and exercise. **Current sports medicine reports**, v. 2, n. 4, p. 213–219, 2003.

PARKER, L.; MCGUCKIN, T. A.; LEICHT, A. S. Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. **Clinical physiology and functional imaging**, v. 34, n. 5, p. 377–83, Oct. 2014.

PASARE, C.; MEDZHITOV, R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. **Science (New York, N.Y.)**, v. 299, n. 5609, p. 1033–1036, 2003.

PASARE, C.; MEDZHITOV, R. Toll-like receptors: linking innate and adaptive immunity. **Microbes and infection / Institut Pasteur**, v. 6, n. 15, p. 1382–1387, 2004.

PAULSEN, G. et al. Can supplementation with vitamin C and E alter physiological adaptations to strength training? **BMC sports science, medicine and rehabilitation**, v. 6, p. 28, 2014.

PAVLOV, V. A. et al. Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. **Proceedings of the National Academy of Sciences of the United States of America**, v. 103, n. 13, p. 5219–5223, 2006.

PAVLOV, V. A. et al. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. **Brain, Behavior, and Immunity**, v. 23, n. 1, p. 41–45, 2009.

PAVLOV, V. A.; TRACEY, K. J. The cholinergic anti-inflammatory pathway. **Brain, behavior, and immunity**, v. 19, n. 6, p. 493–9, Nov. 2005.

PAVLOV, V. A.; TRACEY, K. J. Controlling inflammation: the cholinergic anti-inflammatory pathway. **Biochemical Society transactions**, v. 34, n. Pt 6, p. 1037–1040, 2006.

PEAKE, J. M. et al. Plasma cytokine changes in relation to exercise intensity and muscle damage. **European Journal of Applied Physiology**, v. 95, n. 5-6, p. 514–521, 2005.

PEAKE, J.; NOSAKA, K.; SUZUKI, K. Characterization of inflammatory responses to eccentric exercise in humans. **Exerc Immunol Rev**, v. 11, p. 64–85, 2005.

PEDERSEN, B. K. **Edward F. Adolph distinguished lecture: muscle as an endocrine organ: IL-6 and other myokines.Journal of applied physiology (Bethesda, Md. : 1985)**, 2009.

PEDERSEN, B. K.; FEBBRAIO, M. A. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. **Physiol Rev**, v. 88, p. 1379–1406, 2008.

PEDERSEN, B. K.; FISCHER, C. P. Beneficial health effects of exercise--the role of IL-6 as a myokine. **Trends in pharmacological sciences**, v. 28, n. 4, p. 152–6, Apr. 2007.

PEDERSEN, B. K.; SALTIN, B. Evidence for prescribing exercise as therapy in chronic disease. **Scandinavian journal of medicine & science in sports**, v. 16 Suppl 1, p. 3–63, 2006.

PETERS, E. M. Exercise, immunology and upper respiratory tract infections. **International journal of sports medicine**, v. 18 Suppl 1, p. S69–S77, 1997.

PETERSEN, A. M.; PEDERSEN, B. K. The anti-inflammatory effect of exercise. **J Appl Physiol**, v. 98, n. 4, p. 1154–1162, 2005.

PETERSON, J. M. et al. Ibuprofen and acetaminophen: Effect on muscle inflammation after eccentric exercise. **Medicine and Science in Sports and Exercise**, v. 35, n. 6, p. 892–896, 2003.

PHILLIPS, M. D. et al. Resistance training reduces subclinical inflammation in obese, postmenopausal women. **Medicine and science in sports and exercise**, v. 44, n. 11, p. 2099–110, Nov. 2012.

PICARD, M. et al. Mitochondria: isolation, structure and function. **The Journal of physiology**, v. 589, n. Pt 18, p. 4413–21, 15 Sep. 2011.

PILEGAARD, H. et al. **Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes.The Journal of physiology**. [s.l: s.n.].

PINHO, R. A. et al. Oxidative stress and inflammatory parameters after an Ironman race. **Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine**, v. 20, n. 4, p. 306–11, Jul. 2010.

