UNIVERSIDADE DA REGIÃO DE JOINVILLE – UNIVILLE
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E MEIO AMBIENTE

LARISSA DELMONEGO

EFEITO DO TREINAMENTO AERÓBICO DE ALTA INTENSIDADE E DA SUPLEMENTAÇÃO COM L-CARNITINA SOBRE O ESTRESSE OXIDATIVO, METABOLISMO ENERGÉTICO E INFLAMAÇÃO EM RATOS OBESOS

JOINVILLE – SC
2023

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Tese de doutorado apresentada como requisito parcial para obtenção do título de Doutora em Saúde e Meio Ambiente, na Universidade da Região de Joinville - UNIVILLE.

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JOINVILLE – SC

2023

Catalogação na publicação pela Biblioteca Universitária da Univille

Delmonego, Larissa
D359e Efeito do treinamento aeróbico de alta intensidade e da suplementação com L-Cartinina sobre o estresse oxidativo, metabolismo energético e inflamação em ratos obesos / Larissa Delmonego; orientadora Dra. Daniela Delwing de Lima; coorientadora, Dra. Débora Delwing Dal Magro. – Joinville: Univille, 2023.

227 f.: il.

Tese (Doutorado em Saúde e Meio Ambiente – Universidade da Região de Joinville)

1. Obesidade. 2. Treinamento intervalado de alta intensidade. 3. Estresse oxidativo. 4. Metabolismo energético. I. Lima, Daniela Delwing de (orient.). II. Magro, débora Delwing Dal. III. Título.

CDD 616.398

Elaborada por Ana Paula Blaskovski Kuchnir – CRB-14/1401

Termo de Aprovação

"Efeito do Treinamento Aeróbico de Alta Intensidade e da Suplementação com L-Carnitina sobre o Estresse Oxidativo, Metabolismo Energético e Inflamação em Ratos Obesos".

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Tese julgada para a obtenção do título de Doutora em Saúde e Meio Ambiente, área de concentração Saúde e Meio Ambiente e aprovada em sua forma final pelo Programa de Pós-Graduação em Saúde e Meio Ambiente.

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Joinville, 26 de maio de 2023

Dedico esse trabalho primeiramente a minha família, meus colegas de trabalho e doutorado, minhas orientadoras e colegas de pesquisa pela constante ajuda, compreensão, incentivo e amor nessa jornada.

AGRADECIMENTOS

Em primeiro lugar agradeço a Deus, pela oportunidade de alcançar mais uma realização na minha vida profissional, me ajudando a driblar as dificuldades com sabedoria e discernimento e me amparando nos momentos difíceis onde nada parecia dar certo.

Aos meus pais, Marisa e Saul, e meu irmão Vinícius, por sempre me incentivar, apoiar, e me instigar a seguir em frente em busca dos meus objetivos sem nunca medir esforços para que isso acontecesse. Muito obrigada! Serei eternamente grata a vocês.

Também agradeço a minha orientadora Prof. Dra. Daniela Delwing de Lima, pela dedicação, paciência, compreensão, apoio e incentivo nessa etapa. Você é um exemplo de profissional e me sinto lisonjeada pela oportunidade de ser sua orientanda. Irei levar comigo todos os ensinamentos aprendidos até aqui e espero um dia ser uma profissional tão boa quanto você. Muito obrigada!

Ao grupo de pesquisa Mecanismos de Saúde e Doença da Univille: Thayná, Alessandra, Luana, Gabriela, Júlia, Heloisi, Heloiza, Giovanna, Scheila, Maria Helena, Maria Augusta, Victor, Prof. Carla, Prof. Eduardo e demais colegas que participaram na realização desse trabalho, obrigada pelas tardes a fio nos treinamentos aeróbicos com os ratos e por estarem sempre dispostos a ajudar. Vocês foram essenciais para a realização desse sonho!

Aos meus colegas de doutorado pela troca de experiências, ensinamentos e tardes divertidas de aula e cafezinhos orgânicos. Aprendi muito com vocês. Somos uma turma e tanto!

A todos os professores do doutorado pelos ensinamentos, e a todos aqueles que de alguma forma estiveram ao meu lado durante a execução deste trabalho.

Aos membros da banca, pelas contribuições.

À UNIVILLE, FAPESC e CAPES.

“Não se pode criar experiência. É preciso passar por ela.”

Albert Camus

RESUMO

Estudos mostram que a obesidade está diretamente relacionada a processos inflamatórios e químicos que desencadeiam quadros de estresse oxidativo e alterações no metabolismo energético celular. Além disso, autores relatam que a suplementação de L-Carnitina possivelmente promove efeitos antioxidantes e que o protocolo HIIT promove uma melhora na capacidade oxidativa. Este estudo avaliou os efeitos do protocolo HIIT e da suplementação com L-Carnitina sobre parâmetros de estresse oxidativo, inflamatórios e de metabolismo energético no cérebro, sangue, músculo, fígado, coração, rim e adipócitos de ratos obesos. Para a análise, os animais foram divididos em cinco grupos: Dieta Normal Sedentários (DNL-SED), Dieta Hiperlípidica Sedentários (DHL-SED), Dieta Hiperlípidica Sedentários + L-Carnitina (DHL-SED-C), Dieta Hiperlípidica + Treino Intervalado de Alta Intensidade (DHL-HIIT) e Dieta Hiperlípidica + Treino Intervalado de Alta Intensidade + L-Carnitina (DHL-HIIT-C). Para induzir a obesidade, os animais dos grupos DHL foram alimentados com dieta hiperlipídica por 14 semanas, enquanto os animais dos grupos DNL com dieta padrão. Os animais dos grupos DHL-SED-C e DHL-HIIT-C receberam a suplementação com L-Carnitina por gavagem assim que o protocolo HIIT foi iniciado. O protocolo HIIT aconteceu com frequência de 5 dias por semana e os animais do grupo SED caminharam com intensidade de 40%, duas vezes por semana. Após o término da 10ª semana de treinamento, os animais foram sacrificados por decapitação e o sangue e as estruturas foram separados e preparados de acordo com a técnica. A atividade antioxidante da catalase (CAT), superóxido dismutase (SOD), glutationa peroxidase (GSH-Px), níveis de substâncias reativas ao ácido tiobarbitúrico (TBA-RS), conteúdo total de sulfidrilas e proteínas carboniladas, piruvato quinase, citrato sintase, succinato desidrogenase (SDH), atividades do complexo II e citocromo c oxidase, determinação de interleucina-1β, interleucina-6 e fator de necrose tumoral α, glicose, insulina, triglicerídeos, LDL-colesterol (LDL-c), HDL-colesterol (HDL-c) e colesterol total foram determinados. Os resultados demonstraram que a DHL promoveu estresse oxidativo, como lipoperoxidação, dano proteico, alterações nas atividades das enzimas antioxidantes, promoveu disfunção no metabolismo energético e alteração em parâmetros bioquímicos, e o protocolo HIIT, isolado e algumas vezes associado à L-Carnitina, impediu algumas dessas alterações.

