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EFEITOS DO TREINAMENTO AERÓBICO NO PROCESSO INFLAMATÓRIO E ESTRESSE OXIDATIVO NO CÓRTEX E MÚSCULO E A SUA RELAÇÃO COM EXERCÍCIOS À EXAUSTÃO, BEM COMO AS ADAPTAÇÕES CAUSADAS PELO TREINAMENTO AERÓBICO NO FÍGADO DE RATOS

TESE DE DOUTORADO

Frederico Diniz Lima

Santa Maria, RS, Brasil

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Frederico Diniz Lima

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Orientador: Prof. Dr. Luiz Fernando Freire Royes

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A Comissão Examinadora, abaixo assinada,
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EFEITO DO TREINAMENTO AERÓBICO E POSSÍVEL EFEITO ERGOGÊNICO DA ADMINISTRAÇÃO DE IBUPROFENO NA FADIGA E ADAPTAÇÕES NO FÍGADO EM TESTES À EXAUSTÃO

elaborada por
Frederico Diniz Lima

Como requisito parcial para a obtenção do grau de
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Santa Maria, 08 de janeiro de 2015.

DEDICATÓRIA

Dedico este trabalho à minha esposa Luciane e à minha filha Antonella, que souberam me apoiar e me compreender em todos os momentos.

AGRADECIMENTOS

Abençoa Senhor, meus amigos e minhas amigas e dá-lhes a paz! Não existe outro meio de iniciar um agradecimento, senão dessa forma. Uns dizem que são os experimentos a parte mais difícil de um trabalho, outros falam que é a escrita da tese. Já ouvi que a introdução tira o sono de vários alunos, que o resumo é dificílimo e até, pasmem, que a formulação do título deve ser feita em harmonia com o universo, pois é o cartão de apresentação de um trabalho. Participando de todos os processos pertinentes à confecção dessa tese, e olhando por outro prisma, venho dizer que esta é a parte mais difícil e complicada. "Por que", pode perguntar algum desatento ou que ainda não chegou nesse ponto e eu respondo: porque um filme passa em frente aos olhos. Coisas que estavam lá no fundo da nossa alma quase esquecidas e que, por algum processo inexplicável de evocação, nossa memória traz à tona. O desatento pode, ainda, insistir: "mas se são memórias boas, como isso pode atrapalhar?". Justamente por serem boas, ótimas, engraçadas (umas nem tanto), históricas e homéricas é que torna todo esse processo muito complicado. São muitos, milhares de nomes, lugares, ações, resultados e consequências ao mesmo tempo e que tornam a tarefa de colocar tudo em uma sequencia lógica impossível. São vários fatores que culminam com esse momento! Para, tentar, começar lembro-me do momento em que conheci a minha esposa e, de lá pra cá, tudo o que faço é pensando nela, aquela que tornou a minha vida muito mais alegre, feliz e com significado, principalmente no dia que vi a nossa filha linda, mesmo que tenha sido em uma tela de computador que mais parecia fora do ar (tudo é pra ela também); **como é grande o meu amor por vocês**!!

Lembro-me dos meus pais me dando força para realizar toda e qualquer tarefa, desde que fosse com honestidade e seriedade, pois foi isso que me ensinaram e dos meus irmãos que, do seu jeito, contribuíram para a minha formação como pessoa. Conheço muita gente, tenho vários conhecidos e alguns amigos, aqueles que posso chamar de irmãos e irmãs do coração, os quais sabem quem são, e que sempre estiveram do meu lado, mesmo reclamando quando eu não podia pois "tinha experimento". Tenho, também, aqueles que eu chamo de outra família (nessa hora o pavor de esquecer-me de nomear alguém é inexplicável) e, para esses, deixo mais uma parte da Oração pelos meus amigos: "Gente que sonha junto, gente que brinca e briga e se zanga e perdoa. Um sentimento forte, mais forte que a morte, nos faz ser amigos no riso e na dor. Vidas que fluem juntas, rios que não confluem, mas vão paralelos, aves que voam juntas e sabem que um dia, por força da vida não mais se verão. Resta apenas o sonho que a gente viveu, meus amigos e minhas amigas e eu!

EPÍGRAFE

O corpo humano está séculos à frente dos fisiologistas e pode realizar uma integração entre coração, pulmões e músculos, a qual é muito complexa para os cientistas analisarem. E é o cérebro e não o coração ou os pulmões o órgão crítico, é o cérebro.

Sir Roger Gilbert Bannister

RESUMO

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

EFEITOS DO TREINAMENTO AERÓBICO NO PROCESSO INFLAMATÓRIO E ESTRESSE OXIDATIVO NO CÓRTEX E MÚSCULO E A SUA RELAÇÃO COM EXERCÍCIOS À EXAUSTÃO, BEM COMO AS ADAPTAÇÕES CAUSADAS PELO TREINAMENTO AERÓBICO NO FÍGADO DE RATOS

AUTOR: Frederico Diniz Lima
ORIENTADOR: Luiz Fernando Freire Royes
Local e data da defesa: Santa Maria, 8 de janeiro de 2015.

A prática regular de exercícios físicos está intimamente ligada a uma boa qualidade de vida. Entretanto, cada sessão de treino tem como consequência a produção de espécies reativas de oxigênio (EROs), o processo inflamatório sub-clínico, a dor muscular tardia (DMT), diminuição do desempenho e a fadiga. Esses fatores causam adaptações na musculatura esquelética, cérebro e fígado e, consequentemente, de uma forma crônica, contribuem para a melhora do condicionamento físico e do desempenho esportivo o que protela o aparecimento da fadiga. Além disso, existem evidências do uso de anti-inflamatórios não esteroidais (AINEs) no sentido de diminuir a sensação de dor para atingir o mesmo objetivo. 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 AINEs para evitar perdas em suas provas. Neste sentido, o presente trabalho teve como objetivo investigar os efeitos do treinamento aeróbico em ratos sobre o tempo à exaustão em três testes exaustivos. Foram avaliados os marcadores inflamatórios e de estresse oxidativo, atividade da enzima acetilcolinesterase (AChE), assim como a administração de ibuprofeno age nos mesmos parâmetros e na DMT no córtex e músculo. Outro objetivo desse trabalho foi avaliar as adaptações do treinamento físico no fígado, nos marcadores de estresse oxidativo e viabilidade mitocondrial e como esse órgão reage frente a exercícios à exaustão em ratos. No manuscrito demonstrou-se que o treinamento aeróbico (natação 6 semanas) causou uma diminuição da atividade da enzima acetilcolinesterase (AChE) no córtex e do conteúdo de Fator de Necrose Tumoral – alfa (TNF-α) e Interleucina – 1 Beta (IL-1β) *per se* no músculo. Além disso, verificou-se que a administração de ibuprofeno (15 mg/kg, por 9 dias), *per se* e em combinação com o exercício, aumentou o tempo à exaustão, causou uma diminuição da atividade da AChE no córtex, diminuição do conteúdo TNF-α e IL-1β no músculo e no conteúdo espécies reativas totais nos dois tecidos. No artigo verificou-se que o treinamento aeróbico (natação 6 semanas) causou aumento no conteúdo de glutationa reduzia (GSH) e da razão glutationa reduzida/oxidada (GSH/GSSG), na atividade da enzima superóxido dismutase, diminuiu a peroxidação lipídica e aumentou a viabilidade mitocondrial no fígado. Além disso, as adaptações causadas pelo treinamento mantiveram os níveis elevados de GSH, protegeu do aumento dos níveis de GSSG, e do aumento de peroxidação lipídica e de carbonilação proteica, além de manter a redução de metiltetrazol, aumentar o potencial de membrana e de proteger de um grande aumento de produção de ERO. Considerando os dados apresentados no presente estudo, conclui-se que a administração de ibuprofeno pode ter um papel ergogênico na realização de testes exaustivos, adiando a fadiga e que o fígado sofre adaptações mitocondriais pelo treinamento aeróbico que auxiliam na realização dos mesmos testes.

Palavras-chave: Treinamento aeróbico. Inflamação. Dor muscular. Fadiga. Ibuprofeno. Fígado.

ABSTRACT

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

EFFECTS OF AEROBIC TRAINING AND POSSIBLE ERGOGENIC EFFECT OF IBUPROFENO ADMINISTRATION ON FATIGUE AND ADAPTATIONS ON LIVER IN EXHAUSTIVE TESTS

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ADVISOR: Luiz Fernando Freire Royes
Place and date of defense: Santa Maria, January 8th, 2015.

The regular practice of physical exercise is linked to a good life quality. However, each physical bout has consequences named reactive oxygen species (ROS) production, low-grade inflammation, delayed onset muscle soreness (DOMS), impaired performance and the fatigue. Those factors cause adaptation on skeletal muscle, brain and liver and also, in a chronic practice, contribute to physical condition improvement and sport performance, causing an delay on fatigue. Besides, there are evidences for use of Non-Steroidal Anti-inflammatory Drugs (NSADs) with the objective of diminishing soreness and achieve the same objective. Considering that athletes get training with objective of improve their performance, principally in high intense and short duration exercises and because this could lead to soreness and low-grade inflammation, a great number of athletes uses NSADs to avoid diminishing performance. In this sense, the objective of this thesis was investigating the effects of aerobic training in rats on time to exhaustion in three exhaustive bouts. Inflammatory markers and ROS production acetylcholinesterase (AChE) activity enzyme was investigate and how ibuprofen administration acts on same parameters, on DOMS and in exhaustive bouts in the cortex and muscle. Another objective was evaluated the training adaptations on liver, on oxidative stress markers and mitochondrial viability and how this organ reacts against exhaustive exercise in rats. The manuscript showed that physical exercise (swimming for 6 weeks) mitigates the acetylcholinesterase (AChE) activity enzyme on cerebral cortex and on levels of Tumoral Necrosis Factor alpha (TNF-α) and Interleukin 1 beta (IL-1β) per se on skeletal muscle. Besides, the ibuprofen administration (15 mg/kg, 9 days), per se and in combination with training, increase time to exhaustion on third exhaustive bout, diminished the AChE activity on cerebral cortex and the TNF-α e IL-1β content on skeletal muscle and in total reactive species content in both tissues. On the article, physical exercise (swimming for 6 weeks) increased reduced glutathione (GSH) and reduced/oxidized glutathione ratio (GSH/GSSG) content and **Superoxide dismutases** (SOD) activity, decreased lipidic peroxidation (TBARS) levels and increased mitochondrial viability (MTT) on liver. Besides, the adaptations caused by aerobic training preserved high GSH levels, protected oxidized glutathione (GSSG) levels, against TBARS and Protein carbonilation increases in addition to the maintenance of MTT reduction, increased membrane potential and protects ROS production. Considering data on this thesis, ibuprofen administration could has used as ergogenic aid on exhaustive bouts delaying fatigue and aerobic training causes mitochondrial adaptations on liver that support exhaustive bouts.

Keywords: Aerobic training. Inflammation. Muscle soreness. Fatigue. Ibuprofeno. Liver.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

5-HT	Serotonina
ACh	Acetilcolina
AChE	Acetilcolinesterase
AINES	Anti-inflamatórios Não Esteroidais
AMA	Agência Mundial Antidoping
CAT	Catalase
ChAT	Colina Acetil Transferase
CK	Creatina cinase
COX	Ciclooxigenase
COX-2	Ciclooxigenase – 2
DA	Dopamina
DCFH – DA	Diclorofluoresceina diacetato
EF	Exercício Físico
EO	Estresse Oxidativo
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
FC_{max}	Frequência cardíaca máxima
GPx	Glutationa Peroxidase
GSH	Glutatina reduzida
GSSG	Glutationa oxidada
GSH/GSSG	Razão glutationa reduzida/glutationa oxidada
IBU	Ibuprofeno
IL-6	Interleucina – 6
IL-1β	Interleucina – 1 beta
IL-1βra	Receptor antagonista de Interleucina – 1 beta
iNOS	Óxido nítrco sintase induzida
JNM	Junção neuromuscular
NA	Noradrenalina
ON	Óxido nítrico
PG	Prostaglandina
PGE_2	Prostaglandina E_2
PLA_2	Fosfolipase A_2
RL	Radical livre
SOD	Superóxido dismutase
SNC	Sistema nervoso central
SNP	Sistema nervoso periférico
sTNF-α	Receptor solúvel de fator de necrose tumoral – alfa
$T_{1/2}$	Tempo de meia-vida
TBARS	Espécies reativas ao ácido tiobarbitúrico
TNF-α	Fator de necrose tumoral – alfa
UM	Unidade motora
VO_{2max}	Volume máximo de oxigênio

SUMÁRIO

1 INTRODUÇÃO

1.1 Exercício físico – breve histórico

O Exercício Físico (EF) sempre esteve intimamente ligado à história humana. O homem primitivo vivenciava a atividade física para garantir sua sobrevivência através da caça, luta e fuga. Pode-se citar, também, a presença do EF nos rituais, nos jogos e nas festividades (RAMOS, 1982). Exercício, jogo e saúde eram sempre presentes em diversas civilizações, como da Babilônia, da Pérsia, da Assíria, do Egito, do oriente Médio até a Índia e a China. Porém, foi a civilização da Grécia antiga que mais influenciou a civilização ocidental no que diz respeito ao EF, nos estudos anatômicos de Herodicus (500 a.C.), Hipócrates (460-377 a.C.) e Galeno (131-201 d.C.) (MCARDLE, KATCH e KATCH, 2003). Também foi na Grécia que surgiu a ideia da beleza humana, o que pode ser facilmente observado em obras de arte. Existia, contudo, uma diferença no pensamento dos gregos em relação ao EF: ao passo que em Atenas ele tinha o objetivo de educação corporal, em Esparta era usado como preparação para a guerra. A Grécia também é considerada o berço dos Jogos Olímpicos, que ocorreram por 12 séculos perfazendo um total de 293 eventos (776 a.C-393 d.C) o que demonstra a importância do EF. Pode-se colocar, também, que na Roma antiga o EF era parte da preparação militar dos soldados e dos gladiadores para o combate (RAMOS, 1982).

Na Idade Média, o EF era usado exclusivamente pelos exércitos na preparação dos seus soldados, mas na Idade Moderna, alguns estudiosos contribuíram para o desenvolvimento do conhecimento sobre Fisiologia, Anatomia, Técnicas Desportivas e Pedagogia (RAMOS, 1982; MCARDLE, KATCH e KATCH, 2003). As práticas inglesas no século XVIII até a metade do século XIX também foram importantes, pois neste período não existiam campeões, mas sim vencedores, já que não existiam campeonatos, mas sim disputas em apostas (TUBINO e MOREIRA, 2003).

No período do Esporte Moderno, Thomas Arnold iniciou a estabelecer regras para o esporte, criando organizações, direção e administração quando da sua nomeação para a direção da Rugby School, entre 1820 e 1840. Posteriormente, houve um grande impulso quando da restauração dos Jogos Olímpicos por Pierre de Coubertin. Houve também uma tentativa de ligar o esporte a ideias políticas com Hitler e, depois, na Guerra Fria entre Estados Unidos e União Soviética. O Esporte Contemporâneo surgiu após a edição da carta

Internacional de educação Física e Esporte da UNESCO. Esse documento divide a prática esportiva em Esporte-Educação, Esporte-Lazer e Esporte de rendimento (TUBINO e MOREIRA, 2003).

Atualmente, a prática regular de exercícios físicos moderados está bem consolidada na sociedade em consequência do seu 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 e SPANGENBURG, 2002), redução da pressão sanguínea, e também de efeitos terapêuticos, com alcance em uma gama de problemas como diabetes melittus, obesidade, doenças cardiovasculares e pulmonares, problemas musculares e articulares e depressão (PEDERSEN; SALTIN, 2006; BLAIR e JACKSON, 2001).

Evidências clínicas e experimentais recentes mostram os efeitos benéficos da atividade física sobre o Sistema Nervoso Central (SNC), como: o aumento da capacidade de aprendizado, a diminuição do declínio cognitivo decorrente do envelhecimento, a capacidade de melhorar a memória, as melhoras na resposta neuroimunológica, além de proteger o SNC de insultos, como o acidente vascular encefálico (KIM et al., 2014; INOUE et al., 2014; HU et al., 2014; SPEISMAN et al., 2013) e traumatismo cranioencefálico (LIMA et al., 2009).

1.2 Treinamento Desportivo

A prática regular de exercícios físicos chama-se de treinamento desportivo que se caracteriza pelas adaptações psico-morfofuncionais que se estabelecem durante a sua prática. O treinamento físico depende diretamente de uma organização, estruturação e controle do treinamento com o objetivo de melhorar o desempenho físico (GOMES, 2002). Durante cada sessão de treino, as variáveis como carga, duração, pausa entre os estímulos, ação muscular, velocidade de execução do movimento, frequência dos exercícios por semana, números de exercícios por sessão de treino, amplitude dos movimentos e combinação dos exercícios na sessão devem ser manipuladas para um melhor aproveitamento do planejamento e para alcançar as metas desejadas (TOIGO e BOUTELLIER, 2006).

O treinamento visa um condicionamento atlético e pode ser realizado para aumentar a força, aumentar a capacidade anaeróbica ou a capacidade aeróbica (GOMES, 2002). O treinamento para o aumento da força tem como objetivo aumentar a quantidade de força gerada por determinado grupo muscular. Os exercícios para alcançar os objetivos podem ser

classificados em isométricos, onde existe a aplicação de força, mas nenhum movimento articular, em dinâmicos, onde existe o movimento articular ou em isocinéticos, com a produção de força a uma velocidade constante (POWERS e HOWLEY, 2000). Como consequência desse tipo de treinamento, as adaptações fisiológicas são o aumento da massa muscular e a quantidade de força gerada através da hipertrofia, ou seja, o aumento do diâmetro das fibras musculares, ou através da hiperplasia, que significa o aumento do número de fibras musculares, através de alterações no conteúdo proteico intramuscular, que tem como consequência o aumento da área de secção transversa do músculo esquelético, acarretando o aumento da capacidade de gerar força muscular (PETTE e STARON, 1997); (YAMADA, VERLENGIA e BUENO JUNIOR, 2012).