PIPE, A.; AYOTTE, C. Nutritional supplements and doping. **Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine**, v. 12, n. 4, p. 245–249, 2002.

POLTORAK, A. et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. **Science (New York, N.Y.)**, v. 282, n. 5396, p. 2085–2088, 1998.

PRADHAN, A. D. et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. **JAMA**, v. 286, n. 3, p. 327–34, 2001.

PROKOPCHUK, O. et al. Skeletal muscle IL-4, IL-4Ralpha, IL-13 and IL-13Ralpha1 expression and response to strength training. **Exercise immunology review**, v. 13, p. 67–75, 2007.

RADAK, Z. et al. Exercise, oxidative stress and hormesis. **Ageing Res Rev**, v. 7, p. 34–42, 2008.

RADÁK, Z. et al. Regular training modulates the accumulation of reactive carbonyl derivatives in mitochondrial and cytosolic fractions of rat skeletal muscle. **Arch Biochem Biophys**, v. 383, p. 114–118, 2000.

RADÁK, Z. et al. Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. **FASEB J**, v. 18, p. 749–750, 2004.

RADAK, Z.; CHUNG, H. Y.; GOTO, S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. **Biogerontology**, v. 6, p. 71–75, 2005.

RAHME, E.; PILOTE, L.; LELORIER, J. Association between naproxen use and protection against acute myocardial infarction. **Archives of internal medicine**, v. 162, n. 10, p. 1111–1115, 2002.

RAMM, S.; MALLY, A. Role of drug-independent stress factors in liver injury associated with diclofenac intake. **Toxicology**, v. 312, p. 83–96, 2013.

RASMUSSEN, U. F. et al. The effect of high-intensity exhaustive exercise studied in isolated mitochondria from human skeletal muscle. **Pflugers Arch**, v. 443, p. 180–187, 2001.

RAY, W. A. et al. Non-steroidal anti-inflammatory drugs and risk of serious coronary heart disease: An observational cohort study. **Lancet**, v. 359, n. 9301, p. 118–123, 2002.

REID, M. B. Nitric oxide, reactive oxygen species, and skeletal muscle contraction. **Medicine and science in sports and exercise**, v. 33, n. 3, p. 371–376, 2001.

REID, M. B. **Free radicals and muscle fatigue: Of ROS, canaries, and the IOCFree Radical Biology and Medicine**, 2008.

RICCIOTTI, E.; FITZGERALD, G. A. Prostaglandins and inflammation. **Arteriosclerosis, thrombosis, and vascular biology**, v. 31, n. 5, p. 986–1000, May 2011.

RINNER, I.; KAWASHIMA, K.; SCHAUENSTEIN, K. Rat lymphocytes produce and secrete acetylcholine in dependence of differentiation and activation. **Journal of neuroimmunology**, v. 81, n. 1-2, p. 31–7, Jan. 1998.

RISTOW, M. et al. Antioxidants prevent health-promoting effects of physical exercise in humans. **Proceedings of the National Academy of Sciences of the United States of America**, v. 106, n. 21, p. 8665–8670, 2009.

RISTOW, M.; SCHMEISSER, S. Extending life span by increasing oxidative stress. **Free Radic Biol Med**, v. 51, p. 327–336, 2011.

ROBERTS, C. K.; LITTLE, J. P.; THYFAULT, J. P. Modification of insulin sensitivity and glycemic control by activity and exercise. **Medicine and science in sports and exercise**, v. 45, n. 10, p. 1868–77, Oct. 2013.

ROBERTSON, D. et al. Tolerance to the humoral and hemodynamic effects of caffeine in man. **Journal of Clinical Investigation**, v. 67, n. 4, p. 1111–1117, 1981.

RODRIGUEZ, N. R.; DI MARCO, N. M.; LANGLEY, S. American College of Sports Medicine position stand. Nutrition and athletic performance. **Medicine and science in sports and exercise**, v. 41, n. 3, p. 709–31, Mar. 2009.