Palavras Chave: Obesidade; Treinamento Intervalado de alta intensidade; HIIT; Estresse oxidativo; Metabolismo Energético; L-Carnitina.

ABSTRACT - EFFECT OF HIGH-INTENSITY AEROBIC TRAINING AND L-CARNITINE SUPPLEMENTATION ON OXIDATIVE STRESS, ENERGY METABOLISM AND INFLAMMATION IN OBESE RATS

Studies show that obesity is directly related to inflammatory and chemical processes triggering oxidative stress and alteration in cell metabolism energy. Furthermore, authors report that L-Carnitine supplementation possibly promotes antioxidant effects and that HIIT protocol promotes an improvement in oxidative capacity. This study evaluated the protective effects of HIIT and L-Carnitine supplementation on oxidative stress, energy metabolism and inflammatory parameters in cerebrum, blood, muscle, liver, heart, kidney and adipocytes of obese rats. For the analysis, animals were divided into five groups: Normal Diet-Untrained (ND-UNT), High-Fat Diet-Untrained (HFD-UNT), High-Fat Diet-Untrained + Carnitine (HFD-UNT-C), High-Fat Diet + High-intensity Interval Training (HFD-HIIT) and High-Fat Diet + High-intensity Interval Training + Carnitine (HFD-HIIT-C). To induce obesity, animals in HFD groups were fed with a high-fat diet for 14 weeks, while animals in ND groups with a standard diet. Animals in HFD-UNT-C and HFD-HIIT-C groups received L-Carnitine by gavage as soon as HIIT protocol started. HIIT protocol happened with a frequency of 5 days a week and animals of UNT group walked at 40% intensity, twice a week. After the end of the 10th week of training, animals were sacrificed by decapitation and the blood and structures were separated and prepared according to the technique. The antioxidant activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), levels of thiobarbituric acid reactive substances (TBA-RS), total sulfhydryl and protein carbonyl content, pyruvate kinase, citrate synthase, succinate dehydrogenase (SDH), complex II and cytochrome *c* oxidase activities, interleukin-1β, interleukin-6 and tumor necrosis factor α, glucose, insulin, triglycerides, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c) and total cholesterol were determined. Results showed that HFD promoted oxidative stress, such as lipoperoxidation, protein damage, alterations in the activity of antioxidant enzymes, promoted energy metabolism dysfunction and alterations on biochemical parameters, and the HIIT protocol, isolated and sometimes, associated with L-Carnitine, prevented some of these alterations.

Keywords: Obesity; High Intensity Interval Training; HIIT; Oxidative Stress; Energy metabolism; L-Carnitine.

RESUMEN - EFECTO DEL ENTRENAMIENTO AERÓBICO DE ALTA INTENSIDAD Y LA SUPLEMENTACIÓN DE L-CARNITINA SOBRE EL ESTRÉS OXIDATIVO, EL METABOLISMO ENERGÉTICO Y LA INFLAMACIÓN EN RATAS OBESAS

Los estudios muestran que la obesidad está directamente relacionada con los procesos inflamatorios y químicos que desencadenan el estrés oxidativo y los cambios en el metabolismo energético celular. Además, los autores informan que la suplementación con L-carnitina posiblemente promueva efectos antioxidantes y que el protocolo HIIT promueva una mejora en la capacidad oxidativa. Este estudio evaluó los efectos del protocolo HIIT y la suplementación con L-Carnitina sobre parámetros de estrés oxidativo, inflamación y metabolismo energético en el cerebro, sangre, músculo, hígado, corazón, riñón y adipocitos de ratas obesas. Para el análisis, los animales se dividieron en cinco grupos: Dieta Normal + Sedentario (DNL-SED), Dieta Hiperlipídica + Sedentario (DHL-SED), Dieta Hiperlipídica + Sedentario + L-Carnitina (DHL-SED-C), Dieta Hiperlipídica + Entrenamiento Interválico de Alta Intensidad (DHL-HIIT) y Dieta Hiperlipídica + Entrenamiento Interválico de Alta Intensidad + L-Carnitina (DHL-HIIT-C). Para inducir la obesidad, los animales de los grupos DHL recibieron una dieta rica en grasas durante 14 semanas, mientras que los animales de los grupos DNL recibieron una dieta estándar. Los animales de los grupos DHL-SED-C y DHL-HIIT-C recibieron suplementación de L-carnitina por sonda tan pronto como se inició el protocolo HIIT. El protocolo HIIT ocurrió con una frecuencia de 5 días a la semana y los animales del grupo SED caminaron con una intensidad del 40%, dos veces por semana. Después del final de la semana 10 de entrenamiento, los animales fueron sacrificados por decapitación y lo sangre y las estructuras fueron separadas y preparadas de acuerdo con la técnica. La actividad antioxidante de catalasa (CAT), superóxido dismutasa (SOD), glutatión peroxidasa (GSH-Px), niveles de sustancias reactivas al ácido tiobarbitúrico (TBA-RS), contenido total de sulfhidrilos y proteínas carbonilo, piruvato quinasa, citrato sintasa, succinato deshidrogenasa (SDH), actividades del complejo II y citocromo c oxidasa, determinación de interleucina-1β, interleucina-6 y factor de necrosis tumoral α, glucosa, insulina, triglicéridos, LDL-colesterol (LDL-c), HDL-colesterol (HDL- c) y el colesterol total fueron determinados. Los resultados mostraron que DHL promovió el estrés oxidativo, como la peroxidación lipídica, el daño proteico, cambios en las actividades de las enzimas antioxidantes, promovió la disfunción en el metabolismo energético y cambios en los parámetros bioquímicos, y el protocolo HIIT, aislado y en ocasiones asociado con L-Carnitina, impidió algunos de estos cambios.

Palabras clave: Obesidad; Entrenamiento de intervalos de alta intensidad; HIIT; estrés oxidativo; Metabolismo energético; L-Carnitina.