Outra consequência do treinamento de força é a indução de mudanças no SNC, que pode aumentar o número de unidades motoras (UM), alterar a frequência de disparos dos motoneurônios e a inibição da inibição neural (MORITANI e DEVRIES, 1979); (RUBE e SECHER, 1991); (CARROLL, RIEK e CARSON, 2001); (JENSEN, MARSTRAND e NIELSEN, 2005); (CARROLL et al., 2011), pois adaptações neurais ocorrem nos estágios iniciais do treinamento de força (HAKKINEN e KOMI, 1983). O aumento de força pode ser considerado como um processo de aprendizagem motora, pelo fato de que os atletas aprendem a produzir padrões de recrutamento de fibras musculares para um desempenho cada vez melhor (CARROLL et al., 2011).

O treinamento aeróbico, ou de endurance, tem como objetivo aumentar o volume máximo de oxigênio (VO_{2max}), ou seja, a capacidade do sistema cardiovascular liberar sangue a uma grande massa muscular em exercício dinâmico. Para alcançar tal objetivo, pode-se utilizar o treinamento intervalado, o de longa distância e baixa intensidade ou o contínuo de alta intensidade (POWERS e HOWLEY, 2000).

O treinamento intervalado se caracteriza por períodos de trabalho e recuperação alternados e com intensidades e durações diferentes. Esse tipo de treinamento recebeu variações no decorrer do tempo. As variáveis que devem ser levadas em consideração no planejamento de um trabalho intervalado são a quantidade, a intensidade, os intervalos e o número de repetições onde, geralmente, os intervalos de recuperação são tão longos quanto os intervalos de trabalho (TUBINO e MOREIRA, 2003).

O treinamento de longa distância, que utiliza uma faixa de 55% do VO_{2max} e 70% da frequência cardíaca máxima (FC_{max}), utiliza um volume de trabalho maior do que as distâncias de competição (POWERS e HOWLEY, 2000). Neste tipo de treinamento, com uma

duração maior que dez minutos, a fonte de energia é proveniente do metabolismo aeróbico que utiliza carboidratos ou lipídios, sendo que esses possuem um potencial energético maior que os primeiros (PEREIRA e SOUZA Jr, 2007). Entretanto, evidências vêm mostrando que o trabalho intervalado de alta intensidade possui resultados tanto ou mais significativos do que o trabalho de baixa intensidade (HAZELL et al., 2014); (HAZELL et al., 2012); (HICKSON, BOMZE e HOLLOSZY, 1977); (HICKSON e ROSENKOETTER, 1981), pois esse método possibilita a realização de um volume grande de trabalho em curto período de tempo (POWERS e HOWLEY, 2000).

Outro tipo de treinamento é o exercício contínuo de alta intensidade, o qual se caracteriza pelo uso de uma faixa de 80 a 90% do VO_{2max}, durante um período de 25 a 50 minutos em uma taxa de trabalho pouco acima do limiar de lactato e que poderá produzir um ganho bastante grande no aumento da capacidade aeróbica (ESTEVE-LANAO et al., 2005); (BILLAT e KORALSZTEIN, 1996); (PRIEST e HAGAN, 1987). Esse foi o tipo de treinamento escolhido para o desenvolvimento desse trabalho pela escassez de resultados sobre a influência do treinamento aeróbico na realização de exercício exaustivo e fadiga.

O treinamento de endurance auxilia na manutenção da homeostase durante o exercício, ou seja, uma transição facilitada do repouso a uma exigência metabólica estável, um uso menor das reservas de glicogênio muscular e hepático e causa várias alterações cardiovasculares e termorreguladoras (POWERS e HOWLEY, 2000). Além disso, ocorre o aumento da densidade capilar e da quantidade de mitocôndrias nas células musculares (HOLLOSZY e COYLE, 1984), em consequência do aumento das enzimas oxidativas mitocondriais (WIBOM et al., 1992).

Todos esses tipos de treinamento, que tem por característica serem aeróbicos, podem ser sustentados por um período entre 30 e 180 minutos. Com isso, a taxa de consumo máximo de oxigênio fica entre 60 e 85%, ou até menos.

Além disso, sessões de treinamento, ou mesmo exercícios de alta intensidade podem induzir um estado de estresse oxidativo (URSO e CLARKSON, 2003; BLOOMER, 2008), causar micro lesões teciduais (CLARKSON e NEWHAM, 1995), induzir a um processo de inflamação subclínica (ARMSTRONG, 1990), causar dor muscular e, consequentemente, a diminuição do desempenho esportivo conhecido como fadiga (KAY et al., 2001; NOAKES, 2012).

1.2.1 Estresse oxidativo e treinamento físico

O estresse oxidativo (EO) ocorre quando os sistemas antioxidantes enzimático e não enzimático são incapazes de eliminar uma produção exacerbada de Radicais Livres (RL), Espécies Reativas de Oxigênio (ERO) e Espécies Reativas de Nitrogênio (ERN) levando à oxidação de lipídios, proteínas, DNA (Figura 1) e outras moléculas e, consequentemente, a perda da função celular (BLOOMER e GOLDFARB, 2004; HALLIWELL e WHITEMAN, 2004); (HALLIWELL, 2012). Do ponto de vista químico, um radical livre é definido como qualquer átomo, grupo de átomos ou molécula capaz de existir sob forma independente, que contém um ou mais elétrons desemparelhados (DEL MAESTRO, 1980; SOUTHORN e POWIS, 1988; HALLIWELL e GUTTERIDGE, 1984). As ERO e ERN não são classificados como radicais livres, pois não possuem elétrons desemparelhados em sua última camada, mas podem levar a produção dos radicais livres por meio de várias reações químicas (LIANG, C. H. et al., 2012).

Nos organismos aeróbicos, os produtos do metabolismo de carboidratos, gorduras e proteínas são armazenados para posterior oxidação no Ciclo de Krebs e na Cadeia de Transporte de elétrons para a formação da adenosina trifosfato (ATP) (POWERS e HOWLEY, 2000). Este processo ocorre na mitocôndria e tem como produto final a água. A citocromo C oxidase, complexo IV da cadeia de transporte de elétrons, é responsável por catalisar a redução completa de uma molécula de oxigênio (O_2), junto com quatro elétrons e quatro hidrogênios (H^+) para a formação de água. Esse O_2 representa de 95 a 98% da quantidade processada pela mitocôndria, sendo o restante é reduzido univalentemente (HALLIWELL e GUTTERIDGE, 1984). Durante a atividade física intensa, a quantidade total de oxigênio consumida pela cadeia de transporte de elétrons é aumentada entre dez a vinte vezes, aumentando, assim, a produção de RL (BLOOMER, 2008) os quais podem causar danos ás células, inclusive as células musculares e as do fígado (KAKARLA, VADLURI e REDDY KESIREDDY, 2005; HOENE e WEIGERT, 2010).

O primeiro intermediário formado a partir da redução incompleta do oxigênio molecular para a formação de água é o radical superóxido ($O_2^{\bullet -}$), o qual é pouco reativo, entretanto possui uma grande capacidade de geração de radicais secundários altamente tóxicos como o peróxido de hidrogênio (H_2O_2) (GRISHAM, HERNANDEZ e GRANGER, 1986; HALLIWELL e GUTTERIDGE, 1984). Outro exemplo é radical hidroxila ($OH^{\bullet}$), o mais reativo dos intermediários, e formado a partir da reação de H_2O_2 com cobre (Cu^{+1}) ou ferro

(Fe^{+2}), hemoglobina ou mioglobina, na reação de Fenton, ou a partir do superóxido ($O_2^{-\bullet}$) reagindo com peróxido de hidrogênio na reação de Haber – Weiss (LIANG, HO e PATEL, 2000).

Existem ainda as ERN, formadas tanto na mitocôndria quanto no citosol, e entre as mais representativas estão o óxido nítrico ($NO^{\bullet}$) e o peroxinitrito ($ONOO^-$). Por ação da enzima óxido nítrico sintase (NOS), presente no citosol e na mitocôndria, o $NO^{\bullet}$ é gerado a partir da arginina (RIOBO et al., 2002). Este radical é essencial para a vasorregulação, a agregação plaquetária e a neurotransmissão, mas em excesso, inibe a citocromo-c oxidase levando à consequente formação de $O_2^{\bullet-}$ (FORFIA et al., 1999). O $ONOO^-$ é formado a partir de uma reação controlada entre $NO^{\bullet}$ e $O_2^{\bullet-}$ (RADI et al., 1994) e ele pode se difundir para o meio intra ou extracelular e, então, promover a oxidação de lipídios, proteínas e DNA (BECKMAN e AMES, 1996).

Para combater a formação contínua de ERO e ERN, as células possuem um sistema de defesa para a eliminação desses elementos, classificado como enzimático e composto principalmente pelas enzimas Superóxido Dismutase (SOD), Glutationa Peroxidase (GPx), e Catalase (CAT), e o não-enzimático, representado pelas vitaminas A, C, E e D, e a glutationa reduzida (GSH) (HALLIWELL e GUTTERIDGE, 1984).

Durante a prática de exercício físico, a produção de ERO pode ser resultado do metabolismo aeróbico, pois durante a atividade física a captação de O_2 aumenta de 10 a 20 vezes (BLOOMER, 2008), pela ação da xantina oxidase ou reações catalisadas por ferro e/ou da ativação de células fagocíticas como resposta ao dano muscular (PATTWELL e JACKSON, 2004; CHILDS et al., 2001; HELLSTEN et al., 1997). Esse aumento na formação de ERO está relacionado com o volume e a intensidade do exercício (BLOOMER, 2008), tanto em humanos (TURNER et al., 2011; PINHO et al., 2010; NEUBAUER et al., 2008) quanto em animais (SELMAN et al., 2002) e pode causar um decréscimo na produção de força muscular em consequência de alterações no sarcolema (DUDLEY et al., 2006), a regulação do cálcio e a disposição dos filamentos (CALLAHAN, SHE e NOSEK, 2001), ou seja, causar fadiga (REID, 2001; HOLLANDER et al., 1999). De fato, estudos mostraram que fadiga pode ser postergada com a administração de antioxidantes (MOOPANAR e ALLEN, 2006; SUPINSKI et al., 1997).

Por outro lado, o estresse oxidativo causado pelo exercício causa adaptações no sistema antioxidante endógeno (JI, GOMEZ-CABRERA e VINA, 2006) melhorando a sua capacidade de neutralização de ERO é importante para as adaptações das células musculares

ao exercício (HOENE e WEIGERT, 2010; GOMEZ-CABRERA et al., 2005). Dessa forma, já que o treinamento desportivo crônico aumenta a capacidade antioxidante, espera-se que possa postergar, também o aparecimento da fadiga.

Ao contrário do tecido muscular, o SNC mostra-se resistente ao EO que ocorre durante o exercício físico, como demonstra o trabalho de Ackigoz e colaboradores (ACIKGOZ et al., 2006) onde exercício de corrida em esteira não alterou a atividade das enzimas SOD e GPx ou os níveis de TBARS no hipocampo, córtex pré-frontal e estriado de ratos. Radack e colaboradores (RADAK et al., 1995) já tinham observado resultados semelhantes em exercício de esteira, com maior inclinação. Esses efeitos foram vistos sobre a atividade das enzimas SOD, CAT e GPx e peroxidação lipídica no hipocampo e cerebelo de ratos. Entretanto Somani e colaboradores (SOMANI, RAVI e RYBAK, 1995), não encontraram alterações na atividade da SOD no córtex, estriado, cerebelo, medula e hipotálamo de ratos submetidos à um exercício em esteira com 100% do VO_{2max}. Pouco se sabe sobre a influência do EO no desempenho esportivo em exercícios repetidos de alta intensidade e de curta duração.

Com relação ao EO causado pelo exercício físico, pouca informação existe sobre a sua influência no fígado após exercício exaustivo (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 aeróbico no aparecimento de EO no fígado e a sua relação com exercícios exaustivos agudos se fazem necessários.

Uma fonte secundária de EO é a síntese de prostaglandinas, liberadas pelas células musculares pela contração excessiva. Também intermediam a formação de ERO a lipoxigenase, o aumento de catecolaminas durante o exercício (JACKSON, 2005) e atividade da enzima xantina desidrogenase (pouco ativa). Quando se instala uma depleção de ATP, por isquemia e reperfusão, ocorre acúmulo de ADP e aumento intracelular de Ca^{+2}, a enzima xantina desidrogenase é, então, convertida à xantina oxidase, produzindo radical superóxido (JACKSON, 2005). Esse processo se desenvolve principalmente no fígado, onde existe uma grande quantidade desta enzima (RADAK et al., 1995).

Outra fonte de ERO e ERN em consequência do exercício é o dano tecidual e a invasão de células fagocíticas, como neutrófilos e macrófagos, que podem gerar superóxido e

peróxido de hidrogênio que, em parte, tem como função a degradação do tecido lesado e consequente regeneração (TIDBALL, 2005).

1.2.2 Inflamação e treinamento físico

Um dos pontos mais estudados nas pesquisas de ciência do esporte é o dano muscular causado pelo exercício (KANDA et al., 2014) e muitos estudos demonstram os efeitos do exercício sobre o dano muscular, a dor muscular tardia (DMT) e a resposta inflamatória em humanos (SUGAMA et al., 2012; UCHIDA et al., 2009; NIEMAN, 2003; PEDERSEN et al., 2003) e em animais (CARMICHAEL et al., 2010; CARMICHAEL et al., 2006; CARMICHAEL et al., 2005). Esses danos são chamados de microtraumas adaptativos (MTA), são muito importantes para o aumento da performance (BYRNE, TWIST e ESTON, 2004; SMITH, L. L., 2000) e ocorrem tanto em atletas amadores quanto em atletas profissionais e estão ligadas diretamente à intensidade do treino ou à atividade física realizada (PYNE, 1994).

As microlesões podem acarretar o rompimento do sarcolema, edema ou o rompimento do sistema tubular do retículo sarcoplasmático, comprometimento do aparelho contrátil e danos ao citoesqueleto (TAKEKURA et al., 2001). Com isso, os sintomas que acometem o praticante incluem um pequeno aumento de volume no membro afetado (HOWELL; CHLEBOUN e CONATSER, 1993), uma diminuição no ângulo da articulação quando em repouso (ESTON; PETERS, 1999), diminuição na produção de força (KEHL, TREMPE e HARGREAVES, 2000), extravasamento de proteínas para o sangue, como a mioglobina, lactato desidrogenase, aspartato aminotransferase e creatina cinase (CK) (PAULSEN et al., 2012; BRANCACCIO et al., 2008) e dor, chamada de DMT (ARMSTRONG, 1984).

O trauma mecânico causado pelo exercício resultará em uma resposta inflamatória, na qual as proteínas do plasma as células inflamatórias irão infiltrar o tecido lesado com o objetivo de fazer a “limpeza” do local e preparar a fase de regeneração (MACINTYRE, REID e MCKENZIE, 1995; CLARKSON e HUBAL, 2002). As primeiras populações de células inflamatórias a chegar ao local do tecido lesado são os neutrófilos, podendo apresentar um pico de infiltração de 60 minutos e perdurar até 5 dias. Esse tipo celular é responsável por retirar o tecido lesado por fagocitose (TIDBALL, 2005) e essa ação gera, também ERO, resultado da atividade das enzimas NADPH oxidase e Mieloperoxidase (MPO) (TIDBALL, 2005). Os monócitos são a segunda população a migrar para o tecido lesado e, quando passam

da circulação para o tecido, são chamados de macrófagos. Essa população celular tem por objetivo a remoção e regeneração do tecido lesado e sua migração tem um pico em 24 horas e podendo permanecer no tecido entre 9 e 14 dias (TIDBALL e WEHLING-HENRICKS, 2007).

A infiltração dessas células é organizada por várias proteínas, dentre elas estão as citocinas (PYNE, 1994), responsáveis pelos eventos inflamatórios e seus efeitos (SMITH, 2000; (MOLDOVEANU, SHEPHARD e SHEK, 2001). As citocinas sinalizam outros tecidos como cérebro e fígado ocasionando, assim, a resposta inflamatória (SMITH, 2000). Estudos têm sugerido que essa resposta esteja ligada a alguns pontos negativos como: dor, regeneração muscular deficiente, e déficits de desempenho (SMITH, L. K., 1991); e tanto o dano mecânico quanto o processo inflamatório podem influenciar tanto a musculatura esquelética quanto o SNC (SHEN et al., 2001; DANTZER, 2004b).

Citocinas são pequenos peptídeos que possuem um papel imunoregulador (AKIRA, TAGA e KISHIMOTO, 1993) e são moléculas mensageiras produzidas, não somente pelas células do sistema imunológico, mas também produzidas no cérebro, músculo esquelético e tecidos do sistema endócrino (SCHOBITZ et al., 1993; BARTOCCIONI; MICHAELIS; HOHLFELD, 1994; KELLER ET AL., 2001). A sequência de liberação de citocinas é composta pelo Fator de Necrose Tumoral – alfa (TNF-α), Interleucina 1-beta (IL-1β), Inteleucina 6 (IL-6), receptor antagonista de IL-1 (IL-1ra) e receptores solúveis de TNF-α (sTNF-α) sendo as três primeiras expressas na musculatura esquelética após exercício físico (AKIRA e KISHIMOTO, 1992; AKIRA, TAGA E KISHIMOTO, 1993; DINARELLO, 1991). Entretanto, ainda são necessários estudos sobre a produção de citocinas no SNC e a sua relação com a diminuição do desempenho esportivo.

Elas são classificadas como pró-inflamatórias (tipo Th 1, estimulatórias) ou anti-inflamatórias (tipo Th 2, inibitórias) (MOSMANN et al., 1986) e estão envolvidas, além da resposta imunológica, em uma variedade de processos fisiológicos e imunológicos tanto no Sistema Nervoso Periférico (SNP) quanto no SNC e podem ter sua produção modulada por neurotransmissores (WOICIECHOWSKY et al., 1998; ELENKOV, CHROUSOS e WILDER, 2000).

As principais citocinas pró-inflamatórias são IL-1β e TNF-α e são consideradas como citocinas de alarme, pois são secretadas em resposta a eventos relacionados diretamente à lesão do tecido como a produção de prostaglandinas e ERO. Elas são liberadas por macrófagos, células do endotélio e células musculares lisas e esqueléticas (BASSEL-DUBY e

OLSON, 2006) e sua função é de facilitar a migração de monócitos e neutrófilos para o local da inflamação (MOLDOVEANU, SHEPHARD e SHEK, 2001). Em relação ao exercício, essas citocinas podem causar alterações no apetite, na libido e no humor (SMITH, 2004).