RODRIGUEZ-MIGUELEZ, P. et al. Role of Toll-like receptor 2 and 4 signaling pathways on the inflammatory response to resistance training in elderly subjects. **Age (Dordr)**, v. 36, p. 9734, 2014.

ROSA, J. C. et al. Exhaustive exercise increases inflammatory response via Toll like receptor-4 and NF-κBp65 pathway in rat adipose tissue. **J Cell Physiol**, v. 226, p. 1604–1607, 2011.

ROSE, E. H. DE et al. **Referred use of medication and dietary supplements in athletes selected for doping control in the South-American GamesRevista Brasileira de Medicina do Esporte**, 2006.

RYAN, M. T.; HOOGENRAAD, N. J. Mitochondrial-nuclear communications. **Annual review of biochemistry**, v. 76, p. 701–722, 2007.

SACHDEV, S.; DAVIES, K. J. A. **Production, detection, and adaptive responses to free radicals in exerciseFree Radical Biology and Medicine**, 2008.

SALMINEN, A.; VIHKO, V. Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation in vitro. **Acta Physiol Scand**, v. 117, n. 1, p. 109–113, 1983.

SCHLIENGER, R. G.; JICK, H.; MEIER, C. R. Use of nonsteroidal anti-inflammatory drugs and the risk of first-time acute myocardial infarction. **BRITISH JOURNAL OF CLINICAL PHARMACOLOGY**, v. 54, n. 3, p. 327–332, 2002.

SCULLY, L. J.; CLARKE, D.; BARR, R. J. Diclofenac induced hepatitis. 3 cases with features of autoimmune chronic active hepatitis. **Dig Dis Sci**, v. 38, p. 744–751, 1993.

SEDDON, M.; LOOI, Y. H.; SHAH, A. M. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. **Heart (British Cardiac Society)**, v. 93, p. 903–907, 2007.

SEIBL, R. et al. Expression and regulation of Toll-like receptor 2 in rheumatoid arthritis synovium. **The American journal of pathology**, v. 162, n. 4, p. 1221–1227, 2003.

SELMAN, C. et al. Antioxidant enzyme activities, lipid peroxidation, and DNA oxidative damage: the effects of short-term voluntary wheel running. **Archives of biochemistry and biophysics**, v. 401, n. 2, p. 255–261, 2002.

SHEN, S. et al. Cytochrome P4502C11 is a target of diclofenac covalent binding in rats. **Chemical Research in Toxicology**, v. 10, n. 4, p. 420–423, 1997.

SHEN, S. et al. Metabolic activation of diclofenac by human cytochrome P450 3A4: Role of 5-hydroxydiclofenac. **Chemical Research in Toxicology**, v. 12, n. 2, p. 214–222, 1999.

SIMMONDS, M. J.; MINAHAN, C. L.; SABAPATHY, S. Caffeine improves supramaximal cycling but not the rate of anaerobic energy release. **Eur J Appl Physiol**, v. 109, p. 287–295, 2010.

SINCLAIR, C. J. D.; GEIGER, J. D. **Caffeine use in sports. A pharmacological reviewJournal of Sports Medicine and Physical Fitness**, 2000.

SMITH, C. et al. Metabolism of the Eicosanoids. In: WILKINS, L. W. & (Ed.). . **Mark's Basic Medical Biochemistry: a Clinical Approach**. [s.l: s.n.]. p. 654–667.

SMITH, W. L.; DEWITT, D. L.; GARAVITO, R. M. Cyclooxygenases: structural, cellular, and molecular biology. **Annual review of biochemistry**, v. 69, p. 145–182, 2000.

SÖKMEN, B. et al. Caffeine use in sports: considerations for the athlete. **Journal of strength and conditioning research / National Strength & Conditioning Association**, v. 22, n. 3, p. 978–986, 2008.

SOLOMON, D. H. et al. Nonsteroidal anti-inflammatory drug use and acute myocardial infarction. **Archives of internal medicine**, v. 162, n. 10, p. 1099–1104, 2002.

SOLOMON, S. D. et al. **Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention.The New England journal of medicine**. [s.l: s.n.].