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ADP: Adenosina Difosfato ou Difosfato de Adenosina

ATP: Adenosina Trifosfato ou Trifosfato de Adenosina

AVC: Acidente Vascular Cerebral

CAT: Catalase

CK: Creatinoquinase

CoA: Coenzima A

DM: Diabetes Mellitus

DNA: Ácido Desoxirribonucleico

DNPH: Dinitrofenilhidrazina

DTNB: Ácido Ditionitrobenzóico

ERO: Espécie Reativa de Oxigênio

ERN: Espécie Reativa de Nitrogênio

FAD: Flavina Adenina Dinucleotídeo

GDP: Guanidina Difosfato ou Difosfato de Guanidina

GR: Glutationa Redutase

GSH: Glutationa

GSH-Px: Glutationa Peroxidase

GTP: Guanidina Trifosfato ou Trifosfato de Guanidina

HAS: Hipertensão Arterial Sistêmica

HIIT: High-intensity Interval Training

IAM: Infarto Agudo do Miocárdio

IL: Interleucina

IMC: Indíce de Massa Corporal

$L^{\cdot}$: Radical Lipídico

LH: Ácido Graxo Poliinsaturado

$LOO^{\cdot}$: Radical Peroxila

LOOH: Hidroperóxidos

MDA: Malondialdeído

NADH: Nicotinamida Adenina Dinucleotídeo

NADPH: Nicotinamida Adenina Dinucleotídeo Fosfato

$NO^{\cdot}$: Óxido Nítrico

NOS: Óxido Nítrico Sintase

O_2: Oxigênio Molecular

$O_2^{\bullet -}$: Radical Superóxido

$OH^{\bullet}$: Radical Hidroxil

OMS: Organização Mundial de Saúde

$ONOO^{-}$: Ânion Peroxinitrito

Pi: Fosfato Inorgânico

Pcr: Fosfocreatina

PDH: Piruvatodesidrogenase

Q: Ubiquinona

RNA: Ácido Ribonucleico

SDH: Succinato desidrogenase

SOD: Superóxido Dismutase

SOD-Cu-Zn: Superóxido Dismutase Cobre-Zinco

SOD-Mn: Superóxido Dismutase Manganês

TBA-RS: Substâncias reativas ao ácido tiobarbitúrico

TCA: Ciclo do Ácido Tricarboxílico

TNF-α: Fator de Necrose Tumoral Alfa

UV: Ultravioleta

Vmáx: Velocidade máxima

VO_2: Volume de Oxigênio

SUMÁRIO

1 INTRODUÇÃO

Segundo a Organização Mundial da Saúde (OMS), o termo obesidade é utilizado para descrever um acúmulo excessivo de gordura corporal prejudicial à saúde do indivíduo (WHO, 2020).

Vários estudos já conseguiram associar a obesidade ao desenvolvimento de diversas complicações, dentre elas: cardiovasculares (hipertensão arterial, insuficiência coronariana, aterosclerose), respiratórias, musculoesqueléticas, complicações dermatológicas, distúrbios relacionados ao sistema endócrino-metabólico (Diabetes Mellitus e dislipidemia) e também ao aparecimento de certas neoplasias (CHOI, 2016; DE FRANCISCHI *et al.*, 2000; RAZA; JOHN; HOWARTH, 2015).

Segundo França e colaboradores (2013), pacientes obesos apresentam alterações biológicas que tornam o organismo mais suscetível ao dano oxidativo. Esses indivíduos apresentam um desequilíbrio entre as quantidades de gordura, peso corporal, lipoproteínas e lipídios, desequilíbrio esse que promove aumento das necessidades metabólicas e do consumo de oxigênio e, consequentemente, aumento da produção de espécies reativas de oxigênio (EROs), como superóxido e peróxido de hidrogênio (FRANÇA *et al.*, 2013).

Além do aumento na produção de radicais livres, estudos sugerem que a obesidade está associada à diminuição da atividade de enzimas antioxidantes (NOEMAN; HAMOODA; BAALASH, 2011). Em 2002, Olusi testou 250 indivíduos obesos ao determinar a atividade das enzimas superóxido dismutase (SOD) e glutationa peroxidase (GSH-Px) e concluiu que pacientes obesos apresentam menor atividade enzimática antioxidante eritrocitária. Essa situação pode gerar dano progressivo às células e ser uma das explicações para o fato da obesidade ser um importante fator de risco no desenvolvimento de diversas patologias.

Vários estudos já demonstraram que pacientes obesos apresentam níveis mais elevados de citocinas circulantes, promovendo um estado inflamatório, que também pode estar relacionado à resistência à insulina, hiperlipidemia e síndrome metabólica (DO PRADO *et al.*, 2009). A associação entre obesidade, inflamação e estresse oxidativo acontece por diferentes vias físico-químicas, como aumento dos níveis de

glicose no sangue, aumento na geração e armazenamento de lipídios, estimulação na oxidação de ácidos graxos e também aumento de citocinas pró-inflamatórias, promovendo estresse oxidativo e distúrbios metabólicos (DELWING-DE LIMA *et al.*, 2018).

Em relação às alterações no metabolismo energético, alguns estudos já conseguiram correlacionar a obesidade com alterações no funcionamento da cadeia respiratória mitocondrial e também com efeitos deletérios no metabolismo de carboidratos (SCHMITT; GASPAR, 2023; TAN; NORHAIZAN, 2019; YOKOTA *et al.*, 2009).

A prática diária de exercícios físicos já é comprovadamente uma das melhores formas de promover a saúde física, mental e cognitiva do ser humano, independente da idade e sexo (CARLUCCI *et al.*, 2013). A associação entre exercício físico e geração de radicais livres foi inicialmente estudada na década de 1970, por meio da análise de biomarcadores de peroxidação lipídica no tecido muscular esquelético (BRADY; BRADY; ULLREY, 1979; DILLARD *et al.*, 1978). Em 1983, Quintanilha e Packer mostraram que o treinamento físico aeróbio provocava um aumento na produção de agentes antioxidantes e na expressão de enzimas antioxidantes, promovendo assim, aos sistemas cardiovascular e musculoesquelético, benefícios às suas defesas antioxidantes (QUINTANILHA; PACKER, 1983).

O protocolo de treinamento intervalado de alta intensidade (HIIT) é caracterizado pelo intercalamento de atividades intensas submáximas, máximas ou supramáximas com períodos de descanso ou atividades de baixa intensidade (PAZ; FRAGA; TENÓRIO, 2017). Alguns estudos já trazem dados associando o protocolo HIIT com melhorias na capacidade oxidativa do músculo e na biogênese mitocondrial, como é o caso de Gibala, Terada e seus colaboradores (GIBALA *et al.*, 2006; TERADA *et al.*, 2001).