Das citocinas anti-inflamatórias, a IL-6 é considerada o maior regulador da resposta inflamatória. Ela é produzida pela musculatura esquelética e pelos leucócitos, é liberada em resposta à IL-1β e TNF-α e ERO e está diretamente relacionada à intensidade e duração do exercício físico (PETERSEN e PEDERSEN, 2005).

Esses potentes mediadores regulam uma variedade de processos fisiológicos e patológicos que incluem o início e a coordenação das respostas imunológicas, além de ter um papel importante tanto na saúde quanto em processos patológicos humanos (HENDERSON et al., 2000); (LLOYD E JOHNSTON, 1993). O exercício físico intenso leva à secreção de citocinas (MOLDOVEANU, SHEPHARD e SHEK, 2001; PEDERSEN, 2000), algo análogo à inflamação sistêmica como sepses ou trauma, entretanto a prática de exercício leva a uma concentração menor de citocinas (SHEPHARD, 2001).

As citocinas podem alcançar o SNC atravessando a barreira hemato-encefálica através dos órgãos circuventriculares, mesmo em condições basais (WATKINS; MAIER; GOEHLER, 1995). A ativação de células endoteliais por citocinas liberam segundos mensageiros, como Óxido Nítrico (ON) e prostanóides sintetizados por enzimas como a óxido Nítrico Sintase induzida (iNOS) e a ciclooxigenase – 2 (COX-2), os quais representam uma rota indireta de ação no SNC (HASHIMOTO, 1991; (WONG et al., 1995).

As citocinas podem exercer seu efeito no SNC direta ou indiretamente, podendo ser produzidas por células neuronais no SNC como resposta a infecções, inflamação, isquemia e outras patologias cerebrais (BREDER et al., 1994; STERNBERG, 1997); WOICIECHOWSKY et al., 1998) e, também, podem estar relacionados com a diminuição do desempenho esportivo (NOAKES, 2012; DONATTO et al., 2010; CARMICHAEL et al., 2005).

Os resultados apresentados por Bovorikova e colaboradores (2000) demonstraram que a acetilcolina (ACh) pode ter um papel no combate à inflamação por inibir a produção de TNF-α, IL-1β por macrófagos, os quais possuem expressão de receptores nicotínicos de acetilcolina.

A acetilcolina (ACh) é um neurotransmissor envolvido em várias ações no SNC, no SNP e na Junção Neuromuscular (JNM) (BRUNEAU e AKAABOUNE, 2006). Sua síntese é realizada pela enzima Colina-O-Acetil Transferase (ChAT), a partir da acetil-coenzima A e

colina. Segundo Dale (1914), as ações da acetilcolina podem ser classificadas por como ações nicotínicas e/ou muscarínicas, baseado nos subtipos de receptores colinérgicos capazes de se ligar à nicotina e à muscarina. Os receptores colinérgicos nicotínicos pertencem à família de receptores ionotrópicos, canais iônicos que, quando abertos, são permeáveis ao sódio (Na^+) e ao potássio (K^+), e são responsáveis por uma transmissão sináptica rápida. Esses receptores são formados por cinco subunidades proteicas e estão distribuídos em vários tecidos, incluindo o cérebro e músculos (MILLAR e GOTTI, 2009). Já os receptores muscarínicos também são amplamente distribuídos em diversos sistemas e participam de várias funções vitais como a redução da frequência e da força da contração cardíaca, relaxamento de vasos sanguíneos e a constrição das vias respiratórias (BRADY et al., 2012).

A atividade da ACh é controlada pela ação da Acetilcolinesterase (AChE), enzima responsável pela sua hidrólise (DARVESH et al., 2003). Pode-se especular que um aumento na atividade da AChE, e uma consequente diminuição na concentração de ACh, pode causar uma diminuição do processo anti-inflamatório (TAGLIARI et al., 2011). Com isso, uma alteração na atividade da enzima pode estar ligada, de alguma forma, com a diminuição do desempenho esportivo e, também, a fadiga.

1.3 Fadiga neuromuscular

Fadiga é a diminuição progressiva da capacidade de contração muscular (KAY et al., 2001) ou a incapacidade de manter um nível adequado de produção de força (EDWARDS, R. H., 1981). Esse fenômeno é complexo e multifatorial e tem como causa o tipo de exercício que se está executando (ENOKA e STUART, 1992).

Seu estudo e entendimento começou com o trabalho do fisiologista Angelo Mosso (1846 – 1910) que dizia que a fadiga iniciava assim que se iniciava o exercício (NOAKES, 2012). Os estudos sobre a fadiga levaram a ideias de que o cérebro (MARCORA, STAIANO e MANNING, 2009) e a musculatura esquelética (AMANN et al., 2006; AMANN e DEMPSEY, 2008) alteram suas funções durante o exercício, diminuindo a capacidade de contração, que a fadiga é um estado emocional (ST CLAIR GIBSON et al., 2003) e parte de uma regulação complexa (NOAKES, 2011).

A presença de agentes químicos estimulantes (SWART et al., 2009) como a cafeína (HOGERVORST et al., 2008), analgésicos (SGHERZA et al., 2002; MAUGER, JONES e

WILLIAMS, 2010) e IL-1β (CARMICHAEL, DAVIS e MURPHY, 2006) alteraram a capacidade de realização de exercício bem como o tratamento placebo.

A fadiga também é considerada um fator de proteção contra danos às fibras musculares (RACINAIS et al., 2008). A tentativa da manutenção da produção de força pelo sistema neuromuscular é realizada por uma série de mecanismos no sentido de postergar a interrupção da realização da tarefa (BOYAS e GUEVEL, 2011). Esse evento pode ter duas origens diferentes: a "fadiga central" e a "fadiga periférica".

A fadiga central engloba processos fisiológicos supra espinhais e espinhais caracterizados pela progressiva diminuição da ativação muscular (BOYAS e GUEVEL, 2011). A fadiga se instala em consequência da redução da descarga nos motoneurônios e falha no recrutamento por parte das unidades motoras (TAYLOR, TODD e GANDEVIA, 2006). Também ocorre uma inibição do córtex motor pela estimulação dos nervos aferentes tipo III e IV, quando estimulados por isquemia (LAGIER-TESSONNIER, BALZAMO e JAMMES, 1993) e o acúmulo extracelular de potássio e lactato, causando uma queda na taxa de disparo pelos motoneurônios e uma inibição do comando pelo córtex motor (AMENT e VERKERKE, 2009)

A diminuição do desempenho pode, também, ser influenciada pela ação de neurotransmissores como, por exemplo, a serotonina (5-HT), dopamina (DA) e noradrenalina (NA), que podem influenciar negativamente na continuidade da atividade física, aumentando o sentimento de letargia e cansaço e reduzindo a capacidade de recrutamento de fibras (MEEUSEN et al., 2006). Outros estudos também sugerem que a acetilcolina e o glutamato estão envolvidos no desenvolvimento da fadiga central (ABDELMALKI et al., 1997); (CONLAY, SABOUNJIAN e WURTMAN, 1992). Alguns trabalhos indicam ainda que a produção de citocinas pela musculatura, também pode estar ligada à fadiga central (CARMICHAEL et al., 2006) pois elas são potentes efetores do SNC (DANTZER, 2004a).

Entretanto, o estado biológico da atleta no início do exercício pode não ser o único fator levado em consideração. Existem trabalhos mostrando que a fadiga central pode ser influenciada por fatores psicológicos (RENFREE et al., 2012), como a extensão da sua fadiga mental (MARCORA, STAIANO e MANNING, 2009), a capacidade de motivação ou experiência anterior (CORBETT, BARWOOD e PARKHOUSE, 2009); (FOSTER et al., 2009), a crença em si mesmo (MICKLEWRIGHT et al., 2010) e, até, crenças supersticiosas (DAMISCH, STOBEROCK e MUSSWEILER, 2010).

Na mesma linha de raciocínio, outros fatores são abordados por pesquisadores como uma possível recompensa monetária (CABANAC, 1986), experiência prévia na prova/exercício em sua duração (ANSLEY et al., 2004) e até a presença de outros competidores podem influenciar o estado de fadiga, principalmente se esses competidores estão no mesmo nível de excelência (CORBETT, BARWOOD e PARKHOUSE, 2009).

Já a fadiga periférica é caracterizada pela diminuição da propagação do sinal na junção neuromuscular, problemas no aparato contrátil muscular a regulação do uso do ATP pelas fibras musculares (MACINTOSH e SHAHI, 2011). Alguns autores colocam que a perda da homeostase de Ca^{2+} e, também, o seu aumento no citoplasma pode ser uma das causas da fadiga periférica (BINDER-MACLEOD e RUSS, 1999; SEJERSTED e SJOGAARD, 2000). Há, também, a hipótese da diminuição da disponibilidade de substratos energéticos durante a atividade física como fator predominante para a diminuição da performance (SAHLIN, TONKONOGI e SODERLUND, 1998).

1.4 Anti-inflamatórios não Esteroidais

Muitos treinadores e preparadores físicos trabalham no intuito de preparar seus atletas para performances máximas (TSCHOLL et al., 2010). Entretanto a diferença entre uma prática correta, ética e moral e a prática ilegal é muito tênue, principalmente no que diz respeito ao uso de drogas legais, a efeitos colaterais e, talvez, a efeitos na melhora na performance e/ou aceleração da recuperação (TSCHOLL et al., 2007).

A rotina diária de treinamento dos atletas e as competições que estes participam podem causar danos musculares, inflamação e, também, a diminuição da performance (MCHUGH et al., 1999). Em muitos casos, o uso de Anti-inflamatórios Não Esteroidais (AINEs) é uma prática comum para diminuir a inflamação e a dor muscular em atletas (MAHLER, 2001; LIPPI et al., 2006), as quais são responsáveis pela perda do rendimento esportivo (CIOCCA, 2005; ALARANTA et al., 2006; ALARANTA, ALARANTA e HELENIUS, 2008). Estudos têm mostrado o uso frequente de AINEs por atletas em substâncias que não estão inseridas na lista de proibições da Agência Mundial Anti Doping (AMA) (ALARANTA, ALARANTA e HELENIUS, 2008) e são muito usados por atletas, tanto por prescrição quanto por automedicação (HERTEL, 1997) e, também, por atletas adolescentes (WARNER et al., 2002).

Os AINEs são medicamentos responsáveis pelo combate à inflamação e à dor, agindo na inibição da enzima ciclooxigenase (COX) (RAINSFORD, 2007). A COX catalisa a oxidação do ácido araquidônico (AA), liberado da membrana celular pela ação da PLA_2 em resposta a estímulos térmicos, mecânicos e/ou químicos (LEES et al., 1991), tendo como produto final as prostaglandinas (PG), substâncias químicas que possuem função autócrina e parácrina e são mediadoras do processo de inflamação (CONNOLLY, SAYERS e MCHUGH, 2003) e aumentam a sensibilidade dos nociceptores, ligados a sensação e dor (BEAR, CONNORS e PARADISO).

Em especial, a prostaglandina E_2 (PGE_2) possui capacidade pró-inflamatória e está relacionada à dor e, dessa forma, os AINEs podem reduzir a dor e a inflamação através da inibição da produção de PGE_2 (MENDIAS, TATSUMI e ALLEN, 2004).

Dessa forma, atletas fazem uso de AINEs no intuito de diminuir o processo inflamatório e a dor após exercício de alta intensidade e, assim, diminuir as causas da fadiga (KRENTZ et al., 2008).

1.5 Ibuprofeno

O Ibuprofeno (IBU) é um dos AINEs mais usados entre atletas e pela população, talvez, pelo fácil acesso, baixo preço e baixa incidência de efeitos colaterais quando comparado a outros fármacos similares (ALARANTA et al., 2006). Provavelmente, é o medicamento menos tóxicos e raramente é associado com doses letais pelo consumo (acidental ou auto-medicação) ou com reações adversas (RAINSFORD, 2009).

Apresenta os enantiômeros S(+), o mais ativo como inibidor da síntese de PG, e R(-), menos ativo mas com alguma propriedade relevante à ação anti-inflamatória do medicamento (BROCKS; JAMALI, 1999; GRAHAM e WILLIAMS, 2005). Entre 40 – 60% da forma R (-) é metabolicamente convertida em S(+) no trato intestinal e fígado após a absorção (JEFFREY et al., 1991).

O IBU é rapidamente absorvido e o fármaco tem um pico de concentração plasmática em aproximadamente 1 a 2 horas e essa variação está ligada à formulação farmacêutica e o seu tempo de meia-vida ($t_{1/2}$) é de aproximadamente 2 horas (GRAHAM e WILLIAMS, 2004; HIGTON, 1999). Não existem evidências sobre a formação de metabólitos ativos suficientes para causar alguma modificação do fígado ou de outras proteínas que podem causar toxicidade (GRAHAM e HICKS, 2004).

A droga age em diferentes vias inflamatórias envolvidas na inflamação aguda e crônica (RAINSFORD, 1999) e a principal ação farmacodinâmica é a inibição da COX-1 e COX-2 impedindo, assim, a formação de PGE2 (BURIAN e GEISSLINGER, 2005); (HINZ, DORMANN e BRUNE, 2006). O ibuprofeno pode penetrar no SNC de modo que pode contribuir para o efeito analgésico central (BANNWARTH et al., 1995); (MARTINEZ et al., 2002); (MCCRORY e FITZGERALD, 2004); (KOKKI et al., 2007).

Assim, sabendo que a prática de exercícios de alta intensidade leva a um estado de EO, inflamação sub clínica, sensação de dor muscular e diminuição do desempenho esportivo, afetando tanto a musculatura esquelética quanto o SNC, e atletas fazem uso de AINEs para evitar essas consequências, é de suma importância saber como o treinamento físico crônico influencia esses tecidos quando da prática de exercícios à exaustão.

Dessa forma torna-se importante o entendimento de como o ibuprofeno pode agir no sentido de adiar os efeitos deletérios da realização de exercício repetidos de alta intensidade e curta duração.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos do treinamento aeróbico no processo inflamatório e estresse oxidativo no córtex e músculo e a sua relação com exercícios à exaustão, bem como as adaptações causadas pelo treinamento aeróbico no fígado de ratos.

2.2 Objetivos específicos do manuscrito

Avaliar em músculo esquelético e córtex cerebral:

- O efeito de um treinamento aeróbio de seis semanas de natação nos conteúdos de TNF-α e IL-1β, atividade da enzima acetilcolinesterase, geração de EROs e carbonilação proteica em córtex e músculo de ratos.

- O efeito da administração de ibuprofeno no tempo à exaustão e sensação de dor em ratos treinados e sedentários submetidos a três sessões de exercício exaustivo.

- O efeito da administração de ibuprofeno na atividade da enzima acetilcolinesterase e conteúdo de TNF-α e IL-1β em córtex e músculo de ratos de ratos treinados e sedentários submetidos a três sessões de exercício exaustivo.

- O efeito da administração de ibuprofeno na geração de EROs e carbonilação proteica em córtex e músculo de ratos de ratos treinados e sedentários submetidos à três sessões de exercício exaustivo.

2.3 Objetivos específicos do artigo

Avaliar no fígado:

- O efeito de um treinamento aeróbio de seis semanas de natação nos conteúdos nos marcadores de EO: carbonilação proteica, espécies reativas ao ácido tiobarbitúrico e diclorofluoresceina no fígado de ratos.

- O efeito de um treinamento aeróbio de seis semanas de natação na atividade da enzima SOD e níveis de GSH no fígado de ratos.

- O efeito de um treinamento aeróbio de seis semanas de natação nos conteúdos nos marcadores de Viabilidade mitocondrial: redução de metil-tetrazol (MTT) e potencial de membrana ($\Delta\Psi$) no fígado de ratos.

3 MANUSCRITO – ADMINISTRAÇÃO DE IBUPROFENO AUMENTA O TEMPO À EXAUSTÃO: UM POSSÍVEL PAPEL PARA A PREVENÇÃO DA FADIGA INDUZIDA PELO EXERCÍCIO.

Título Original

Ibuprofen intake increases exercise time to exhaustion: a possible role for preventing exercise-induced fatigue

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IBUPROFEN INTAKE INCREASES EXERCISE TIME TO EXHAUSTION: A POSSIBLE ROLE FOR PREVENTING EXERCISE-INDUCED FATIGUE

Abstract

Background: Although Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) intake by athletes prevents soreness after exhaustive exercise, little is known concerning their role on exercise performance.

Purpose: Assess the effects of ibuprofen intake on an exhaustive protocol test after 6 weeks of swimming training in rat cortex and muscle.

Study Design: Controlled laboratory study.

Methods: Animals were divided in Sedentary and Training groups. Training group performed 6 weeks of swimming training and, after, groups were then divided in two subsets: sedentary saline/or ibuprofen, training saline/ or ibuprofen. Afterwards, three repeated swimming bouts were performed by groups. Ibuprofen (15 mg/kg) was administered after each swimming bout. Pain measurements were performed, and inflammatory and oxidative stress parameters were assayed in cerebral cortex and gastrocnemic muscle of rats.

Results: Training, ibuprofen administration or both combined ($p<0,05$; 211 ± 18s, 200 ± 31s and 279 ± 23s respectively) increased exercise time to exhaustion. Training decreased acetylcholinesterase (AChE) activity ($p<0,05$;149 ± 11) in cerebral cortex. Ibuprofen intake decreased the AChE activity after the exhaustive protocol test in trained and sedentary rats ($p<0,05$; 270 ± 60; 171 ± 38 and 273 ± 29 respectively), and also prevented neuronal tumor necrosis factor-α (TNF-α) and interleukin (IL 1β) increase.

Conclusion: Fatigue elicited by this exhaustive protocol may involve disturbances of the central nervous system (CNS).

Clinical relevance: Additive anti-inflammatory effects of exercise and ibuprofen intake support the hypothesis that this combination may constitute a more effective approach, as ergogenic aids may be a useful means to prevent exercise-induced fatigue.

Keywords: Exercise training, NSAIDs intake, exhaustive exercise, inflammation, AChE activity.

INTRODUCTION

Evidence accumulated over many decades illustrates the beneficial role of physical activity in maintaining and improving neural and muscular functions in humans and animals[28]. However, high-intensity exercise is a known cause of microtrauma in the muscle, leading to inflammation, reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation[36]. Considering that tissue damage occurs after infiltration of phagocytic cells[49], it is not surprising that a variety of biochemical markers as pro inflammatory and ROS production are related to impaired exercise performance and fatigue[37].