SOLTOW, Q. A. et al. Ibuprofen inhibits skeletal muscle hypertrophy in rats. **Medicine and Science in Sports and Exercise**, v. 38, n. 5, p. 840–846, 2006.

SPEED, C.; JAQUES, R. High-performance sports medicine: an ancient but evolving field. **British journal of sports medicine**, v. 45, n. 2, p. 81–3, Feb. 2011.

SPEISMAN, R. B. et al. **Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging ratsBrain, Behavior, and Immunity**, 2013.

SPRIET, L. L. et al. Caffeine ingestion and muscle metabolism during prolonged exercise in humans. **Am J Physiol**, v. 262, p. E891–8, 1992.

STARKIE, R. et al. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. **The FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, v. 17, p. 884–886, 2003.

STEENSBERG, A. et al. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. **J Physiol**, v. 529 Pt 1, p. 237–242, 2000.

STEENSBERG, A. et al. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. **American journal of physiology. Endocrinology and metabolism**, v. 285, n. 2, p. E433–7, Aug. 2003.

STEPPAN, J. et al. Alagebrium in combination with exercise ameliorates age-associated ventricular and vascular stiffness. **Experimental Gerontology**, v. 47, p. 565–572, 2012.

STEWART, L. K. et al. Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. **Brain, Behavior, & Immunity**, v. 19, n. 5, p. 389–397, 2005.

ST-PIERRE, J. et al. Topology of superoxide production from different sites in the mitochondrial electron transport chain. **The Journal of biological chemistry**, v. 277, n. 47, p. 44784–44790, 2002.

SUN, L. et al. Endurance exercise causes mitochondrial and oxidative stress in rat liver: effects of a combination of mitochondrial targeting nutrients. **Life Sci**, v. 86, p. 39–44, 2010.

SUPINSKI, G. S. et al. N-acetylcysteine administration alters the response to inspiratory loading in oxygen-supplemented rats. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 82, n. 4, p. 1119–25, Apr. 1997.

TAGLIARI, B. et al. Chronic variable stress alters inflammatory and cholinergic parameters in hippocampus of rats. **Neurochemical research**, v. 36, n. 3, p. 487–93, Mar. 2011.

TAKEDA, K.; AKIRA, S. TLR signaling pathways. **Semin Immunol**, v. 16, n. 1, p. 3–9, 2004.

TAKEDA, K.; AKIRA, S. Toll-like receptors in innate immunity. **Int Immunol**, v. 17, n. 1, p. 1–14, 2005.

TAKEUCHI, O.; AKIRA, S. Toll-like receptors; their physiological role and signal transduction system. **International immunopharmacology**, v. 1, n. 4, p. 625–635, 2001.

TANAKA, H. et al. Aging, habitual exercise, and dynamic arterial compliance. **Circulation**, v. 102, p. 1270–1275, 2000.

TANG, W. et al. Roles of human hepatic cytochrome P450s 2C9 and 3A4 in the metabolic activation of diclofenac. **Chem Res Toxicol**, v. 12, p. 192–199, 1999.

TARNOPOLSKY, M. A. Caffeine and endurance performance. **Sports Med**, v. 18, p. 109–125, 1994.

TARNOPOLSKY, M. A. Effect of caffeine on the neuromuscular system--potential as an ergogenic aid. **Appl Physiol Nutr Metab**, v. 33, p. 1284–1289, 2008.

TARNOPOLSKY, M. A. Caffeine and creatine use in sport. **Ann Nutr Metab**, v. 57 Suppl 2, p. 1–8, 2010.

TARNOPOLSKY, M.; CUPIDO, C. Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers. **J Appl Physiol**, v. 89, p. 1719–1724, 2000.

TEGEDER, I.; PFEILSCHIFTER, J.; GEISSLINGER, G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. **The FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, v. 15, n. 12, p. 2057–2072, 2001.

THOMPSON, P. D. et al. The acute versus the chronic response to exercise. **Medicine and science in sports and exercise**, v. 33, p. S438–S445; discussion S452–S453, 2001.