A L-Carnitina (3-hidroxi-4-N-trimetilaminobutirato) é uma amina quaternária com função fundamental na geração de energia pela célula, pois atua nas reações de transferência de ácidos graxos livres da cadeia longa do citosol para a mitocôndria, facilitando sua oxidação e geração de adenosina trifosfato (ATP) (COELHO *et al.*, 2005). Aumentar o fluxo de substratos através do Ciclo de Krebs pode resultar em uma produção e uso mais efetivos de oxigênio, além de melhorar a capacidade de

realizar tarefas físicas (COELHO *et al.*, 2005). Tem sido frequentemente utilizada como tratamento coadjuvante para dislipidemias, pois atua como cofator importante na oxidação de ácidos graxos de cadeia longa, aumentando a utilização de triglicerídeos para fornecimento de energia, e também por indivíduos ativos como coadjuvante na redução de gordura corporal (COELHO *et al.*, 2005). Além disso, por se tratar de uma substância produzida no organismo em condições normais e com boa tolerabilidade, a suplementação de L-Carnitina vem sendo estudada quanto aos seus possíveis efeitos antioxidantes, tanto em indivíduos saudáveis quanto naqueles com necessidades especiais, como portadores de doenças isquêmicas e neuropatia diabética (COELHO *et al.*, 2005).

Considerando que a obesidade está relacionada à quadros inflamatórios e de estresse oxidativo, à alterações no metabolismo energético e em parâmetros bioquímicos, e que estudos indicam que o protocolo HIIT e a suplementação com L-Carnitina promovem melhora na capacidade oxidativa celular, este estudo teve como objetivo avaliar os efeitos do treinamento intervalado de alta intensidade (HIIT) e da suplementação de L-Carnitina sobre parâmetros de estresse oxidativo, metabolismo energético, inflamatórios e bioquímicos no sangue, fígado, rins, coração, cérebro, adipócitos, músculo plantar, sóleo e gastrocnêmio de ratos obesos.

2 OBJETIVOS

2.1 Objetivo Geral

Verificar a influência do treinamento físico aeróbico intervalado de alta intensidade (HIIT) e da suplementação com L-Carnitina sobre as alterações metabólicas, bioquímicas, de estresse oxidativo e de inflamação em tecidos/órgãos e sangue de ratos obesos.

2.2 Objetivos Específicos

1) Verificar a influência do protocolo HIIT e da suplementação com L-Carnitina sobre TBA-RS, conteúdo total de sulfidrilas e conteúdo total de proteínas carboniladas em músculos (sóleo, plantar e gastrocnêmico), adipócitos, plasma, fígado, rins, coração e estruturas cerebrais (córtex cerebral e cerebelo) de ratos submetidos à dieta hiperlipídica;
2) Verificar a influência do protocolo HIIT e da suplementação com L-Carnitina sobre a atividade das enzimas antioxidantes catalase (CAT), glutationa peroxidase (GSH-Px) e superóxido dismutase (SOD) em músculos (sóleo, plantar e gastrocnêmico), adipócitos, eritrócitos, fígado, rins, coração e estruturas cerebrais (córtex cerebral e cerebelo) de ratos submetidos à dieta hiperlipídica;
3) Verificar a influência do protocolo HIIT e da suplementação com L-Carnitina, sobre a atividade das enzimas piruvato quinase, citrato sintase, complexo II e succinato desidrogenase, complexo IV (citocromo *C* oxidase) em músculos (sóleo, plantar e gastrocnêmico) e estruturas cerebrais (córtex cerebral e cerebelo) de ratos submetidos à dieta hiperlipídica;
4) Verificar a influência do protocolo HIIT e da suplementação com L-Carnitina sobre o fator de necrose tumoral alfa (TNF-α), interleucina 6 (IL-6) e interleucina 1 beta (IL-1β) em soro de ratos submetidos à dieta hiperlipídica;
5) Verificar a influência do protocolo HIIT e da suplementação com L-Carnitina sobre as dosagens de glicose, insulina, triglicerídeos, colesterol total, colesterol LDL,

colesterol HDL e sobre a gordura intra abdominal, de ratos submetidos à dieta hiperlipídica.

3 REVISÃO

3.1 Obesidade e Sedentarismo

3.1.1 Obesidade

De acordo com a Organização Mundial de Saúde (OMS), o termo obesidade é empregado para descrever um acúmulo excessivo de gordura corporal prejudicial à saúde do indivíduo (WHO, 2020). Para se realizar o diagnóstico e a classificação da obesidade, utiliza-se um parâmetro conhecido mundialmente como Índice de Massa Corporal (IMC), no qual o peso de uma pessoa (em quilogramas) é dividido pelo quadrado da sua altura (em metros) (WANDERLEY; FERREIRA, 2010; WHO, 2020). Indíviduos que apresentam um IMC superior ou igual a 30 kg/m^2 são considerados obesos (WANDERLEY; FERREIRA, 2010; WHO, 2020).

Vários estudos já foram capazes de associar a obesidade ao desenvolvimento de diversas complicações, entre elas: complicações cardiovasculares (hipertensão arterial, insuficiência coronariana, aterosclerose), respiratórias, osteomusculares, dermatológicas, distúrbios relacionados ao sistema endócrino-metabólico (Diabetes Mellitus e dislipidemia) e também ao aparecimento de determinadas neoplasias (DE FRANCISCHI *et al.*, 2000).

A associação entre obesidade e quadros de estresse oxidativo e alterações no metabolismo energético celular já foi descrita por pesquisadores. De acordo com França e colaboradores (2013), pacientes obesos possuem alterações biológicas que tornam o organismo mais susceptível à lesões oxidativas. Esses indivíduos possuem um desequilíbrio entre as quantidades de gordura, peso corporal, lipoproteínas e lipídeos, desequilíbrio esse que promove aumento da necessidade metabólica e do consumo de oxigênio e consequentemente, uma produção aumentada de espécies reativas de oxigênio (EROs), como superóxidos e peróxidos de hidrogênio (FRANÇA *et al.*, 2013).

Além do aumento na produção de radicais livres, estudos sugerem que a obesidade está associada à uma redução na atividade das enzimas antioxidantes. Em 2002, Olusi testou 250 indivíduos obesos através das medidas das enzimas

superóxido dismutase (SOD) e glutationa peroxidase (GSH-Px) e por meio desse estudo, chegou à conclusão de que pacientes obesos apresentam uma menor atividade enzimática antioxidante eritrocitária, situação essa que pode gerar danos progressivos às células e ser uma das explicações para a obesidade ser um importante fator de risco no desenvolvimento de diversas patologias.