Fatigue is characterized by performance decrease linked with an increase in the real and/or perceived difficulty to overcome a task and/or exercise [33], and it has been divided in central – metabolic, circulatory, neurotransmitter, thermodynamic changes or others disturbances of Central Nervous System (CNS)[22] and peripheral – linked to depletion of substrates, accumulation of metabolites and changes at the neuromuscular junctions (NMJs)[43]. From this perspective, considerable body of evidence suggests that inflammation and immunological regulation exerted by exercise are involved in alterations on neuroendocrine and neurotransmitter activity[59].

In fact, studies have demonstrated that aerobic exercise decreases the production of pro inflammatory cytokines[48], leading to beneficial health effects in patients with chronic diseases[21]. Furthermore, the ability of exercise to increase parasympathetic tone and heart rate variability and to enhance acetylcholine levels in the brain may promote additional benefits in these diseases[14], since acetylcholine (ACh) has been proved to significantly attenuate the release of pro inflammatory cytokines [46].

Nevertheless, although integrative physiology studies have shown potential link between oxidative stress and immune system interplay towards behavioral modulation[40] little

is known regarding the role of pro inflammatory cytokines, ROS production, and neuronal cholinergic pathway on experimental fatigue-induce sets after exercise training[39]. In this context, several procedures have been undertaken in an attempt to investigate pathways related to exercise-induced fatigue. In this scenario, ergogenic aids have been tested in order to increase performance outcomes (for a review see [58]).

Previous surveys on the use of non-steroidal anti-inflammatory drugs (NSAIDs) by athletes who participate in international sports events have demonstrated this sort of drugs to be the most frequently used among high-class athletes[11]. The NSAIDs are drugs commonly used to reduce acute pain[18] and muscle injuries, related to inflammation caused by stress generated by both acute and chronic exercise[67]. The ibuprofen, is among the most commonly used NSAIDs by athletes[67].

The anti-inflammatory action of this NSAID is based on the inhibition of prostaglandin endoperoxide synthase (PTGS) which responds to the synthesis of endoperoxidase prostaglandins (PGs)[52]. Consequently, the production of cytokines secreted by macrophages at the injury site is hampered, reducing the inflammatory response. Furthermore, researchers have shown that ibuprofen reduces the activity of inducible isoform of nitric oxide synthase (iNOS), which produces changes in transmembrane ion flux[31] and therefore is also involved in inflammatory processes[16]. Interestingly, an elegant study described by Da Silva and col leagues[13] revealed that athletes who participated in the anti-doping control of the XV Pan-American Games reported high NSAIDs intake during competition when compared to out-of-competition athlete, suggesting that these medicaments may be used as ergogenic aid.

However, the role of these medicaments is not completed defined. Thus, the purpose of this study was to investigate the effects of ibuprofen administration on pain, inflammatory

markers, and ROS production in cerebral cortex and gastrocnemic muscle of trained rats submitted to repeated exhaustive swimming bouts.

MATERIALS AND METHODS

Animals

Male Wistar rats (180-250g) were 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 and water *ad libitum*. All experiments were conducted in accordance with national and international legislation and with the approval of the Ethics Committee for Animal Research of the university. Assay reagents were purchased from Sigma (St Louis, MO, USA).

Study design

In this study animals were randomly divided into training and sedentary groups. The training group performed a 6-weeks swimming training with body weight overload. After 24 h of the last training session, both training and sedentary groups performed a lactate threshold (LT) test to assay training adaptations. Subsets of training and sedentary groups were sacrificed, after LT test, in order to assess possible training effects upon the biomarkers herein assayed. After LT test, trained and sedentary groups were subdivided in saline or ibuprofen administration groups, and rats performed 3 exhaustive swimming bouts. Each bout was separated for a 72 h time period. Pain measurements were performed before each exhaustive

swimming bout. Rats were then sacrificed and cerebral cortex and gastrocnemic muscle were immediately removed for further biochemical assays. Figure 1 depicts the study design.

Water Adaptation

Rats were adapted to the water before the beginning of the experiment. The adaptation consisted on keeping the animals in shallow water at 31 ± 1°C between 9:00 to 11:00 a.m. The adaptation period was carried out during the week before the swimming training onset. The purpose of the water adaption was to reduce stress without promoting exercise training adaption.

Training protocol and lactate threshold test

Animals were weighed and randomly assigned to the following groups: (1) sedentary and (2) training. The exercise training protocol consisted of 6 weeks, 5 sessions per week of 60 min each. The training tank used in this study was 80 cm in length, 50 cm in width and 90 cm in depth, and the swimming was always performed in water temperature of 31 ± 1°C between 10 to 16 h am. Animals were subjected to swimming training with a 5% body weight overload attached to the back to improve endurance[23]. Along with the training session, sedentary rats 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.

After 6 weeks of swimming training, a test protocol was used to determine the lactate threshold test (LT) in sedentary (n=8) and trained rats (n=8). The LT test was carried out according to the protocol described by Marquezi et al.[34] with few modifications. The test consisted on 3 swimming bouts with progressive overload corresponding to 5%, 7% and 9%

of each animal body weight for a period of 3 min for each load, with a 1 min resting period between bouts. During the resting periods, 25 µL of blood were collected from the tail vein for lactate concentration assay, resulting in a total of 4 blood samples, measured with a lactimeter (Accutrend® Plus, Roche Diagnostics GmbH, Germany). The LT for each animal was calculated based on the graphic inflection point when plotting lactate concentration against the corresponding exercise workload. Twenty four hours after the LT assay, sedentary and trained animals' subsets were killed by decapitation and cerebral cortex and gastrocnemic muscle were immediately removed and immediately frozen at -80 °C for further biochemical assays.

Exhaustive protocol test

Three days after the LT test, all four groups (sedentary and training groups subdivided in saline or ibuprofen) performed the exhaustive protocol test according to de Araujo et al.[15] with few modifications. The protocol consisted on three repeated exhaustive swimming bouts: 72 h, 144 h, and 216 h after the LT test (Figure 1). Animals swam individually in the tank with an overload of 13% of body weight until exhaustion in order to determine the time to exhaustion. Exhaustion was characterized by the moment at which animals were no longer able to maintain themselves in the water surface, reaching 10 s submerged[15]. When exhaustion was reached animals were taken out from the tank, dried, and, after the third bout, sacrificed and cerebral cortex and gastrocnemic muscle were immediately removed and immediately frozen at -80 °C for further biochemical assays.

Ibuprofen administration

In order to evaluate the effect of ibuprofen administration on time to exhaustion in sedentary (n=7) and trained (n=7) rats submitted to the exhaustive protocol test, a subset of animals were supplemented with ibuprofen or saline. Ibuprofen was dissolved in water and, as saline, injected via intragastric gavage after LT test until 24 before last exhaustion bout at a dose of 15 mg/kg[31].

Hyperalgesia Test

For measuring the thermal hyperalgesia the Plantar Test (Ugo Basile, Varese, Italy) was used according to Hargreaves et al[25]. Briefly, 1 h before each exhaustive swimming bout rats were accustomed to the place of observation and an infrared beam generated by a 60 W lamp was focused on the animal's right hind leg. The time required for the animal to withdraw the paw of the incident ray was recorded automatically and used as an index of nociception. Significant decreases in paw withdrawal time compared to baseline are considered as hyperalgesia.

Nociception assessment

The mechanical allodynia is considered an indicator of nociception and was herein assessed as previously described by Chaplan et al[9]. Rats were placed in Plexiglas boxes (9 cm x 7 cm x 11 cm) on elevated, wire-mesh platforms in order to access the ventral surface of the hind paws that were in contact with one of seven von Frey hairs (6-100 g). The von Frey hairs were applied perpendicularly to the paw’s surface in order to cause a slight buckling for

approximately 2 s. The 50% withdraw threshold was determined using the up and down method of Dixon[17]. In this scenario, nociception assessment was initiated with the 15 g hair. Stimuli were continuously consecutive whether ascending or descending. Paw withdrawal thresholds were verified before each bout of the exhaustive protocol test.

Acetylcholinesterase (AChE) activity

The AChE activity was determined by the method of Ellman et al.[20]. Cerebral cortex and gastrocnemic were homogenized 1:20 with 10mM Tris HCl, pH 7.4. The homogenates were centrifuged at 1000 x g for 20 min at 4 ºC and the supernatant was used as enzymatic source. The mixture assay contained 1.04 mM DTNB (5,5'-dithiobis-2-nitrobenzoic acid), 24 mM PBS (pH 7.2) and 100 μL of enzymatic material. It was pre-incubated for 2 min at 28 °C and the reaction was started with the addition of 0.83 mM acethiolcholine. The product from the thiocholine reaction with DTNB was determined at 412 nm every 30 s during 2 min with an absorption coefficient of 0.0136 M-1cm-1 for the TNB anion. The specific activity was expressed as nmol acethiolcholine hydrolyzed/h/mg protein.

Interleukin 1beta (IL-1β) and tumor necrosis factor alpha (TNF-α) content

The cerebral cortex and gastrocnemic were homogenized in a solution containing bovine serum albumin (BSA 10 mg / ml), 2 mM EGTA, 2 mM EDTA and 0.2 mM PMSF in PBS (pH 7.4) for the IL-1β and TNF-α assays. Cytokine content were measured according to the protocol of the manufacturer, using a commercially available ELISA kit from R & D Systems (Minneapolis, MN, USA). The results are expressed as pg/mg protein for tissue homogenate.

Estimation of ROS production

Production of ROS was estimated with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), as described by Ali et al[2]. Briefly, tissues were homogenized in 2.5 ml of saline solution (0.9% NaCl). Aliquots of 2.5 ml were incubated in the presence of DCFH-DA (5 μM) at 37 °C for 60 min. The DCFH-DA is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was established with standard DCF (0.1 nM to 1 μM), and ROS levels were expressed as percentages of control.

Protein carbonyl levels.

Protein oxidation in cerebral cortex, gastrocnemic was measured as concentration of protein carbonyls formed, and the levels were determined using 2,4 dinitrophenylhydrazine (DNPH) assay[30]. The cortex were divided into two portions containing 1 mg prot/mL in each. To one portion, 1 ml of 2 N HCl was added and incubated at room temperature shaking intermittently for 1 h. The other portion was treated with 1 ml of 10 mM DNPH in 2 N HCl and incubated by shaking intermittently for 1 h at room temperature. After incubation the mixture was precipitated with 10% TCA and centrifuged. The precipitate was washed thrice with 1 ml of ethanol: ethyl acetate (1:1). The final protein precipitate was dissolved in denaturation buffer (3% SDS and 150 mM NaH_2PO_4; pH 6.8) and the absorption at 370 nm

(DNPH-treated sample minus sample blank) was determined. Carbonyl content was calculated using the molar extinction coefficient of 22,000 and expressed as nmol carbonyls/mg protein.

Protein determination

The protein content was measured colorimetrically by the method of Bradford[8] using bovine serum albumin (1 mg/ml) as standard.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, Ins, Chigaco, IL) version 17 was used for all analyses. Data were expressed as mean ± standard error of means (SEM). Significance was assessed by one- or two-way analysis of variance (ANOVA), followed by Newman–Keuls's Test for post-hoc comparison when appropriate. Statistical significance was set at $p<0.05$.

RESULTS

In the present study, a significant increase in total body weight in sedentary versus trained rats along the 6 weeks of swimming training was found [$F(1,12)=20.58$; $P<0.05$; Figure 2A]. In addition, statistical analysis revealed a clear stabilization of the blood lactate concentration in trained group for the lactate threshold assay [$F(1,12)=22.56$; $P<0.05$; Figure 2B].

Figure 3 shows the effect of training, ibuprofen intake or both combined on time to exhaustion. Swimming training increased time to exhaustion, as expected, on the first [$F(3,26)=24.31$; $P<0.01$; Figure 3A] and second [$F(3,26)=7,03$; $P<0.05$; Figure 3B] bouts of exhaustive exercise protocol when compared to sedentary saline group. At same time, the experimental findings also showed that ibuprofen administration in sedentary rats as well as the combination of training and ibuprofen administration exhibited an higher increase on time to exhaustion on the third bout [$F(1,26)=10,28$; $P<0.05$; Figure 3C] of exhaustive protocol test when compared to the other groups, showing that this combination may have a synergistic effect on fatigue-related exercise.

The results presented in this report revealed that this fatiguing protocol and/or ibuprofen administration had no effect on total body weight in sedentary or trained rats after the exhaustive protocol test [$F(3,25)=21,79$; $P<0.05$; Figure 4].

Considering that analgesic drugs are commonly consumed to reduce or prevent pain and soreness after exhaustive exercise[61,3], the increase on time to exhaustion induced by training and/or ibuprofen intake was investigated. Statistical analysis revealed that repeated swimming bouts preceded by training and/or ibuprofen intake had no effect on hyperalgesia in Hargreaves or mechanical allodynia tests. These results indicated that effect of training and/or ibuprofen intake on time to exhaustion was not due to an antinocipetive effect of this drug (data not shown).

In the present study 6 weeks of swimming training decreased the neural AChE activity [$F(1,12)=18,40$; $P<0.05$; Figure 5A]. At same time, after three bouts of exhaustive exercise, both aerobic training as ibuprofen prevented the increase of AChE activity [$F(3,25)=10,68$;$P<0,05$; Figure 5B] when compared with sedentary/saline group. At same time, there was no statistical difference of AChE activity in muscle (data not shown).

Since biochemical markers as pro-inflammatory cytokines are related to fatigue[34], the effect of exercise training and ibuprofen treatment on pro-inflammatory cytokines TNF-α and IL-1β was investigated in cerebral cortex and gastrocnemic muscle of rats after the exhaustive protocol test.

The 6 weeks of swimming training did not elicit detectable alterations in pro-inflammatory cytokine TNF-α (Figure 6A) but after three bouts of exhaustive exercise, just ibuprofen administration decreased the TNF-α levels in cerebral cortex [$F(3.24)=11,03$; $P<0.05$; Figure 6B]. Unlike, in gastrocnemic muscle, aerobic training decreased the content of TNF-α [$F(1,13)=30,78$; $P<0.05$; Figure 6C] but three bouts of exhaustive exercise did not elicit any changes in this cytokine levels (Figure 6D).

Likewise, there were no statistical differences in pro-inflammatory cytokines IL-1β levels (Figure 7A) in cortex after aerobic training. After last bout of exhaustive exercise, statistical analysis shown a decrease in IL-1β levels with ibuprofen administration and an higher decrease with the combination with exercise [$F(3,26)=22,57$; $P<0.05$; Figure 7B] after the exhaustive protocol test. In the meantime, swimming training decreased the IL-1β content in muscle [$F(1,13)=19,99$; $P<0.05$; Figure 7C] but exhaustive test also did not changed the IL-1β content (Figure 7D).

Considering that tissue damage occurs after infiltration of phagocytic cells, MPO activity was analyzed in cortex and muscle. After three bouts of exhaustive exercise, both aerobic training as ibuprofen prevented the increase of MPO activity [$F(3,25)=5,26$; $P<0,05$; Figure 8] when compared with sedentary/saline group. At same time, there was no statistical difference of AChE activity in cerebral cortex (data not shown).

Given the possible relationship between inflammation and oxidative stress, DCFH-DA oxidation and protein carbonylation were assayed in cerebral cortex and gastrocnemic muscle after the swimming training and exhaustive protocol test. Training did not elicit any changes

in neuronal DCFH-DA oxidation nor in cortex nor in muscle (Figures 8A and C). But, at same time, both exercise and ibuprofen administration decreased neuronal DCFH-DA oxidation in cortex [$F(3,24)=10,015$; $P<0.05$; Figure 8B] and in muscle [$F(3,25)=23,83$; $P<0.05$; Figure 8D].

In the other hand there were no statistical differences in protein carbonylation after aerobic training and exhaustive exercise (data not shown).

DISCUSSION

The results presented in this report show for the first time that training or its combination with a worldwide NSAID (ibuprofen) increased time to exhaustion, decreased neural AChE activity, and protected against neuronal inflammation as well as oxidative stress insults after an exhaustive protocol test. Of note, in this study increased time to exhaustion induced by swimming training and/or ibuprofen intake was not due the reduction of pain as speculated in the literature.

The NSAIDs are routinely used during and after competition on injury treatments to suppress minor soreness symptoms in muscle and joint stiffness associated with overexertion. On the same line, it is generally agreed that delayed onset muscle soreness after eccentric exercise peaks roughly at 2 to 3 days post exercise[4,10]. In line of this view, our experimental findings revealed that combination of training and ibuprofen intake significantly increased time to exhaustion on the third day of the exhaustive protocol test when compared with trained group, indicating that this combination has additive effects on exercise-related fatigue onset.

It is important to underline that the trained group herein studied performed a high intensity training protocol according to Ravi Kiran et al.[54]. High-intensity exercise training

promotes body weight reduction in humans due to intense lipid metabolism activation during recovery after high-intensity glycogen-depleting exercise[66]. In this way, muscle glycogen resynthesis is of high metabolic priority resulting in preferential use of intramuscular triacylglycerol and circulating lipids by the recovering muscle[9] and these mechanisms were also reported in rats [6]. In line with this, lactate level was lower and time to exhaustion was higher in the trained group, corroborating previous findings[15,32].

In athletes, most pain reports present a mechanical origin, which may be related to muscle strains[1]. For this reason it has been observed a high prevalence of NSAIDs intake by athletes from different sports modalities[65,62]. A possible reason for this high is the fact that NSAIDs help to carry on training or even competitions without the need of a recovery window when minor injuries take place[11]. Although the analgesic drugs are commonly consumed to reduce or prevent pain and soreness after exhaustive exercise[61,3], the results herein found showed that repetitive exhaustive exercises induced nociceptive response and training and ibuprofen intake did not suppress pain. It is interesting to consider that the exhaustive protocol test was designed in order to mimic as close as possible individual sports competitions (swimming, for example) during important worldwide events, such as the Olympics. In this kind of championship athletes are asked to perform all-out trials during consecutive days without proper rest, for what metabolic byproducts may accumulate to the last day and thus influence on performance outcomes. In this scenario, our experimental results suggest that improvement in performance elicited by ibuprofen intake in trained rats is not due to antinociceptive response.