THUYNE, W. V; DELBEKE, F. T. Declared use of medication in sports. **Clin J Sport Med**, v. 18, p. 143–147, 2008.

TIDBALL, J. G.; VILLALTA, S. A. Regulatory interactions between muscle and the immune system during muscle regeneration. **American journal of physiology. Regulatory, integrative and comparative physiology**, v. 298, n. 5, p. R1173–R1187, 2010.

TRACEY, K. J. The inflammatory reflex. **Nature**, v. 420, p. 853–859, 2002.

TRACEY, K. J. Physiology and immunology of the cholinergic antiinflammatory pathway. **The Journal of clinical investigation**, v. 117, n. 2, p. 289–96, Feb. 2007.

TRAPPE, T. A. et al. Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults. **American journal of physiology. Regulatory, integrative and comparative physiology**, v. 300, n. 3, p. R655–R662, 2011.

TSCHOLL, P. et al. The use and abuse of painkillers in international soccer: data from 6 FIFA tournaments for female and youth players. **Am J Sports Med**, v. 37, p. 260–265, 2009.

TSCHOLL, P.; JUNGE, A.; DVORAK, J. The use of medication and nutritional supplements during FIFA World Cups 2002 and 2006. **British journal of sports medicine**, v. 42, n. 9, p. 725–730, 2008.

TUNNICLIFFE, J. M. et al. Consumption of dietary caffeine and coffee in physically active populations: physiological interactions. **Appl Physiol Nutr Metab**, v. 33, p. 1301–1310, 2008.

TURNER, J. E. et al. Prolonged depletion of antioxidant capacity after ultraendurance exercise. **Medicine and science in sports and exercise**, v. 43, n. 9, p. 1770–6, 2011.

URSO, M. L. Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment? **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 115, n. 6, p. 920–8, 15 Sep. 2013.

VAN HECKEN, A. et al. **Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers.Journal of clinical pharmacology**. [s.l: s.n.].

VAN PRAAG, H. et al. Running enhances neurogenesis, learning, and long-term potentiation in mice. **Proceedings of the National Academy of Sciences of the United States of America**, v. 96, n. 23, p. 13427–13431, 1999.

VANE, J. R.; BOTTING, R. M. Mechanism of action of anti-inflammatory drugs. **Scand.J.Rheumatol.Suppl.**, v. 102, p. 9–21, 1996.

VASSILAKOPOULOS, T. et al. **Antioxidants attenuate the plasma cytokine response to exercise in humans.Journal of applied physiology (Bethesda, Md. : 1985)**. [s.l: s.n.].

VAYNMAN, S.; GOMEZ-PINILLA, F. Revenge of the “sit”: how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. **Journal of neuroscience research**, v. 84, n. 4, p. 699–715, Sep. 2006.

VENEROSO, C. et al. Melatonin reduces cardiac inflammatory injury induced by acute exercise. **J Pineal Res**, v. 47, p. 184–191, 2009.

VILLA, J. G. et al. Impairment of bile secretion induced by exhaustive exercise in the rat. Protective effects of S-adenosyl-L-methionine. **Int J Sports Med**, v. 14, p. 179–184, 1993.

VINA, J. et al. Exercise acts as a drug; the pharmacological benefits of exercise. **British Journal of Pharmacology**, v. 167, n. 1, p. 1–12, 3 Sep. 2012.

VINCENT, H. K.; TAYLOR, A. G. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. **International journal of obesity (2005)**, v. 30, n. 3, p. 400–18, Mar. 2006.

WANG, H. et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. **Nature**, v. 421, n. 6921, p. 384–388, 2003.

WANG, H. et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. **Nat Med**, v. 10, p. 1216–1221, 2004.

WANNMACHER, L.; BREDEMEIER, M. **Antiinflamatórios não-esteróides: uso indiscriminado de inibidores seletivos de cicloxigenase-2Uso Racional deMedicamentos**, 2004.