No que diz respeito as alterações no metabolismo energético, diversos estudos já conseguiram correlacionar a obesidade às alterações no funcionamento da cadeia respiratória mitocondrial e também à efeitos deletérios ao metabolismo de carboidratos. Em 2013, Christe e colaboradores, ao analisar a atividade da enzima Citrato Sintase (participante do Ciclo do Ácido Tricarboxílico) em 90 pacientes obesos e em 45 pacientes não-obesos, detectaram que os indivíduos obesos apresentaram um decréscimo na atividade e na expressão proteica dessa enzima, e como consequência, apresentaram uma redução na capacidade oxidativa de suas mitocôndrias. Entretanto, mais estudos se fazem necessários para comprovação desse mecanismo, uma vez que outros estudos não demonstraram essa alteração mitocondrial (FISHER-WELLMAN, 2014).

Em relação aos efeitos da obesidade sobre o metabolismo de carboidratos, estudos conduzidos por Beck-Nielsen (2012), Velho (1996) e colaboradores, demonstraram em indivíduos portadores de doenças relacionadas à obesidade, uma redução no conteúdo de glicogênio, tanto muscular quanto hepático, sugerindo um possível estresse metabólico. É de conhecimento científico que a glicose armazenada em forma de glicogênio (hepático e muscular) é acionada em momentos de maior necessidade de aporte energético do organismo (CEPERUELO-MALLAFRÉ, 2016). Por outro lado, Lu e colaboradores (2014) encontraram um aumento no conteúdo de glicogênio hepático em ratos alimentados com dieta hiperlipídica, comprovando que o mecanismo responsável pela interferência no metabolismo do glicogênio ainda necessita de maiores estudos para avaliação dos possíveis efeitos.

Outra questão importante relacionada à obesidade é a sua capacidade de desencadear processos inflamatórios crônicos. Pacientes obesos apresentam níveis circulantes elevados de citocinas e proteínas de fase aguda associadas à inflamação, como IL-6, TNF-α e leptina, o que pode ser explicado pela secreção dessas adipocinas pelo tecido adiposo, presente em maior quantidade nos indivíduos obesos (DO

PRADO *et al.*, 2009; FRANCISQUETI; NASCIMENTO; CORRÊA, 2015). Os níveis circulantes elevados dessas adipocinas interferem em diversos processos metabólicos e biológicos, como controle da ingestão alimentar e balanço energético, sistema imunológico, sensibilidade à insulina, angiogênese, controle da pressão arterial, metabolismo de lipídeos e homeostase corporal, processos esses que, em desequílibrio, estão fortemente associados ao desenvolvimento de doenças cardiovasculares (DO PRADO *et al.*, 2009).

3.1.2 Sedentarismo

A prática diária de exercícios físicos já é comprovadamente uma das melhores maneiras de promover saúde física, mental e cognitiva ao ser humano, independente da faixa etária e do gênero (CARLUCCI *et al.*, 2013). Estudos demonstram que exercícios físicos, sejam eles esportivos ou recreacionais, se realizados com frequência e constância, têm a capacidade de auxiliar no processo de emagrecimento, seja pela promoção de ganho de massa magra ou perda de gordura corporal, contribuindo para a prevenção de inúmeras patologias, principalmente aquelas atribuídas ou favorecidas pela obesidade (CARLUCCI *et al.*, 2013).

Mesmo diante de todos esses benefícios já comprovados cientificamente, dados obtidos pelo Instituto Brasileiro de Geografia e Estatística (IBGE) por meio da Pesquisa Nacional de Saúde (PNS), realizada em 2019, demonstraram que 40,3% da população brasileira que se encontra na faixa de idade entre 18 ou mais anos de idade, são classificados como insuficientemente ativos, uma vez que não praticam atividade física ou praticam menos do que 150 minutos por semana. Além disso, a PNS mostra que, no Brasil, as mulheres são menos ativas fisicamente que os homens, correspondendo a 49,5% e 32,1%, respectivamente. Entre as faixas etárias, a PNS mostra que 59,7% das pessoas do grupo de 60 anos ou mais de idade é insuficientemente ativa, enquanto o grupo menos sedentário é o grupo que se encontra na faixa etária de 18 a 24 anos de idade (32,8%), seguido pelo grupo de 25 a 39 anos (32,9%) (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE, 2020).

Segundo a Organização Pan-Americana de Saúde, o sedentarismo constitui um importante fator de risco para o desenvolvimento de doenças crônico-degenerativas não transmissíveis, com hipertensão arterial sistêmica (HAS), Diabetes Mellitus (DM) e câncer (OPAS, 2003). Dessa maneira, a obesidade e o sedentarismo, isolados ou em associação, possuem papel importante no planejamento da saúde pública, uma vez que são considerados fatores de risco desencadeadores de diversas patologias, as quais se tornam dispendiosas ao sistema público de saúde (DA COSTA; VASCONCELOS; DA FONSECA, 2014).

3.2 Radicais Livres

Os radicais livres são moléculas altamente reativas e instáveis, que contêm elétrons não pareados em sua última camada eletrônica, o que os tornam capazes de reagir com outras moléculas, como proteínas, carboidratos, lipídeos e DNA, modificando suas estruturas moleculares ao se comportarem como receptores (oxidantes) ou doadores (redutores) de elétrons (ANDRADE JÚNIOR *et al.*, 2005; BARBOSA *et al.*, 2010; FERREIRA; MATSUBARA, 1997).

A geração de radicais livres é um processo fisiológico e de extrema importância para o correto funcionamento do organismo (BARBOSA *et al.*, 2010). Um exemplo de situação na qual se faz importante a geração de radicais livres é no processo de defesa contra infecções, quando os neutrófilos são estimulados a produzir radicais livres com o intuito de destruir um microrganismo (FERREIRA; MATSUBARA, 1997).

Outras situações nas quais a produção de radicais livres se torna benéfica são: no processo fagocitário, na regulação do crescimento celular, na sinalização intercelular, na geração de energia (ATP), na fertilização dos óvulos e na ativação genética (BARBOSA *et al.*, 2010; ENGERS; BEHLING; FRIZZO, 2011; RAHMAN, 2007). Desta forma, em níveis baixos ou moderados, as espécies reativas de oxigênio e de nitrogênio exercem efeitos benéficos sobre as respostas celulares e a função imunológica.

Entretanto, quando em concentrações elevadas, os radicais livres encontram-se associados diretamente com o aparecimento de inúmeras doenças, como câncer e uma variedade de alterações degenerativas. Saeidnia e Abdollahi (2013) atribuem

esse papel devido a capacidade dos radicais livres em iniciar processos de apoptose e necrose celular.

Em um estudo de 2014, Blake e Trounce relataram que um período de hiperglicemia prolongada causa alterações de vias metabólicas e à superprodução de radicais livres pela mitocôndria, processo esse que pode estar relacionado ao desencadeamento de complicações do DM.