The discovery that cytokines are related to a range of disease conditions has opened a whole new investigation field on the physiological mechanisms to maintain and control health by restraining or counter-regulating cytokines release[41]. More recently, this scenario has evolved to suggest that exercise may also control inflammatory responses within the CNS[42,45].

In this context, the CNS is able to inhibit cytokine release through an inflammatory reflex of the vagus nerve, thereby preventing tissue injury and death[60]. Furthermore, it has been demonstrated that ACh, a major parasympathetic neurotransmitter, inhibits proinflammatory cytokines such as IL-1β and TNF-α from macrophages[5] and microglia[56]. Accordingly, our experimental findings showed that 6 weeks of swimming training decreased the neural AChE activity but have no effect on IL-1β and TNF-α. At same time, in muscle, there was no effect on AChE activity, but a decrease on IL-1β levels but had no effect on TNF-α content. Training has also reduced neural AChE activity and protected against IL-1β and TNF-α increase after the repeated exhaustive bouts. Considering that motor functions can be modulated by signals originating in the cerebral cortex and that blockade of cholinergic pathway accelerate the exercise-induced fatigue[12], it is plausible to propose that the neuronal cholinergic pathway maintenance induced by exercise training protects against IL-1β and TNF-α increase after repeated exhaustive exercise bouts. In addition, we could speculate that a lower content of pro-inflammatory cytokines induced by cholinergic pathway activation could be responsible for a continuous communication between cerebral cortex and neuromuscular junctions maintaining muscle contraction.

Although exercise training has long been known to regulate immune responses[64], recent studies suggest that exercise may also modulate inflammatory and ROS production responses within the CNS[51]. In this context, several studies have pointed out that exercise-induced modulation of the redox status is an important means by which exercise may benefit brain function, increasing the resistance against oxidative stress, and facilitating recovery from oxidative stress[50],[53],[57]. The results presented in this report demonstrated parameters related to the antioxidant status and ROS production in cortical homogenates were affected after repeated exhaustive swimming bouts. The DCFH-DA oxidation increase suggests that fatigue elicited by the exhaustive protocol test was accompanied by overproduction of ROS

and nitrogen species (ROSN). These results agree with several studies that have shown total antioxidant status upregulation of the main enzymes (superoxide dismutase, catalase, and glutathione peroxidase) after 14 days of swimming both in serum and hypothalamus. In line with this, several studies have investigated the stress-induced oxidative modification in rat brain[55]. Intensive stress has been shown to bring about changes in antioxidant defense system in rats[26]. Moreover, increased production of ROS in the tissues cause lipid peroxidation (especially in membranes) and plays an important role in tissue injury[27]. One of the possible causes for ROS production during swimming stress is the increased catecholamine metabolism represented by dopamine and norepinephrine. Increased catecholamine as a result of stress-induced activation of sympathetic-adrenal system may cause auto-oxidation in which electrons are generated and in turn may produce ROS[38]. Oxidative stress initiated by imbalance in oxidants and antioxidants in the hypothalamus might mediate cell damage and may be responsible for the neuronal disorders during stress[24].

On the other hand, the adaptive response to exercise training characterized here by DCFH-DA decrease, lead to the development of compensatory responses to oxidative stress[63] and pro-inflammatory cytokines generation[47] after the exhaustive protocol test. In addition, the effective protection exerted by ibuprofen intake on the fatiguing protocol in trained and sedentary rats reinforces the assumption that fatigue and impaired recovery from exercise share a common link with inflammation and ROS overproduction[19]. In line of this view, considering that ibuprofen reduces ROS production after a repeated exhaustive exercise protocol a mild indirect ergogenic effect may be suggested. However, the role of these drugs in sports is not completed understood. While some authors revealed that prophylactic ibuprofen significantly attenuated the decline in quadriceps isometric contraction and eccentric torque at 24 h post-exercise[29], others have not found oral ibuprofen intake to be better than placebo in this setting[7] and its use has no beneficial effect on the relief of muscle

damage and pain perception after ultramarathon[44] or downhill running[35]. The determining factor for such a discrepancy is not known, but one might argue that methodological differences may account for it. An interesting possibility is that ibuprofen intake effect on exercise-induced fatigue may vary with the model of training performed.

In conclusion, the present study reports that swimming training, ibuprofen intake or both combined increases time to exhaustion induced by an exhaustive protocol test. Our experimental findings also revealed that the neuronal cholinergic pathway maintenance elicited exercise training and its combination with ibuprofen administration protects against overproduction of pro-inflammatory cytokines and DCFH-DA oxidation increase after an exhaustive protocol test. These specific molecular systems modulated by ibuprofen in trained and sedentary rats provide a framework to guide further studies to examine the mechanisms by which NSAIDs may alter neuronal functions related to exercise-induced fatigue.

FIGURE CAPTIONS

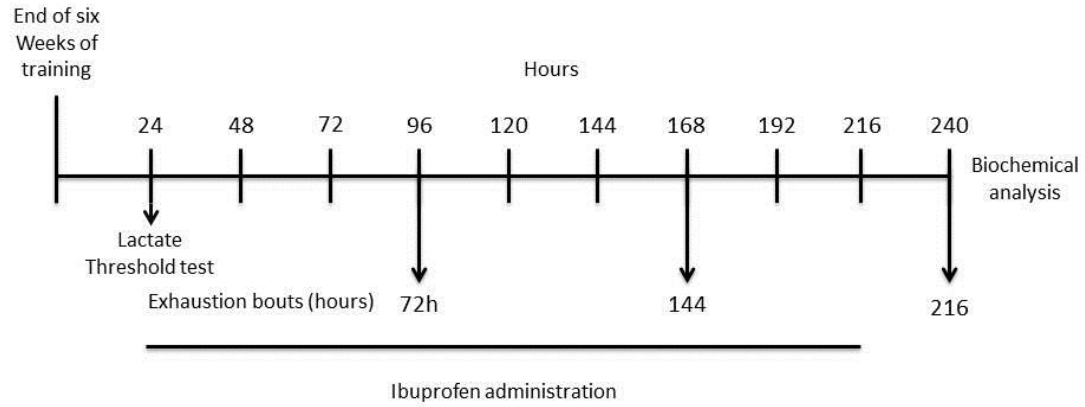

Fig. 1 – Timeline of the exercise training schedule and exhaustive protocol test data collection

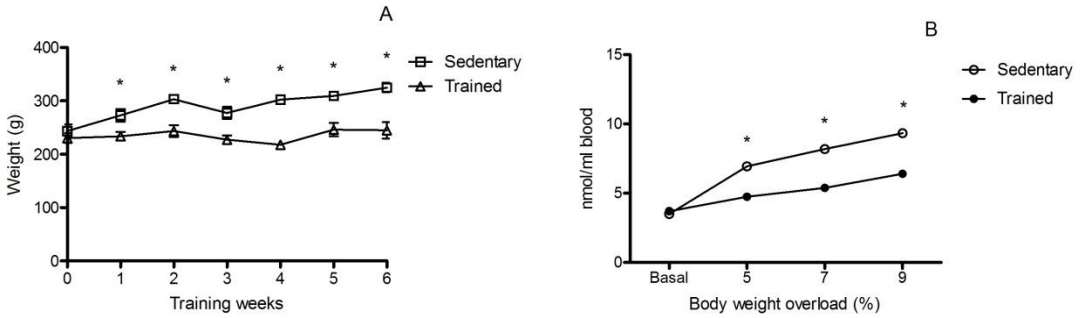

Fig. 2 – Effect of six weeks of exercise training on body weight (A), and lactate threshold (B). *p<0.05 when compared with sedentary group (F test for simple effect). Data mean + S.E.M. for n=7 in each group

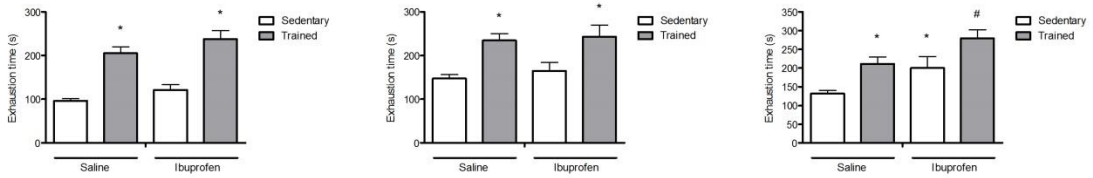

Fig. 3 – Effect of six weeks of exercise training and/or ibuprofen intake on exhaustion time on first (A), second (B), and third (C) bouts of the exhaustive protocol test. *p<0.05 when compared with sedentary group (F test for simple effect); #p<0.05 when compared with training group (F test for simple effect). Data mean + S.E.M. for n=7 in each group

Fig. 4 – Body weight of animals after exhaustive test period. *p<0.05 when compared with sedentary group (F test for simple effect). Data mean + S.E.M. for n=7 in each group

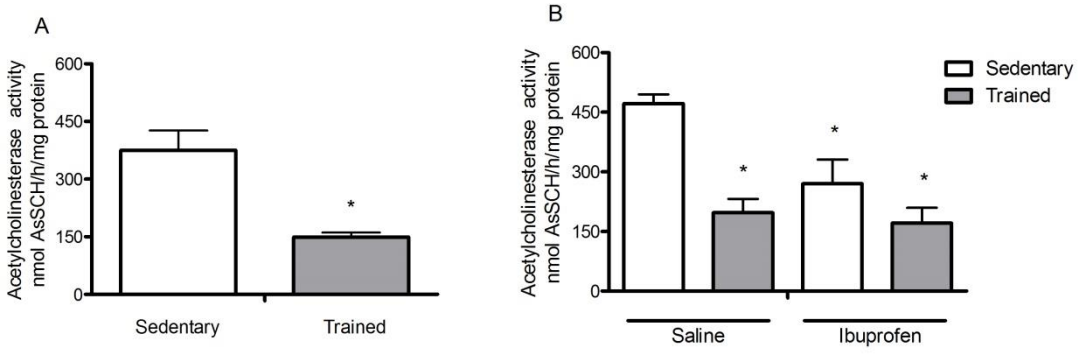

Fig. 5 – Effect of six weeks of exercise training (A) and exhaustive protocol test (B) on acetylcholinesterase activity. *p<0.05 when compared with sedentary-saline group (F test for simple effect (A) and Student-Newman-Keuls test (B)). Data mean + S.E.M. for n=8 – 9 in each group

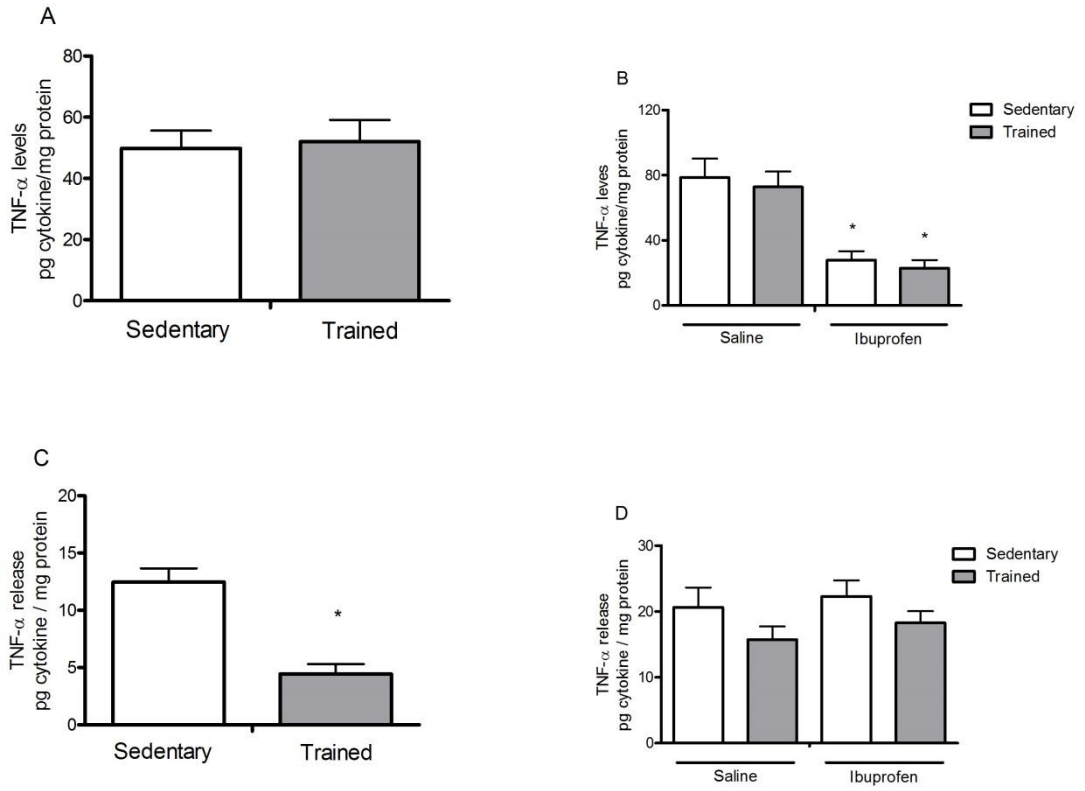

Fig. 6 – Effect of six weeks of exercise training on TNF-α levels in cortex and muscle (A and C respectively) and after exhaustive protocol test with or without ibuprofen intake (B and D, respectively). *p<0.05 when compared with sedentary-saline group; #p<0.05 when compared

with sedentary-ibuprofen group (Student-Newman-Keuls test (B and D). Data mean + S.E.M. for n=8 – 9 in each group. TNF-α: tumor necrosis factor alpha

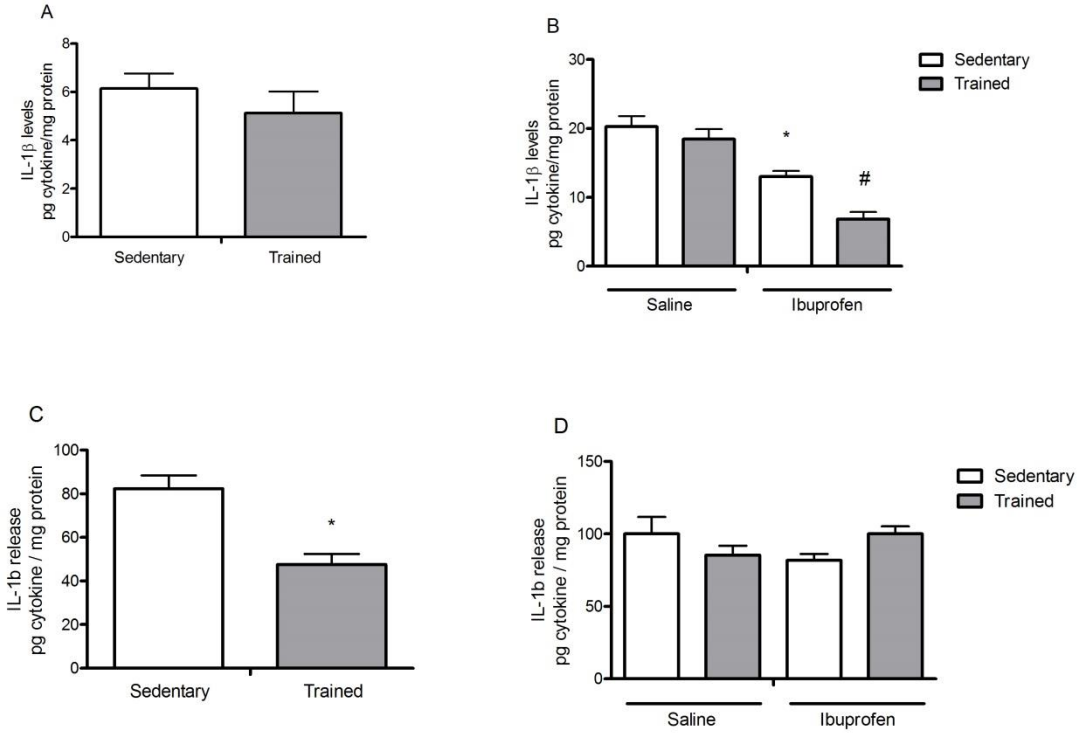

Fig. 7 – Effect of six weeks of exercise training on IL-1β levels in cortex and muscle (A and C respectively) and after exhaustive protocol test with or without ibuprofen intake (B and D, respectively). *p<0.05 when compared with sedentary-saline group; #p<0.05 when compared with sedentary-ibuprofen group (F test for simple effect (C); Student-Newman-Keuls test (B)). Data mean + S.E.M. for n=8 – 9 in each group. IL-1β: interleukine 1 beta.