WARBURTON, D. E. R.; NICOL, C. W.; BREDIN, S. S. D. Health benefits of physical activity: the evidence. **CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne**, v. 174, n. 6, p. 801–809, 2006.

WARNER, D. C. et al. Prevalence, attitudes, and behaviors related to the use of nonsteroidal anti-inflammatory drugs (NSAIDs) in student athletes. **J Adolesc Health**, v. 30, p. 150–153, 2002.

WARNER, T. D. et al. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. **Proceedings of the National Academy of Sciences of the United States of America**, v. 96, n. 13, p. 7563–7568, 1999.

WATANABE, Y. et al. **Expression of acetylcholine in lymphocytes and modulation of an independent lymphocytic cholinergic activity by immunological stimulationBiogenic Amines**, 2002.

WATKINS, P. B.; SEEFF, L. B. Drug-induced liver injury: summary of a single topic clinical research conference. **Hepatology (Baltimore, Md.)**, v. 43, n. 3, p. 618–31, Mar. 2006.

WATSON, D. J. et al. Lower risk of thromboembolic cardiovascular events with naproxen among patients with rheumatoid arthritis. **Archives of internal medicine**, v. 162, n. 10, p. 1105–1110, 2002.

WEINHEIMER, E. M. et al. Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis. **American journal of physiology. Regulatory, integrative and comparative physiology**, v. 292, n. 6, p. R2241–R2248, 2007.

WEST, A. P.; KOBLANSKY, A. A.; GHOSH, S. Recognition and signaling by toll-like receptors. **Annual review of cell and developmental biology**, v. 22, p. 409–437, 2006.

WESTHOFF, T. H. et al. The cardiovascular effects of upper-limb aerobic exercise in hypertensive patients. **Journal of Hypertension**, v. 26, n. 7, p. 1336–1342, Jul. 2008.

WHARAM, P. C. et al. NSAID use increases the risk of developing hyponatremia during an Ironman triathlon. **Med Sci Sports Exerc**, v. 38, p. 618–622, 2006.

WIENS, K. et al. Dietary supplement usage, motivation, and education in young, Canadian athletes. **International journal of sport nutrition and exercise metabolism**, v. 24, n. 6, p. 613–22, Dec. 2014.

WILLIS, W. T.; JACKMAN, M. R. Mitochondrial function during heavy exercise. **Med Sci Sports Exerc**, v. 26, p. 1347–1353, 1994.

WILSON, D. O.; JOHNSON, P. Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 88, p. 1791–1796, 2000.

WILUND, K. R. Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? **Clinical science (London, England : 1979)**, v. 112, n. 11, p. 543–555, 2007.

WORLD ANTI DOPING. **The 2015 prohibited list international standard**. Disponível em: <https://www.wada-ama.org/en/what-we-do/prohibited-list>.

YAMAMOTO, M. et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. **Science (New York, N.Y.)**, v. 301, n. 5633, p. 640–643, 2003.

YANG, J. N.; CHEN, J. F.; FREDHOLM, B. B. Physiological roles of A1 and A2A adenosine receptors in regulating heart rate, body temperature, and locomotion as revealed using knockout mice and caffeine. **Am J Physiol Heart Circ Physiol**, v. 296, p. H1141–9, 2009.

ZBINDEN-FONCEA, H. et al. TLR2 and TLR4 activate p38 MAPK and JNK during endurance exercise in skeletal muscle. **Med Sci Sports Exerc**, v. 44, p. 1463–1472, 2012.

ZEIDÁN-CHULIÁ, F. et al. Major Components of Energy Drinks (Caffeine, Taurine, and Guarana) Exert Cytotoxic Effects on Human Neuronal SH-SY5Y Cells by Decreasing Reactive Oxygen Species Production. **Oxid Med Cell Longev**, v. 2013, p. 791795, 2013.

ZELENKA, M.; SCHÄFERS, M.; SOMMER, C. Intraneural injection of interleukin-1β and tumor necrosis factor-alpha into rat sciatic nerve at physiological doses induces signs of neuropathic pain. **Pain**, v. 116, n. 3, p. 257–263, 2005.