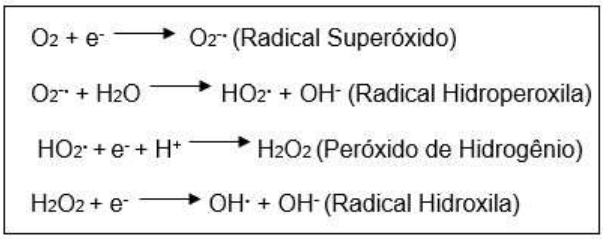
Agarwal e colaboradores (2012), por sua vez, relacionaram a superprodução de radicais livres mitocondrial com a alteração na geração de ATP, o qual é essencial para o desenvolvimento dos óocitos e embriões, e com o desencadeamento de uma série de doenças reprodutivas, como Síndrome do Ovário Policístico, endometriose e infertilidade.

Entre as principais espécies de radicais livres, encontram-se as Espécies Reativas de Oxigênio e as Espécies Reativas de Nitrogênio, nos quais os elétrons desemparelhados estão ligados aos átomos de oxigênio e nitrogênio, respectivamente (BARREIROS; DAVID; DAVID, 2006).

3.2.1 Espécies Reativas de Oxigênio (EROs)

As EROs são encontradas em todos os organismos vivos. Em condições normais, na cadeia de transporte de elétrons, o O_2 sofre redução tetravalente, ou seja, recebe 4 elétrons, formando duas moléculas de H_2O (FERREIRA; MATSUBARA, 1997). Ao decorrer desse processo, tem-se a formação de EROs, sendo as principais o radical superóxido ($O_2^{-\bullet}$), o radical hidroperoxila ($HO_2^{\bullet}$), o peróxido de hidrogênio (H_20_2), o radical hidroxila ($OH^{\bullet}$) e o oxigênio singlet ($^1O_2$) (BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997), conforme Figura 1.

Figura 1 – Formação de EROs



Fonte: Adaptado de Koury e colaboradores (2003).

3.2.1.1 Radical Superóxido ($O_2^{-\cdot}$)

O $O_2^{-\cdot}$ é formado a partir da primeira redução do O_2 com um elétron e produzido basicamente nas mitocôndrias, microssomas e peroxissomas e ocorre em quase todas as células aeróbicas (ANDRADE JÚNIOR *et al.*, 2005; FERREIRA; MATSUBARA, 1997). Possui uma meia vida longa, e apesar de possuir pouca reatividade em meios aquosos, suas reações podem desencadear a formação de $OH^{\cdot}$ e $HO_2^{\cdot}$, e quando em meio ácido, do H_2O_2 (ANDRADE JÚNIOR *et al*., 2005; FERREIRA; MATSUBARA, 1997).

3.2.1.2 Radical Hidroperoxila ($HO_2^{\cdot}$)

O $HO_2^{\cdot}$ é resultado da protonação do $O_2^{-\cdot}$ com um próton do íon hidrogênio em meio aquoso (FERREIRA; MATSUBARA, 1997; VELLOSA *et al.*, 2021).

Trata-se de uma ERO mais reativa que o superóxido, devido a sua alta capacidade de penetrar nas membranas lipídicas, gerando danos como lipoperoxidação (FERREIRA; MATSUBARA, 1997; VELLOSA *et al.*, 2021).

3.2.1.3 Peróxido de Hidrogênio (H_20_2)

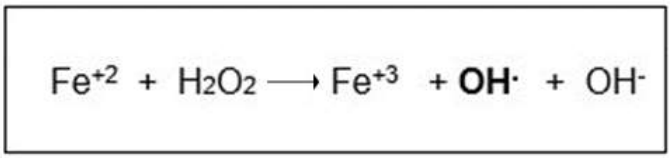
O H_2O_2 é gerado pela protonação do $O_2^{-\cdot}$ em meio ácido (ANDRADE JÚNIOR *et al.*, 2005). Embora não seja considerado um radical livre, é altamente tóxico para as células. Essa toxicidade se deve a sua meia vida longa, ao fato de não possuir elétrons desemparelhados na última camada e a sua capacidade de atravessar membranas lipídicas, reagindo também com a membrana eritrocitária e proteínas ligadas ao ferro, produzindo então danos às moléculas através da geração de novas ERO, como o $OH^{\cdot}$ (ANDRADE JÚNIOR *et al.*, 2005; BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997).

3.2.1.4 Radical Hidroxila ($OH^{\cdot}$)

O $OH^{\cdot}$ é a ERO mais reativa de todas, grande parte devido à sua meia vida curta e maior instabilidade eletrônica, características essas que lhe conferem maior rapidez e capacidade de retirar elétrons de outras moléculas, como ácidos orgânicos, fosfolipídeos, ácido desoxirribonucleico (DNA), ácido ribonucleico (RNA) e aminoácidos (ANDRADE JÚNIOR *et al.*, 2005; BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997).

A geração do $OH^{\cdot}$ pode acontecer principalmente por duas vias de reação química. A primeira reação é denominada Reação de Fenton, observada pela primeira vez em 1894, e baseia-se na reação do H_2O_2 com o sal de ferro, conforme pode ser analisado na Figura 2 (ANDRADE JÚNIOR *et al.*, 2005; FERREIRA; MATSUBARA, 1997).

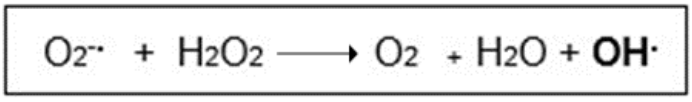
Figura 2 – Formação de $OH^{\cdot}$ pela Reação de Fenton



Fonte: Adaptado de ANDRADE JÚNIOR *et al.* (2005).

Outra via com papel importante na formação do $OH^{\cdot}$ é a reação de Haber-Weiss, na qual ocorre a interação do H_2O_2 com o $O_2^{-\cdot}$, conforme descrito na Figura 3 (ANDRADE JÚNIOR *et al*., 2005).

Figura 3 - Formação de $OH^{\cdot}$ pela Reação de Haber-Weiss



Fonte: Adaptado de ANDRADE JÚNIOR *et al.* (2005).

3.2.1.5 Oxigênio Singlet ($^1O_2$)

O $^1O_2$ é uma ERO que não possui elétrons desemparelhados em sua última camada e consiste na forma excitada do oxigênio molecular (O_2) (FERREIRA; MATSUBARA, 1997). O $^1O_2$ possui importância fundamental nos processos químicos e biológicos, grande parte devido à sua alta reatividade e envolvimento na geração de processos fisiológicos e patológicos (DI MASCIO *et al.*, 2016).