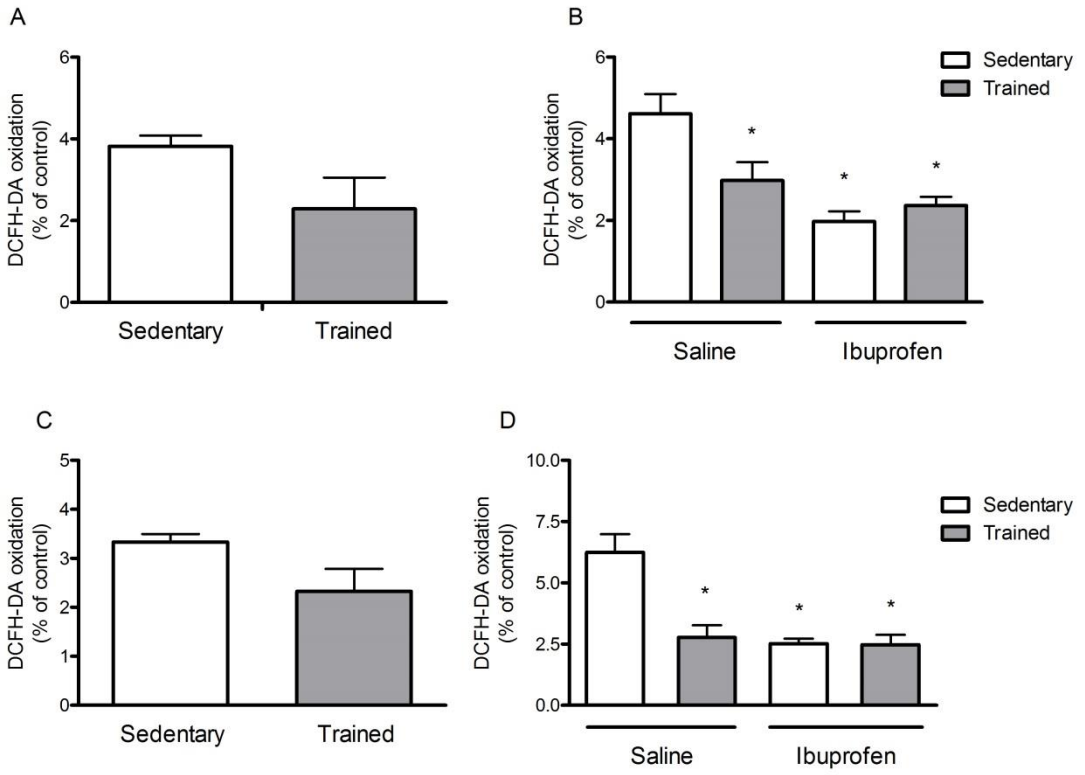

Fig. 8 – Effect of six weeks of exercise training on DCFH-DA in cortex and muscle (A and C respectively) and after exhaustive protocol test with or without ibuprofen intake (B and D, respectively) *p<0.05 when compared with sedentary and sedentary-saline group (Student-Newman-Keuls test (B and D)). Data mean + S.E.M. for n=8 – 9 in each group. DCFH-DA: 2′,7′-dichlorofluorescein diacetate

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4 ARTIGO – TREINAMENTO DE NATAÇÃO INDUZ ADAPTAÇÕES MITOCONDRIAIS AO ESTRESSE OXIDATIVO EM FÍGADO DE RATOS SUBMETIDOS A REPETIDOS EXERCÍCIOS EXAUSTIVOS DE NATAÇÃO

Título original

Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts

Autores

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Swimming Training Induces Liver Mitochondrial Adaptations to Oxidative Stress in Rats Submitted to Repeated Exhaustive Swimming Bouts

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Abstract

Background and Aims: Although acute exhaustive exercise is known to increase liver reactive oxygen species (ROS) production and aerobic training has shown to improve the antioxidant status in the liver, little is known about mitochondria adaptations to aerobic training. The main objective of this study was to investigate the effects of the aerobic training on oxidative stress markers and antioxidant defense in liver mitochondria both after training and in response to three repeated exhaustive swimming bouts.

Methods: Wistar rats were divided into training (n = 14) and control (n = 14) groups. Training group performed a 6-week swimming training protocol. Subsets of training (n = 7) and control (n = 7) rats performed 3 repeated exhaustive swimming bouts with 72 h rest in between. Oxidative stress biomarkers, antioxidant activity, and mitochondria functionality were assessed.

Results: Trained group showed increased reduced glutathione (GSH) content and reduced/oxidized (GSH/GSSG) ratio, higher superoxide dismutase (MnSOD) activity, and decreased lipid peroxidation in liver mitochondria. Aerobic training protected against exhaustive swimming ROS production herein characterized by decreased oxidative stress markers, higher antioxidant defenses, and increases in methyl-tetrazolium reduction and membrane potential. Trained group also presented higher time to exhaustion compared to control group.

Conclusions: Swimming training induced positive adaptations in liver mitochondria of rats. Increased antioxidant defense after training coped well with exercise-produced ROS and liver mitochondria were less affected by exhaustive exercise. Therefore, liver mitochondria also adapt to exercise-induced ROS and may play an important role in exercise performance.

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Introduction

Exercise represents a physical stress that transiently disrupts homeostasis [1], and the working skeletal muscle is clearly the organ most directly affected during physical activity [2]. Studies indicate that exercise may induce structural damage to muscle cells [3], and the production of metabolic by-products, such as lactate [4], and reactive oxygen species (ROS) [5,6]. There is consistent evidence that increased ROS production induced by acute intense exercise may cause an imbalance between oxidative intermediates and antioxidant systems, enhancing muscle lipid and protein oxidation, and the development of oxidative stress [7,8], which was first defined by Helmut Sies in the 1980's [9].

The metabolic adaptations to exercise are not restricted to the working muscle; exercise is also a major challenge to other organs such as cardiac muscle, stomach or brain [10,11]. This is particularly relevant to the liver due to its central role in the maintenance of energy supply to the exercising muscle [12]. Studies aiming to evaluate the effects of the acute exercise on oxidative stress in the liver have shown increased lipid peroxidation [13–15], and protein carbonylation [16,17], and decreased antioxidant defenses [18,19]. Aerobic exercise performance demands energy supply which is mainly attended by increases in oxygen consumption. In the mitochondria, the oxygen consumed partially undergoes a one electron reduction, giving rise to the superoxide radical ($O_2^{\cdot-}$) [20], which is generated in different rates according to the assayed tissue [21]. Additionally, it is known that

strenuous exercise causes a number of marked metabolic changes that may impair mitochondrial function in several ways [22], one major factor being mitochondrial ROS formation [23]. Interestingly, mitochondrial dysfunction appears to be a key issue during exhaustive exercise, and may cause oxidative damage and tissue injury to liver, among others organs [24].

ROS can also activate signal-transduction pathways to induce a stress-resistance response that protects against some of the toxic outcomes of ROS generation [25]. Indeed, exercise training has been reported to produce adaptive responses to oxidative stress, as studied primarily on skeletal muscles [26], but also in the liver. Thus, 8 weeks of aerobic training on treadmill increased the reduced/oxidized glutathione (GSH/GSSG) ratio [27], and 10 weeks of running upregulated superoxide dismutase (SOD) and catalase (CAT) liver enzyme activities in rats [28,29]. Antioxidant capacity increases in SOD, CAT, glutathione reductase (GR), and GSH levels were also found after a 12-week exercise training in the liver of rats [30]. These experimental evidences point out to antioxidant regulation mechanisms in the liver driven by exercise training in rodents [12].

While different studies have investigated the response of the working skeletal muscle to acute exercise and training, considerably less is known about liver adaptations during and after increased physical activity [12]. Liver plays an important role during exercise through glucose release to the bloodstream and gluconeogenesis, and mitochondria are clearly important in exercise performance due to aerobic energy production. Of note, while exercise training seems to improve oxidative metabolism modulation, acute exercise bouts challenge the body's antioxidant defenses with ROS production and exercise performance impairment. Competitive and tournament situations characterized by short recovery intervals between demanding exercise bouts may increase short-term ROS production confronting training adaptations [31]. In this sense, studies aiming to identify liver mitochondria adaptations to exercise-related oxidative stress in repeated stressful stimuli after training are still incipient. Therefore, the aim of this study was to evaluate the impact of swimming training on rat liver mitochondria oxidative stress modulation after training and repeated exhaustive swimming bouts.

Materials and Methods

Ethics Statement

The laboratory experiments were conducted in accordance with 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) and approved by the Ethics Committee for Animal Research of the Universidade Federal de Santa Maria (UFSM; Permit number: 020848). Indeed, animal handling and laboratory assays were conducted in such a way that all efforts were made to minimize suffering.

Animals and Reagents

Male Wistar rats (180–250 g) were kept in plastic boxes containing a maximum of five animals per cage, under controlled environment (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).

Study Design

In this study animals were randomly divided into training and control groups. The training group performed a 6-week swimming training and 24 h after the last training session both groups performed a lactate threshold (LT) test. Subsets of control and training groups were sacrificed in order to assess training effects upon the biomarkers herein assayed. To study the effects of exhaustive exercise seventy two hours afterwards, rats from both groups performed 3 repeated exhaustive swimming bouts with each bout separated for a 72 h time period. Rats were sacrificed after the last bout and liver was immediately removed and prepared for mitochondria isolation. Antioxidant status, oxidative stress markers, and mitochondria potential viability were measured in liver mitochondria within different groups. Figure 1 depicts the study design.

Water Adaptation

Rats were adapted to the water before the beginning of the experiment. The adaptation consisted on keeping the animals in shallow water at 31±1°C between 9:00 to 11:00 a.m. The adaptation period was carried out during the week before the swimming training onset. The purpose of the water adaption was to reduce stress without promoting physical training adaption.

Training Protocol and Lactate Threshold Assay

The use of regular swimming exercise shows advantages over the treadmill protocol, because swimming is a natural ability of rats and is widely used [32–34]. For exercise training, animals were weighed and randomly assigned to the following groups: training and control. The training period consisted of 6 weeks, 60 min per day and 5 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 always performed in water temperature of 31±1°C (70 cm depth) between 10 to 12 h a.m. The training group begun the swimming training with a 5% body weight overload attached to the back to improve endurance [4]. The control group was 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.

After 6 weeks of swimming training, a test protocol was used to determine the LT in control (n = 14) and training groups (n = 14). The LT test was carried out according to the protocol described by Marquezi et al. [35] with few modifications. The test consisted on 3 swimming bouts with progressive overload corresponding to 5%, 7% and 9% of each animal body weight for a period of 3 min for each load. A 1 min resting period was allowed between bouts. During the resting periods, 25 µl of blood were collected from the tail vein for lactate concentration assay, resulting in a total of 4 blood samples measured with a lactimeter (Accutrend® Plus, Roche Diagnostics GmbH, Germany). The LT for each animal was calculated based on the graphic inflection point when plotting lactate concentration against the corresponding exercise workload. Twenty four hours after the LT assay, control and trained animals subsets (n = 7) were killed by decapitation.

Exhaustive Protocol Test

Three days after the LT test, an exhaustive protocol test was carried out according to de Araujo et al. [36] with few modifications. The protocol consisted in 3 repeated exhaustive swimming bouts: first bout took place 72 h after the LT test; second bout at 144 h after the LT test; and the third 216 h after the LT test (Figure 1). Animals swam individually in the tank with an overload of 13% of body weight until exhaustion in order to determine the time to exhaustion. Exhaustion was characterized by the moment at which animals were no longer able to maintain themselves in the water surface, reaching 10 s submerged [36].

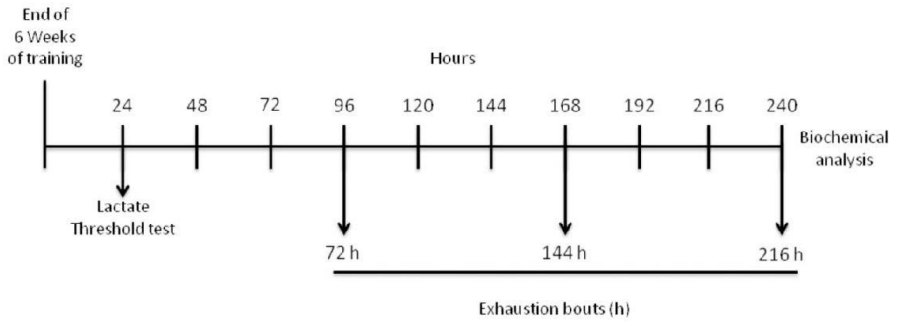

Figure 1. Timeline of the swimming training schedule and exhaustive protocol test data collection.
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When exhaustion was determined animals were taken out of the tank, dried and sacrificed.

Mitochondrial Isolation

Liver mitochondria were isolated as previously described by Bhattacharya et al. [37], with few modifications. The liver was rapidly removed and immersed in ice-cold isolation buffer I (100 mM sucrose, 10 mM EDTA, 100 mM Tris–HCl, 46 mM KCl, at pH 7.4). The tissue was homogenized, and the resulting suspension was centrifuged for 3 min at 2000×g in a Hitachi CR21E centrifuge. After centrifugation, the supernatant was recentrifuged for 10 min at 12000×g. The pellet was resuspended in isolation buffer II (100 mM sucrose, 10 mM EDTA, 100 mM Tris–HCl, 46 mM KCl, and 0.5% bovine serum albumin (BSA) free of fatty acids, at pH 7.4) and recentrifuged at 12000×g for 10 min. The supernatant was decanted, and the final pellet was gently washed and resuspended in 125 µl of isolation buffer III (270 mM mannitol, 70 mM sucrose, 0.02 mM EDTA, 20 mM Tris–HCl, 1 mM K_2HPO_4, at pH 7.4).

Reduced (GSH) and Oxidized Glutathione (GSSG) Content

GSH and GSSG levels were determined with fluorescence detection after reaction of the supernatants from deproteinized mitochondria containing H_3PO_4/NaH_2PO_4–EDTA or H_3PO_4/NaOH, respectively, with *O*-phthalaldehyde (OPT) [38]. In brief, freshly isolated liver mitochondria (0.5 mg prot/ml) resuspended in 1.5 ml phosphate buffer (100 mM NaH_2PO_4, 5 mM EDTA, pH 8.0) and 500 µl H_3PO_4 4.5% were rapidly centrifuged at 100000×g (Hitachi, TL-100 ultracentrifuge) for 30 min. For GSH determination, 100 µl of supernatant was added to 1.8 ml phosphate buffer and 100 µl OPT. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette and the fluorescence was measured at 420 and 350 nm emission and excitation wavelengths, respectively. For GSSG determination, 250 µl of the supernatant was added to 100 µl of *N*-ethylmaleimide and incubated at room temperature for 30 min. After the incubation, 140 µl of the mixture was added to 1.76 ml NaOH (100 mM) buffer and 100 µl OPT. After mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette and the fluorescence was measured at 420 and 350 nm emission and excitation wavelengths, respectively. GSH and GSSG contents were determined from comparisons with a linear GSH or GSSG standard curve, respectively.

Manganese Superoxide Dismutase (MnSOD) Activity

The MnSOD enzyme activity was determined in liver mitochondria according to the method proposed by Misra and Fridovich [39]. This method is based on the capacity of MnSOD in inhibiting autoxidation of adrenaline to adrenochrome. In brief, the supernatant fraction (100 µl) was added to a medium containing sodium bicarbonate–carbonate buffer (50 mM; pH 10.2) and adrenaline (0.4 mM). The kinetic analysis of MnSOD was started after adrenaline addition, and the color reaction was measured at 480 nm.

Thiobarbituric Acid Reactive Substances (TBARS) Levels

Lipid peroxidation was estimated by measuring TBARS according to the method of Ohkawa et al. [40,41]. In this method, malondialdehyde (MDA), an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. In brief, the supernatant fraction of liver mitochondria was incubated at 100°C for 60 min in acid medium containing 8.1% sodium dodecyl sulfate, 0.5 ml of acetic acid buffer (500 mM, pH 3.4) and 0.6% TBA. TBARS levels were measured at 532 nm, and expressed as nmol TBARS/mg mitochondrial protein.

Protein Carbonyl Levels

Protein oxidation in liver mitochondria was measured as concentration of protein carbonyls formed, and the levels were determined using 2,4 dinitrophenylhydrazine (DNPH) assay [42]. The mitochondria were divided into two portions containing 1 mg protein/ml each. To one portion, 1 ml of 2 N HCl was added and incubated at room temperature shaking intermittently for 1 h. The other portion was treated with 1 ml of 10 mM DNPH in 2 N HCl and incubated by shaking intermittently for 1 h at room temperature. After incubation the mixture was precipitated with 10% TCA and centrifuged. The precipitate was washed three times with 1 ml of ethanol:ethyl acetate (1:1). The final protein precipitate was dissolved in denaturation buffer (3% SDS and 150 mM NaH_2PO_4; pH 6.8) and the absorption at 370 nm (DNPH-treated sample minus sample blank) was determined. Carbonyl content was calculated using the molar extinction coefficient of 22,000 and expressed as nmol DNPH/mg mitochondrial protein.

Methyl-Tetrazolium (MTT) Reduction Levels

MTT assays were carried out with a modification [43] of the method described by Berridge and Tan [44], except that the respiration buffer was used as the medium. MTT reduction levels were determined as an index of the dehydrogenase enzymes functions, which are involved in the cellular viability. Samples were incubated in buffer containing glutamate/succinate (5 mM each) and MTT (0.5 mg/ml) for 30 min at 37°C, and MTT reduction reaction was stopped by the addition of 1 ml of dimethylsulphoxide (DMSO). The formed formazan levels were determined spectrophotometrically, reported as the difference in absorbance between 570 and 630 nm and the results were corrected by the protein content. Individual samples were expressed as a percent of the mean control value in the experiment.

Mitochondrial Membrane Potential ($\Delta\psi$) Determination

The mitochondrial $\Delta\psi$ determination was assayed according to Akerman and Wikstron [45]. Briey, the mitochondria samples (150 μg protein/ml) were incubated in a medium containing KCl (65 mM), sucrose (100 mM), EGTA (0.05 mM), BSA (0.2%), HEPES (10 mM, pH 7.2), safranine O (10 μM) and the respiratory substrates glutamate (5 mM) and succinate (5 mM). The reaction was started with the mitochondria addition and the medium was kept at constant stirring during the assay period. The uorescence analysis was performed at 495 nm for excitation and 586 nm for emission, with slit widths of 5 nm. Results are presented as arbitrary units of fluorescense units per second relative to % control.

Estimation of ROS Production

Production of ROS was estimated in liver mitochondria with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), as described by [46,47]. Briefly, tissues were homogenized in 2.5 ml of saline solution (0.9% NaCl). Aliquots of 2.5 ml were incubated in the presence of DCFH-DA (5 μm) at 37°C for 60 min. The DCFH-DA is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was established with standard DCF (0.1 nm to 1 μm), and ROS levels were expressed as percentages of control.

Protein Determination

The protein content was measured colorimetrically by the method of Bradford [48] using bovine serum albumin (1 mg/ml) as standard.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS, Ins, Chigaco, IL) version 17 was used for all analyses. Data were expressed as mean ± standard error of means (SEM). Significance was assessed by one- or two-way analysis of variance (ANOVA), followed by Newman–Keuls's Test for post-hoc comparison when appropriate. Statistical significance was set at $p<0.05$.

Results

Swimming Training Effects on Lactate Threshold, Exhaustion time, and Body Weight

Lactate threshold, exhaustion time, and body weight were previously demonstrated to be altered by swimming training [49–51]. Statistical analysis revealed that blood lactate concentration increased progressively for both trained and control rats, though the trained group presented lower lactate concentrations comparing to the control group [$F(1,12)=23.41$; $p<0.05$; Fig. 2A]. The trained group showed a significantly higher time to exhaustion in comparison to the control group in day 1 [$F(1,14)=33.68$; $p<0.05$; Figure 2B], day 2 [$F(1,14)=42.57$; $p<0.05$; Figure 2B] and day 3 [$F(1,14)=27.71$; $p<0.05$; Figure 2B] of the trial. A significant increase in total body weight of control rats comparing to trained rats was observed after the 6 weeks of swimming training [$F(1,12)=34.58$; $p<0.05$; Figure 2C].

GSH and GSSG Content, and GSH/GSSG Ratio

The trained group showed significantly higher liver mitochondria GSH levels [$F(1,20)=52.56$; $p<0.05$; Figure 3A] and GSH/GSSG ratio [$F(1,20)=32.01$; $p<0.05$; Figure 3C] comparing to the control group after 6 weeks of swimming training. The exhaustive exercise protocol test also resulted in increased GSH levels and GSH/GSSG ratio in the trained group, while GSSG levels were significantly higher in the control group [$F(1,20)=9.39$; $p<0.05$; Figure 3B].

MnSOD Activity, and TBARS and Protein Carbonyl Levels

The statistical analysis showed that swimming training increased MnSOD activity [$F(1,29)=22.97$; $p<0.05$; Figure 4A] and decreased TBARS level [$F(1,30)=45.70$; $p<0.05$; Figure 4B] in comparison to control rats. Training prevented TBARS [$F(1,30)=42.52$; $p<0.05$, Figure 4B] and protein carbonyl increases [$F(1,22)=14.66$; $p<0.05$ Figure 4C] induced by the exhaustive exercise protocol in the control group, suggesting the aforementioned GSH upregulation and MnSOD increase with training may protect against TBARS and protein carbonyl increase after the exhaustive protocol test.

Mitochondria MTT Reduction and Membrane Potential, and ROS Production

Statistical analysis showed that training resulted in a higher liver mitochondria MTT reduction both after training and to the exhaustive protocol test in comparison to the control group [$F(1,26)=25.90$; $p<0.05$; Figure 5A]. On the same line, the $\Delta\psi$ also increased in trained group after the exhaustive protocol test [$F(1,27)=16.94$; $p<0.05$; Figure 5B]. The exhaustive protocol test induced increases in DCFH oxidation both in trained and control rats, with values significantly lower for trained animals [$F(1,26)=83.95$; $p<0.05$; Figure 5C].

Discussion

In the current study we have shown a liver mitochondria adaptive response to exercise training characterized by increases in GSH/GSSG ratio, MnSOD activity, mitochondrial viability and $\Delta\psi$, and decreases in TBARS levels in trained animals. To the best of our knowledge this is the first study to point out that swimming training protects against liver mitochondria oxidative damage after repeated bouts of forced swimming. The results presented in this report also support the hypothesis that acute exercise causes oxidative and mitochondrial stress [17], with increased TBARS and protein carbonyl levels and higher ROS production after an

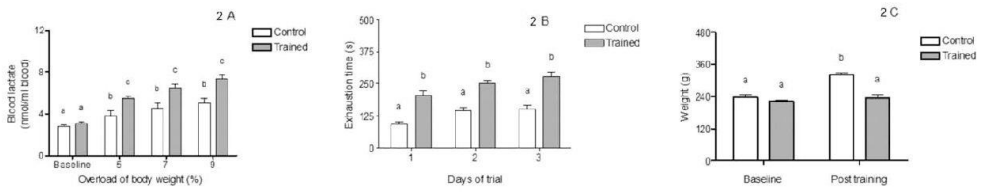

Figure 2. Effect of 6 weeks of swimming training on lactate threshold, time to exhaustion, and body weight. (A–C) Values are mean ±SEM (n = 14). Means without a common letter differ, $p<0.05$.
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exhaustive exercise bout. These experimental findings suggest that acute exercise causes mitochondrial ROS generation and that this oxidative disruption may benefit by activating antioxidant defense systems during exercise training programs.

We found a body weight plateau in trained rats during the 6 week training period when compared to the control group. Other authors have also reported that swimming training stabilizes body weight in rats [52,53]. According to Ravi Kiran et al [52], who investigated different training intensities and durations in 4 and 12 months-old rats, the training protocol intensity applied in our study can be considered as high intensity (5% overload, 60 min/day –5 days/week). A high intensity swimming protocol demands high energy matching capacities so as to overcome the task. Body weight reduction in humans promoted by high-intensity exercise training originates from intense lipid usage during recovery periods following high-intensity glycogen-depleting exercise [54]. When exercise results in glycogen depletion, muscle glycogen resynthesis is of high metabolic priority, resulting in the preferential use of intramuscular triacylglycerol and circulating lipids by the recovering skeletal muscle [55]. These same mechanisms also seem to take place in rats [28]. In accordance, in our study lactate level was lower and time to exhaustion was higher in the trained group, corroborating previous findings [36,49–51].

Concerning antioxidant effects of exercise, a substantial body of evidence suggests that regular exercise plays an important preventive and therapeutic role in oxidative stress-associated diseases, including ischemic heart disease, type II diabetes, and Alzheimer's disease [56–58]. Accordingly, studies have shown that animals and humans clearly undergo significant adaptive responses to regular endurance exercise that involve greatly increased endurance capacity, which is permitted by dramatic mitochondrial biogenesis, reduction in oxidant production and increased antioxidant defenses [59,60]. In this context, the liver plays a key role in exercise-induced oxidative stress. For instance, liver is the major organ for *de novo* GSH synthesis, supplying 90% of the circulating GSH, which is one of the most important endogenous antioxidants [61] and plays an important role as a reducing agent [62], protecting the organism against hydrogen peroxide (H_2O_2) and lipid peroxides [63]. Sun et al. [61] found increased liver mitochondria GSH after 4 weeks of endurance training in rats, which was attributed to an increased antioxidant activity. Navarro et al. [64] also reported that chronic moderate exercise increases MnSOD activity and decreases mitochondrial oxidation products (TBARS and protein carbonyls) in trained rat liver, suggesting that changes were consistent with a faster mitochondria turnover and biosynthesis.

In the present study we found an increase in MnSOD activity and GSH/GSSG ratio, concomitant with decreased TBARS and protein carbonyl levels in trained rats. On the same line, GSH level presented a 2-fold increase after training and levels were maintained after the exhaustive protocol test. This is a remarkable finding since GSH depletion in cells is involved in metabolic limitations such as lower exercise capacity and cell membrane disruption/apoptosis that may lead to oxidative stress [18]. In agreement to this view, Botezelli et al. [28] have recently demonstrated that 8 weeks of swimming training decreased lipid peroxidation, a fact partially attributed to an improved antioxidant system with greater SOD enzyme activity. Our data show the same tendency, with decreased TBARS levels and higher MnSOD activity in trained rats comparing to the control group, suggesting that the MnSOD activity increase in liver mitochondria may be an antioxidant response to the oxidative injury caused by exercise. Together with higher GSH levels, augmented MnSOD activity after training may cope with ROS production and thus prevent from lipid peroxidation and further oxidative stress. These experimental findings indicate a clear adaptation of liver mitochondria towards an enhanced antioxidant system after swimming training.

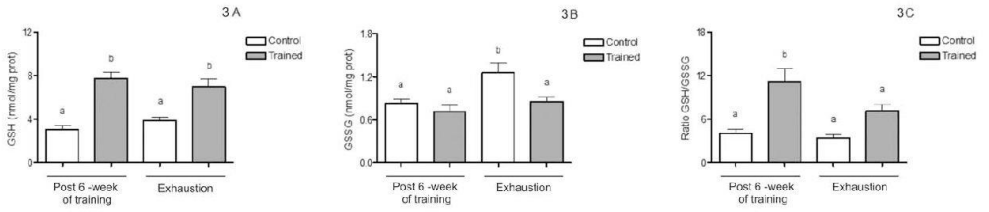

Figure 3. GSH, GSSG, and GSH/GSSG ratio levels both after 6 weeks of swimming training and an exhaustive protocol test. (A–C) Values are mean ± SEM (n = 7). Means without a common letter differ, $p<0.05$. GSH: reduced glutathione; GSSG: oxidized glutathione.
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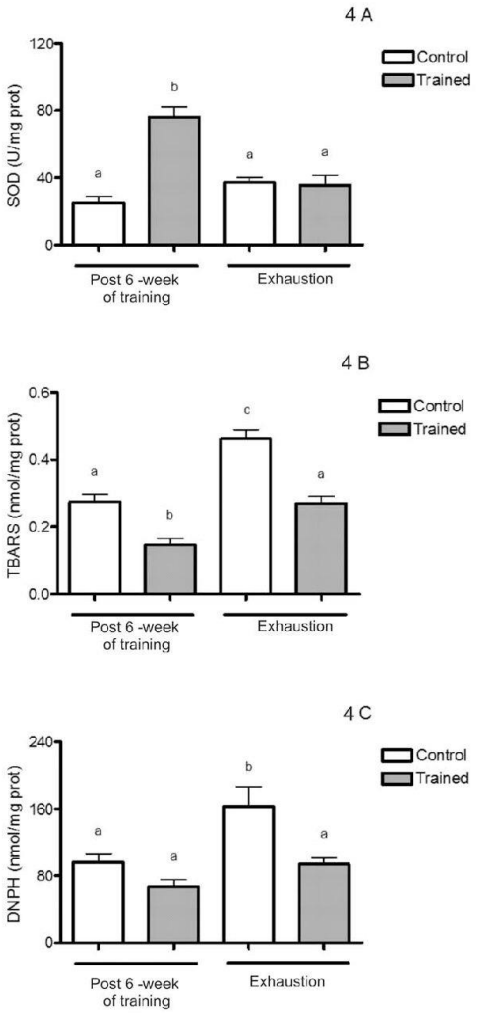

Figure 4. MnSOD activity, TBARS and protein carbonyls levels both after 6 weeks of swimming training and an exhaustive protocol test. (A–C) Values are mean ±SEM (n = 7). Means without a common letter differ, $p<0.05$. MnSOD: manganese superoxide dismutase; TBARS: thiobarbituric acid reactive substances; DNPH: dinitrophenylhydrazine.
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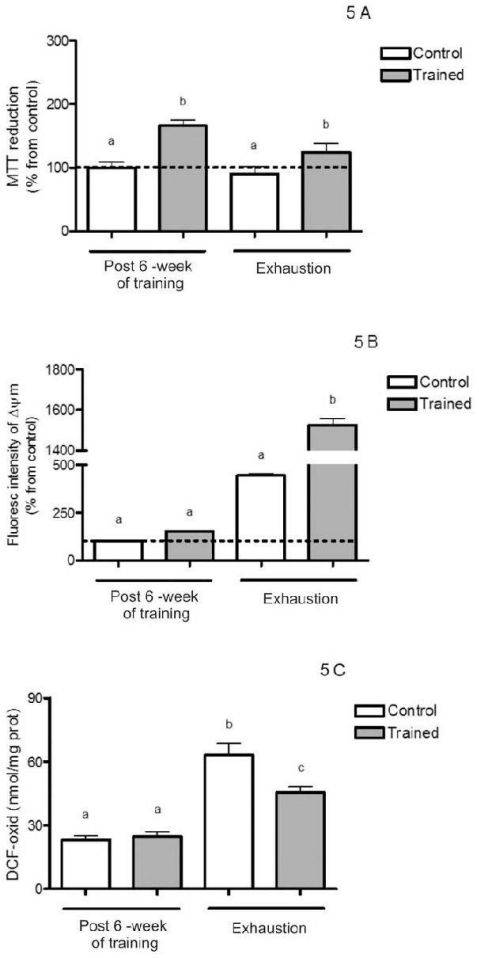

Figure 5. MTT reduction and membrane potential, and ROS production both after 6 weeks of swimming training and an exhaustive protocol test. (A–C) Values are mean ±SEM (n = 7). Means without a common letter differ, $p<0.05$. ROS: reactive oxygen species; MTT: Methyl-tetrazolium; DCF-oxid: oxidized dichlorofluorescein diacetate; $\Delta\psi$: membrane potential.
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Considering that several expressed enzyme systems contribute to ROS formation in the liver and that SOD efficiently removes excessive ROS to maintain the normal cell homeostasis [65], it is plausible to propose that biochemical training adaptation as upregulation of antioxidant enzymes (MnSOD) reflects on decreased markers of lipid and protein peroxidation. Therefore, the increased mitochondrial chain respiratory function (characterized here by mitochondrial electron flow and $\Delta\psi$) in trained rats suggests that mitochondrial redox status elicited by exercise-related oxidative stress may influence on a long lasting exercise performance. Our data showed that the trained group presented diminished TBARS content and higher MnSOD activity comparing to the control group. Swimming training enhanced antioxidant defenses to repeated exhaustive swimming bouts and enforced antioxidant defenses to adapt to these repeated stimuli.

Elevated TBARS levels after the exhaustive protocol test indicate increased membrane lipid peroxidation in the liver [16].

On the same line, ROS interaction with enzymes and structural proteins may cause thiol oxidation and protein carbonyls introduction, affecting activity and function of such molecules [66,67]. In the present study we found a significant increase in TBARS and protein carbonylation in the control group of animals after 3 exhaustive swimming bouts. Liu et al. [17] also reported an increase in liver TBARS levels of rats submitted to an acute exercise bout. An interesting finding in our research is that swimming training prevented TBARS and protein carbonyls increases after the exhaustive protocol test in comparison to the control group. It has been shown that increased TBARS and protein carbonyls in the mitochondria membranes impair membrane-bound enzyme activities leading to mitochondrial dysfunction [64]. These data suggest that swimming training may provide protection to acute insult in the liver mitochondria herein measured by oxidative stress markers. In this context, our data show an adaptation of liver mitochondria towards an enhanced antioxidant system after swimming training.

Considering the energy supply by mitochondria, its dysfunction appears to play a key role in exercise performance [68]. We isolated liver mitochondria to demonstrate MTT reduction, $\Delta\psi$, and ROS production across DCFH oxidation. The MTT reduction depends on the functionality of the oxidoreductase enzyme pool, such as the dehydrogenases [44]. Considering most of these are mitochondrial enzymes [44,69], functional impairment may be related to mitochondrial functional impairment. In this sense, experimental findings revealed that swimming training increased MTT reduction and this step up was associated with higher $\Delta\psi$ and lower increases on ROS production after the exhaustive protocol test comparing to the control group. On the same line, the control group registered higher ROS production after the exhaustive exercise protocol. Of note, the trained group presented higher time to exhaustion during the exhaustive protocol test. Taken together these findings suggest that liver mitochondria dysfunction may be related to exercise-induced fatigue in the control group. It is plausible to suggest a mitochondrial role on exercise-induced fatigue considering that muscle mitochondria are responsible for metabolic stability improvement following endurance training [70].

Noteworthy a major mitochondrial enzyme here analyzed was dampened by the exhaustive protocol test. Our data suggest that MnSOD inhibition combined with higher DCFH oxidation after the exhaustive protocol test may be related to accumulative stress produced across three exhaustive swimming bouts, as indicated by high TBARS and protein carbonyls levels in the control group. In line with this view, we found that swimming training induced a significant increase in liver mitochondrial GSH content, MnSOD activity, and MTT reduction in the trained group, suggesting that ROS production is of key importance in the modulation of signaling pathways involved in the liver adaptation to exercise training. In fact, H_2O_2, the byproduct of O_2^- dismutation, has been reported to activate several signaling pathways across interactions with different molecules [25]. Data herein presented confirm this hypothesis, with trained rats showing higher antioxidant status, enhanced antioxidant enzyme activity, lesser mitochondrial oxidation, and increased mitochondrial viability.

It has been described that exercise training produces remarkable changes in liver metabolism [13,17,19]. Even though we did not characterized the hepatocytes prior to mitochondria isolation, a recent study using a similar swimming protocol (8 weeks, 1 h/day –5.2% body weight overload) showed no differences in shape and size of hepatocytes and nuclei, and no changes in hepatic protein/DNA ratio (a marker of hyperplasia in the liver) after swimming training both in diabetic and normal rats [71]. Moreover, the swimming training applied did not induce changes in the amount of mitochondria present in liver cells or the mitochondria matrix [71]. Another issue that may be addressed is the possible difference in total liver parameters compared to isolated mitochondria measurements. Although we did not perform total homogenate measurements, previous reports have failed to find significant differences among tissue and isolated mitochondria. Thus, no differences between GSH and TBARS in tissue homogenate and isolated mitochondria have been observed following endurance exercise in rat [61].

In summary, the present study reports that swimming training induces positive adaptations in liver mitochondria of rats, characterized by increases on GSH/GSSG ratio, MnSOD activity, MTT reduction, $\Delta\psi$, and decreases on TBARS and protein carbonyl levels. For the first time a response to accumulative exercise-induced stress was reported in liver mitochondria, with control rats presenting higher ROS production associated with an increased TBARS and protein carbonyl levels after repeated exhaustive swimming bouts. On the other hand, increased antioxidant defense induced by swimming training coped well with exercise-produced ROS and thus preserved liver mitochondria redox status after the exhaustive protocol test. Data showing specific molecular systems modulation by physical exercise also provide a framework to guide further studies aimed to examine mechanisms by which regular exercise may alter hepatic mitochondrial metabolism and protect against exercise-induced stress. Considering the importance of liver mitochondria in energy supply and antioxidant defenses, it becomes clear that this organelle may play a role in exercise performance and further studies to determine the participating signaling pathways are of interest.

Author Contributions

Conceived and designed the experiments: FAS LFFR JBR GB. Performed the experiments: FDL DNS IDDP FD NRdC. Analyzed the data: FDL LFFR FAS GB. Contributed reagents/materials/analysis tools: LFFR FAS JBR. Wrote the paper: LFFR JBR JGG GB.

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5 DISCUSSÃO

A regularidade da prática de exercícios físicos traz muitos fatores benéficos para seus praticantes (HOLLOSZY, 1993); (PETERSEN e PEDERSEN, 2005) com expressiva associação entre estilo de vida ativo e melhor qualidade de vida. Os malefícios do sedentarismo superam em muito as eventuais complicações decorrentes da prática de exercícios físicos, os quais apresentam uma interessantíssima relação risco/benefício. Considerando a alta prevalência, aliada ao significativo risco relativo do sedentarismo referente às doenças crônico-degenerativas, o incremento da atividade física de uma população contribui decisivamente para a saúde pública, com forte impacto na redução dos custos com tratamentos, inclusive hospitalares, uma das razões de seus consideráveis benefícios sociais (DUNN e DISHMAN, 1991; VAN PRAAG et al., 1999, MEEUSEN, WATSON e DVORAK, 2006).