Estudos têm demonstrado que essa ERO pode ser gerada por sistemas biológicos, como em reações catalisadas por peroxidases, lactoperoxidases e mieloperoxidases, reação do H_2O_2 com hipoclorito ou peroxinitrito, pela reação de ozônio com biomoléculas ou através da fagocitose (DI MASCIO *et al.*, 2016; RONSEIN *et al.*, 2006).

Há evidências de que o $^1O_2$ é responsável pela oxidação de lipídeos, aminoácidos, proteínas, ácidos nucleicos e carboidratos, ocasionando doenças como cataratas e porfirias (DI MASCIO *et al*., 2016; RONSEIN *et al.*, 2006).

3.2.2 Espécies Reativas de Nitrogênio (ERNs)

Dentre os principais radicais livres derivados da geração de ERNs encontram-se o óxido nítrico ($NO^{\cdot}$), óxido nitroso (N_2O_3), ácido nitroso (HNO_2), nitritos (NO_2^-) e peroxinitritos ($ONOO^-$) (BARREIROS; DAVID; DAVID, 2006).

O $NO^{\cdot}$ é constituído por sete elétrons de nitrogênio e oito elétrons de oxigênio, possuindo um elétron desemparelhado na sua última camada (DUSSE; VIEIRA; CARVALHO, 2003). É produzido pelo organismo por meio da ação da enzima óxido nítrico sintase e também pela estimulação dos fagócitos humanos (BARREIROS; DAVID; DAVID, 2006).

A formação ou metabolização de uma ERN pode desencadear a geração de outras ERNs. O nitrato, por sua vez, pode se transformar em NO_2^-, que ao entrar em contato com os ácidos gástricos, leva a formação do HNO_2, radical livre esse que promove a retirada dos grupos amino das bases do DNA (BARREIROS; DAVID; DAVID, 2006).

Para que o $NO^{\cdot}$ seja capaz de atacar diretamente o DNA, é necessário que o mesmo reaja com o $O_2^{-\cdot}$, formando $ONOO^-$, uma ERN produtora de agentes capazes de introduzir grupamentos amino em aminoácidos aromáticos (BARREIROS; DAVID; DAVID, 2006).

3.3 Estresse Oxidativo

O quadro de estresse oxidativo instala-se quando existe um desequilíbrio no processo de geração dos radicais livres, seja pelo aumento na formação desses compostos ou pela redução da atividade dos sistemas antioxidantes (BARBOSA *et al.*, 2010; BARREIROS; DAVID; DAVID, 2006; ENGERS; BEHLING; FRIZZO, 2011).

O estresse oxidativo provoca alterações em diversas biomoléculas, as quais podem perder suas funções biológicas ao sofrer danos como peroxidação lipídica, danos oxidativos ao DNA e a proteínas (BARBOSA *et al.*, 2010; SILVA; FERRARI, 2011).

Caso o processo descompensatório entre a geração de radicais livres e sua eliminação aconteça por um período prolongado, podem ser desencadeadas várias patologias, entre elas: aterosclerose, artrite, choque hemorrágico, distúrbios cardiovasculares, DM, obesidade, câncer e transtornos neurodegenerativos (BARBOSA *et al*., 2010; BARREIROS; DAVID; DAVID, 2006).

3.4 Mecanismo de Danos às Moléculas

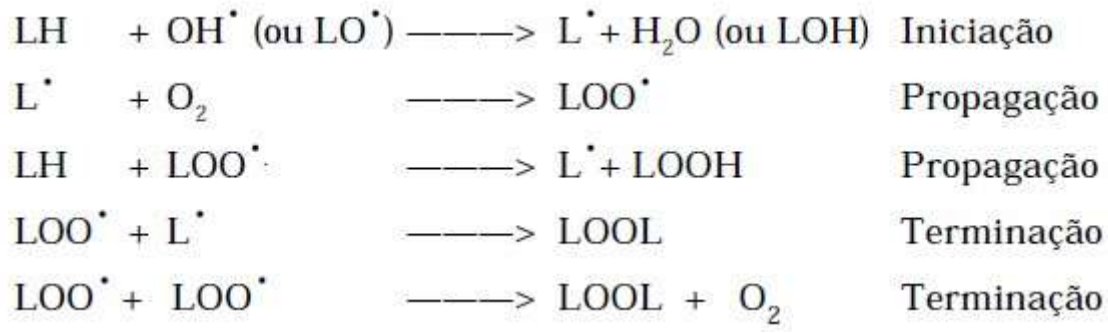
3.4.1 Peroxidação lipídica ou lipoperoxidação

As membranas plasmáticas são as estruturas celulares mais susceptíveis aos efeitos deletérios dos radicais livres, uma vez que a peroxidação lipídica altera sua estrutura, reduzindo a seletividade no transporte iônico e a sinalização transmembrana, prejudicando sua permeabilidade e consequentemente, o transporte celular (FERREIRA; MATSUBARA, 1997; SILVA; FERRARI, 2011). Essas alterações podem resultar em morte celular, uma vez que há a liberação de enzimas hidrolíticas

dos lisossomas e formação de produtos citotóxicos, como o malondialdeído (ANDRADE JÚNIOR *et al.*, 2005; FERREIRA; MATSUBARA, 1997).

O processo de peroxidação lipídica envolve três etapas: iniciação, propagação e terminação, as quais estão descritas na Figura 4.

Figura 4 – Reações de Lipoperoxidação, onde L representa o lipídeo.



Fonte: FERREIRA *et al.* (1997).

Na etapa de iniciação, o radical hidroxila (OH·) ou radical alcoxila (LO·) sequestra o hidrogênio do ácido graxo polinsaturado (LH), levando a formação do radical lipídico (L·). Na primeira reação de propagação, o radical L· reage com o O_2, gerando um radical peroxila (LOO·), que por sua vez sequestra novo hidrogênio do LH, e na segunda reação de propagação, forma mais um radical L·. O processo de peroxidação lipídica se encerra quando os radicais L· e LOO· se propagam até a autodestruição ou pelo compartilhamento de elétrons, através de ligação covalente (ANDRADE JÚNIOR *et al.*, 2005; FERREIRA; MATSUBARA, 1997).

Os íons ferro também participam do processo de lipoperoxidação por meio do desencadeamento de reações de iniciação. Esses íons atuam como catalisadores da conversão de LOOH em radicais altamente reativos como LO·, OH· e LOO·, dando início a uma nova cadeia de reações, que recebe o nome de ramificação (Figura 5) (BARBOSA *et al.*, 2010; FERREIRA; MATSUBARA, 1997).