Da mesma forma que o exercício físico traz benefício para a saúde, também é responsável em aumentar o consumo de oxigênio e, consequentemente gerar espécies reativas de oxigênio (ERO) (BOVERIS e NAVARRO, 2008). Neste contexto, tem sido demonstrado que o exercício físico extenuante pode manifestar um desbalanço entre a geração de radicais livres e as defesas antioxidantes (MEYDANI et al., 1993; JENKINS, 1988); SJODIN, HELLSTEN e WESTING, 1990; JI, 1995). Estudos *in vivo* utilizando tecidos coletados logo após o exercício demonstram que a taxa de produção de ERO aumenta significativamente após exercício exaustivo em ratos jovens e adultos (BEJMA; JI, 1999). A presente geração de ERO não só se eleva como resultado do consumo de O_2, mas pode contribuir para a fadiga (REID et al., 1994) onde a produção de radicais livres pode ser proporcional ao desenvolvimento de tensão muscular (O'NEIL, HYNAK-HANKINSON e GORMAN, 1986). Além da geração de ERO, estudos experimentais e clínicos relatam que determinados tipos de exercício físico, como o excêntrico, causam micro traumas na musculatura esquelética e aumentam as citocinas pró inflamatórias no músculo (CARMICHAEL et al., 2010; CARMICHAEL et al., 2006; CARMICHAEL et al., 2005) e regiões do córtex (CHENNAOUI, DROGOU e GOMEZ-MERINO, 2008; NYBO et al., 2002). O aumento na concentração de IL-1β em determinadas regiões do cérebro estão associadas com diminuição de desempenho esportivo (CARMICHAEL et al., 2005) em modelo experimental. Desta forma, tanto a rotina diária de treinamento como o período competitivo trará os efeitos agudos da prática de exercícios de alta intensidade. Na intenção de minimizar a magnitude desses

efeitos e, consequentemente a perda de desempenho, os atletas fazem uso de medidas protetivas como o uso de AINEs (DA SILVA et al., 2011); (TSCHOLL et al., 2010). Neste contexto, foi observado nos Jogos Olímpicos de Sidney em 2000 que 20% dos atletas fizeram uso desse tipo de medicamento (CORRIGAN e KAZLAUSKAS, 2003), nas Copas do Mundo de 2002 e 2006, mais de 40% dos atletas utilizaram AINEs (TSCHOLL et al., 2010) e nos Jogos pan-americanos de 2007, 18,3% dos atletas selecionados para os exames antidoping utilizaram AINEs antes da competição e 81,7% durante a competição (DA SILVA et al., 2011). Devido a grande variedade de condições associadas à fadiga, há um considerável interesse no desenvolvimento e posterior aplicação de marcadores bioquímicos que relacionem a sua gravidade com o prejuízo no desempenho. Além disso, a alta frequência do uso de AINEs por atletas sugere que os mesmos podem ser usados como auxílio ergogênico, entretanto não existem evidências de que o seu uso aumente o desempenho esportivo em atletas (DA SILVA et al., 2011). Desta forma, o presente trabalho teve como objetivo, no primeiro momento, investigar se a administração de ibuprofeno durante o protocolo de exaustão retardara o aparecimento da fadiga em animais treinados e sedentários.

Os dados experimentais apresentados no manuscrito revelaram que o treinamento físico aeróbico prévio bem com a administração deste AINE durante o protocolo de exaustão aumentou o tempo de nado dos animais treinados quando comparados com sedentários. Cabe salientar que a escolha do protocolo de natação como ferramenta de treinamento físico aeróbico se deve as vantagens que o mesmo possui sobre a corrida, uma vez que o nado caracteriza-se por ser uma habilidade natural dos roedores (ARIDA et al., 2011). Além disso, no protocolo experimental de esteira rolante além da dificuldade na manutenção da velocidade, a presença de estímulos elétricos pode influenciar nos resultados (GOBATTO et al., 2001) uma vez que esses fatores podem gerar um estresse no animal (RADAK et al., 1999). Apesar de a maioria dos trabalhos com ratos e camundongos utilizarem roda de correr ou esteira, nosso grupo de pesquisa tem obtido resultados interessantes utilizando o protocolo de natação. Entretanto o protocolo de treinamento físico utilizado (6 semanas de natação) estabilizou os níveis de lactato sanguíneo em animais treinados quando comparados com o grupo sedentário no teste de limiar de lactato. A manutenção dos níveis sanguíneos de lactato observados no presente estudo corrobora com a premissa de que adaptações aeróbicas musculares responsáveis por uma baixa produção e/ou uma alta remoção do lactato (JONES e CARTER, 2000). Além disso, nossos dados estão de acordo com a literatura que sugere que o

treinamento de natação leva a adaptações musculares em roedores, como àquelas observadas em humanos (VOLTARELLI, GOBATTO e DE MELLO, 2002).

Uma das justificativas para o uso de AINEs como agentes ergogênicos no treinamento físico e competições esportivas encontra-se na capacidade desses medicamentos reduzirem substancialmente a sensação de dor. Diante da experiência dolorosa aguda promovida por determinados tipos de exercício, parece haver um consenso entre atletas, treinadores e pesquisadores de que a dor limita o desempenho em determinadas modalidades esportivas. Para Anshel e Russel (1997), a habilidade de um atleta tolerar a dor induzida pelo exercício é um fator crítico para uma *performance* esportiva de sucesso. Neste sentido, apesar de existirem evidências de que o efeito analgésico dos medicamentos é capaz de alterar a sensação subjetiva de esforço dos atletas, reduzindo o desconforto promovido pelo exercício (GARCIN et al., 2005; AMMAN, 2009), os resultados apresentados no primeiro artigo da Tese revelaram que o protocolo de exercícios exaustivos repetitivos induziu uma resposta nociceptiva e que o treinamento físico prévio bem como a ingestão de ibuprofeno não suprimiu a dor.

Apesar da extensa literatura investigando uso de AINEs no desempenho esportivo, até onde nos sabemos, este é o primeiro trabalho que utiliza este protocolo de exaustão para revelar um possível efeito do ibuprofeno. Cabe salientar que o presente protocolo tentou mimetizar o mais próximo possível de competições desportivas individuais (natação, por exemplo) onde os atletas realizam provas durante dias consecutivos. O resultado disto é o acumulo de subprodutos metabólicos que podem influenciar nos resultados de desempenho. Neste cenário, os nossos resultados experimentais sugerem que a melhoria de desempenho provocada pela ingestão de ibuprofeno em ratos treinados não é devido à sua ação antinoceptiva. Cabe salientar que animais sedentários que receberam o ibuprofeno por nove (9) dias, apresentaram um tempo à exaustão igual ao grupo treinado. Entretanto, tendo em vista o elevado número de substâncias classificadas como AINEs, o presente estudo não pode afirmar categoricamente que todo e qualquer tipo de AINE é capaz de incrementar o desempenho físico e futuros estudos devem ser realizados no intuito de responder tal questão.

Os resultados bioquímicos obtidos nesta primeira etapa do estudo revelaram que o treinamento físico aeróbico de seis semanas bem como os testes de exaustão não alteraram a atividade da enzima acetilcolinesterase (AChE) no músculo gastrocnêmico dos animais. Entretanto o presente protocolo de treinamento físico prévio diminui a atividade da AChE no córtex *per se*. Já a administração de ibuprofeno em animais treinados, apesar de não exercer

efeito na atividade da enzima AChE muscular, manteve diminuída sua atividade no córtex quando comparados com seus respectivos controles após o teste de exaustão. Considerando que a resposta inflamatória está envolvida no desenvolvimento de efeitos negativos do exercício físico intenso (CARMICHAEL et al., 2006) e que atividade colinérgica no cérebro modula a interação entre o SNC e o sistema imunológico (BERCHTOLD, KESSLAK e COTMAN, 2002; DAS, 2007), é plausível propor que um aumento na concentração de acetilcolina no córtex represente um efeito anti-inflamatório do presente protocolo de exercício físico aeróbico. De fato, o protocolo treinamento físico proposto diminuiu os níveis de citocinas pró-inflamatórias (TNF-α e IL-1β) no musculo. Nossos resultados também endossam a hipótese de que o SNC tem a função de manter a homeostase durante o exercício físico (NOAKES, ST CLAIR GIBSON e LAMBERT, 2005; ST CLAIR GIBSON e NOAKES, 2004) e que a perda da capacidade de desempenho esportivo ocorra quando seus limites fisiológicos e bioquímicos forem excedidos (EDWARDS, J. E. et al., 1993). Nossos dados corroboram com esta hipótese uma vez que o protocolo de exaustão proposto levou a um aumento nos níveis de TNF-α e IL-1β no músculo e córtex em animais sedentários. Estes dados sugerem que uma alteração do sistema colinérgico no SNC é seguido por um aumento de processos inflamatórios e perda de rendimento em exercícios físicos intensos. Entretanto, a relação entre o SNC com aparecimento e propagação da dor bem como a regeneração muscular deficiente e/ou diminuição do desempenho ainda precisa ser mais bem elucidada (CARMICHAEL et al., 2006).

No presente estudo pode-se observar que a administração de ibuprofeno além de retardar o aparecimento da fadiga em animais sedentários, manteve diminuída a atividade neuronal da enzima AChE e protegeu contra o aumento nos níveis de TNF-α e IL-1β após a último teste de exaustão. Em animais treinados, a administração de ibuprofeno aumentou a latência para exaustão, manteve a atividade neuronal da enzima AChE e protegeu contra o aumento nos níveis de TNF-α e IL-1β quando comparados com seus respectivos controles. Esses dados reforçam o envolvimento de processos inflamatórios no aparecimento da fadiga e sugerem que o efeito anti-inflamatorio exercido pelo ibuprofeno, especificamente no SNC possa ter efeito benéfico com o objetivo de diminuir o tempo de regresso à competição por atletas. Cabe salientar que os AINEs, considerados pela Agência Mundial Antidoping (AMA) como substâncias de uso permitido, são amplamente utilizados com a finalidade de redução das manifestações excessivas do processo inflamatório decorrente da lesão, como a exemplo o controle da dor muscular tardia (CIOCCA, 2005). Entretanto, há que se questionar se o

consumo excessivo destes compostos ocorre por excesso de treinamento, pela forma como o treinamento é feito, por situações de fragilidade fisiológica ou por outras variáveis que poderiam ser alteradas.

A inflamação é considerada um processo altamente benéfico e necessário quando relacionada ao treinamento físico regular e sistematizado, uma vez que em conjunto com a ação de hormônios e outras moléculas sinalizadoras é responsável pela regeneração e reparo das estruturas danificadas. Entretanto, esta quimiotaxia, embora seja uma reação desejável, quando não bem regulada, pode ser uma das causas de inflamações agudas devido ao aumento na produção de mediadores pró- inflamatórios (IL-1, IL-6, IL-8 e TNF-α) e prostaglandinas, levando à indução e à intensificação de processo inflamatório seguido pelo aumento da produção de ERO (MASTALOUDIS et al., 2004). Considerando que todos esses fatores, juntos ou em parte, podem levar à um declínio reversível na geração de força pelo sistema neuromuscular (BIGLAND-RITCHIE et al., 1983) foi investigado o efeito da administração de ibuprofeno durante o protocolo de exaustão sobre o estado redox das células em animais sedentários e treinados. A produção de ERO expressa na geração de peróxido de hidrogênio no córtex e musculo foram monitoradas quantitativamente por citometria de fluxo usando o reagente 2’7’diacetato de diclorofluoresceína (DCFH-DA). Nossos resultados revelaram que embora o treinamento físico prévio não altere este estado redox das células no córtex e músculo, o mesmo protegeu do aumento da oxidação DCFH-DA muscular após o teste de exaustão. Cabe salientar que o presente protocolo de exaustão não alterou a oxidação DCFH-DA no córtex de animais sedentários reforçando a hipótese de uma alteração do estado redox das células a partir de um processo inflamatório caracteriza-se por ser uma a resposta local e geralmente acompanhada por uma resposta sistêmica, chamada de resposta de fase aguda (GRUYS et al., 2005). Além disso, a efetiva proteção exercida pela ingestão de ibuprofeno no protocolo de exaustão em ratos treinados e sedentários reforça a suposição de que recuperação prejudicada após um exercício físico intenso e resultado de excessiva produção de ERO e citocinas pró-inflamatórias (DROGE, 2002).

Além das alterações musculares, o treinamento físico impõe mudanças a outros tecidos (VENEROSO et al., 2009; CAKIR et al., 2010). Dentre os diversos órgãos que apresentam alterações causadas pelo exercício físico destaca-se o fígado, um órgão importante na manutenção do suprimento energético para o sistema muscular durante um exercício físico de longa duração (HOENE e WEIGERT, 2010). Cabe salientar que durante o exercício físico ocorrem diversas adaptações fisiológicas no intuito de compensar e manter o esforço

realizado. Neste contexto, sabe-se que 95% do total é usado para formar energia por diferentes processos enzimáticos e que 5% de oxigênio participam da formação de radicais livres. O exercício físico aumenta o consumo de oxigênio e, consequentemente, o aumento da produção de radicais livres. 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 a função mitocondrial de várias formas (RASMUSSEN et al., 2001; RADAK et al., 2009), dentre as quais destaca-se a geração de ERO e ERN. 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 esta totalmente estabelecida (HOENE e WEIGERT, 2010).

Os resultados apresentados no segundo estudo da presente Tese demonstraram que 6 semanas de protocolo de treino de natação proporciona alteração no estatus antioxidante no fígado de aninais treinados. A presente alteração foi caracterizada pelo aumento na razão glutationa reduzida/oxidada (GSH / GSSG), atividade da enzima superóxido dismutase (MnSOD), manutenção da viabilidade mitocondrial e potencial de membrana ($\Delta\psi$) bem como diminuição dos níveis de espécies reativas ao ácido tiobarbitúrico (TBARS). O treinamento físico também protegeu do aumento da formação mitocondrial de TBARS, carbonilação de proteínas, oxidação DCFH-DA induzido pelo protocolo de exaustão utilizado. Apesar da extensa literatura investigando o papel do estresse oxidativo em diferentes tipos e intensidade de exercício físico, até onde nós sabemos, este é o primeiro trabalho que revela uma resposta acumulativa de estresse oxidativo em fígado de ratos submetidos a repetidos sets de natação intensa.

Sabe-se que o fato do exercício físico intenso aumentar 10 a 20 vezes o consumo total de oxigênio do organismo eleva também de 100 a 200 vezes a captação de oxigênio, induzindo a formação excessiva de ERO associada ao metabolismo energético acelerado. Essas espécies podem contribuir para danos tissulares e celulares, incluindo modificação oxidativa do DNA, prejudicando o desempenho do atleta. Entretanto, respostas adaptativas claramente significativas induzidas pelo protocolo de treinamento aeróbico utilizado neste estudo pode alterar positivamente a homeostase oxidativa das células por diminuir os níveis basais de agentes oxidantes aumentarem a resistência ao estresse oxidativo. Neste contexto, o fígado desempenha um papel chave no estresse oxidativo induzido pelo exercício. Por exemplo, o fígado é o órgão essencial na síntese de glutationa uma vez que é responsável pelo

fornecimento de 90% do GSH circulante. Sun e colaboradores (2010) encontraram, após quatro (4) semanas de treinamento aeróbico, um aumento no conteúdo de GSH mitocondrial em fígado de ratos. Da mesma forma um estudo de Navarro e colaboradores (2004) encontrou um aumento na atividade da MnSOD mitocondrial bem como diminuição nos marcadores de estresse oxidativo TBARS e carbonilação proteica em mitocôndria de fígado de ratos treinados. Desta forma, é plausível propor que uma alteração na atividade mitocondrial no fígado de ratos destreinados pode estar relacionada com o aparecimento da fadiga em exercícios físico intensos. Por outro lado, um aumento da atividade de enzimas antioxidantes como a enzima SOD bem como a diminuição dos produtos mitocondriais de oxidação (TBARS e carbonilação de proteínas) no fígado de ratos treinados pode manter preservado o estado redox das mitocôndrias do fígado após os testes exaustivos. Além disso, um aumento na função respiratória mitocondrial, representada pelo fluxo de elétrons e potencial de membrana em ratos treinados altera o estado redox mitocondrial, uma alteração metabólica que pode estar relacionada com a melhora no desempenho dos animais evidenciado nos testes de exaustão.

6 CONCLUSÃO

De acordo com os resultados obtidos nesta tese, o protocolo de seis semanas de treinamento aeróbico, além de induzir as adaptações fisiológicas esperadas, diminuiu a atividade AChE no córtex, bem como o conteúdo de TNF-α e IL-1β no músculo dos animais treinados. Por outro lado, a administração por nove dias de ibuprofeno teve como consequência um aumento no tempo à exaustão nos animais sedentários e quando em combinação com o treinamento, esse tempo foi ainda maior após três testes exaustivos. Além disso, a administração da droga diminuiu a atividade da AChE bem como o conteúdo das citocinas pró-inflamatórias no córtex, atentando para uma ação conjunta com o treinamento no conteúdo de IL-1β e, ainda causou uma diminuição na produção de ERO tanto no córtex quanto no músculo dos animais submetidos aos três testes de exercício exaustivo.

Outros resultados desse trabalho mostraram que protocolo de seis semanas de treinamento aeróbico aumentou os níveis de GSH, da razão GSH/GSSG e aumentou a atividade da MnSOD, bem como diminuiu a produção de TBARS e aumentou a viabilidade celular no fígado dos animais treinados. A realização de três testes exaustivos, os níveis de GSSG aumentaram nos animais sedentários, o treinamento protegeu do aumento de TBARS e da carbonilação proteica e da produção de ERO, a viabilidade mitocondrial se manteve aumentada e o potencial de membrana aumentou.

Com esses resultados, pode-se concluir que a administração de ibuprofeno na dose de 15mg/kg pode agir de forma sinergética com o treinamento físico no sentido de adiar o aparecimento da sensação de fadiga. Além disso, o treinamento aeróbico causa adaptações no fígado, o que não causou nenhum prejuízo na realização dos testes exaustivos, mostrando que esse órgão possui um papel importante do desempenho esportivo. Entretanto mais estudos se fazem necessários para estabelecer o real papel dos AINEs na realização de exercícios de alta intensidade e de curta duração.

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