Figura 5 – Reação de ramificação

$$LOOH + Fe^{++} \text{ —rápida—> } LO^{\bullet} + OH^{\bullet} + Fe^{+++}$$
$$LOOH + Fe^{+++} \text{ —lenta——> } LOO^{\bullet} + H^{+} + Fe^{++}$$

Fonte: FERREIRA *et al.* (1997).

3.4.2 Danos às proteínas

As proteínas são essenciais para o correto funcionamento do organismo e desempenham inúmeras funções biológicas como regeneração dos tecidos, catalisação de reações enzimáticas, hormonais e imunológicas. São compostas por cadeias de aminoácidos ligados entre si por ligações peptídicas (NELSON; COX, 2014).

O processo oxidativo induzido pelas EROs gera mudanças físicas nas proteínas, aumentando sua fragmentação, agregação e suscetibilidade à degradação por proteossomos (proteases multicatalíticas) (ANDRADE JÚNIOR *et al.*, 2005; SILVA; FERRARI, 2011). Além disso, as EROs inibem a atividade enzimática responsável por editar e corrigir o RNA transportador, levando a síntese anômala de proteínas (NELSON; COX, 2014; SILVA; FERRARI, 2011).

Segundo Andrade Júnior *et al.* (2005), proteínas como albumina e colágeno, são fragmentadas em prolina (pelo radical $OH^{\cdot}$) e em aminoácidos como histidina e arginina, que se associam com metais de transição. A agregação proteica acontece principalmente pela atividade oxidativa do $OH^{\cdot}$, capaz de formar ligações cruzadas entre as proteínas e a degradação ocorre devido a formação de proteínas anômalas, favorecendo sua remoção pelos proteossomos (ANDRADE JÚNIOR *et al.*, 2005).

3.4.3 Danos ao DNA

O DNA é composto de uma sequência de nucleotídeos e bases nitrogenadas, presente em cromossomos e plasmídeos, e é responsável por armazenar todas as informações genéticas e hereditárias de um ser vivo, além de produzir e codificar corretamente todas as proteínas presentes no organismo (LEWIN *et al.*, 2011; MARTINEZ *et al*., 2006).

Os danos oxidativos ao DNA são ocasionados principalmente pela ação do radical OH·, por meio de uma série de reações que consistem na formação de ligações cruzadas de DNA e suas proteínas, oxidação da desoxirribose e quebra das dupla hélices (NELSON; COX, 2014; SILVA; FERRARI, 2011). Uma das estruturas celulares mais susceptíveis aos danos oxidativos ao nível de DNA é a mitocôndria, por se tratar de uma das fontes mais importantes de produção de EROs no organismo (ANDRADE JÚNIOR *et al*., 2005). A lesão mitocondrial consiste em alterações de bases e quebras moleculares, onde as bases nitrogenadas mais acometidas pelo estresse oxidativo são a timina e citosina (ANDRADE JÚNIOR *et al.*, 2005).

A ação dos radicais livres sobre o DNA, principalmente do radical OH·, leva a alterações na expressão gênica do mesmo, propiciando o desenvolvimento de inúmeros processos patológicos crônicos, como câncer e doenças neurodegenerativas (MARTINEZ *et al*., 2006; SILVA; FERRARI, 2011).

3.5 Sistemas de Defesas Antioxidantes

Os sistemas antioxidantes são sistemas criados pelo organismo para inibir e/ou reduzir os efeitos tóxicos do excesso de radicais livres às células (BARBOSA *et al*., 2010; KOURY; DONANGELO, 2003). Para que essa proteção ocorra por completo, diferentes mecanismos de ação podem ser usados, dentre eles: sistemas de prevenção, os quais impedem a formação de radicais livres; sistemas varredores, que impedem a ação dos radicais livres; e/ou por meio de sistemas de reparo, os quais são responsáveis por acelerar o reparo e a reconstituição das células e estruturas biológicas danificadas (BARBOSA *et al.*, 2010; KOURY; DONANGELO, 2003).

Os sistemas antioxidantes podem ser divididos em dois grupos: o sistema enzimático e não-enzimático, sendo o último sistema composto por inúmeras substâncias antioxidantes que podem ser de origem endógena ou adquiridas pela dieta, como vitaminas, polifenóis e minerais (Tabela 1) (BARBOSA *et al*., 2008, 2010; BARREIROS; DAVID; DAVID, 2006).

Tabela 1. Ações/mecanismos das principais substâncias antioxidantes.

Antioxidantes	Ação	Referências
Não enzimáticos (de origem dietética)		
Vitamina A (Betacaroteno)	Proteção contra oxidação de lipideos e DNA.	Rodrigo *et al.*, 2007.
Vitamina C (Ácido Ascórbico)	Inibição das ERO (Agente redutor), regeneração de alfa-tocoferol.	Rodrigo *et al.*, 2007.
Vitamina E (Alfa-tocoferol)	Proteção contra a peroxidação dos ácidos graxos insaturados da membrana celular.	Rodrigo *et al.*, 2007.
Cu, Zn, Mn, Se	Cofatores das enzimas antioxidantes SOD-Cu/Zn, SOD-Mn e GSH-Px.	Vincent *et al.*, 2007.
Outros carotenoides (licopeno)	Proteção contra a oxidação de lipideos, LDL, proteinas e DNA.	Visioli *et al.*, 2003.
Fitoquimicos (Resveratrol, catequinas, quercetinas, ácidos fenólicos e outros)	Proteção contra a oxidação de lipideos e DNA.	Fito *et al.*, 2007.
Enzimáticos		
Superóxido Dismutase (SOD)	SOD-Cu/Zn (citoplasma), SOD-Mn (mitocôndria). Catalisa a conversão do radical $O_2^{\cdot-}$ em H_2O_2.	Vincent *et al.*, 2007.
Catalase (CAT)	Catalisa a conversão de H_2O_2 em O_2 e H_2O.	Vincent *et al.*, 2007.
Glutationa Peroxidase (GSH-Px)	Catalisa a redução de H_2O_2 em H_2O.	Vincent *et al.*, 2007.

Fonte: Adaptado de BARBOSA *et al.* (2010).

3.5.1 Sistema Enzimático

O Sistema Enzimático é um grupo de defesa antioxidante composto por três importantes enzimas presentes no citosol e mitocôndrias das células: Superóxido Dismutase (SOD), Catalase (CAT) e Glutationa Peroxidase (GSH-Px) (ANDRADE JÚNIOR *et al.*, 2005; BARBOSA *et al*., 2010).

3.5.1.1 Superóxido Dismutase (SOD)

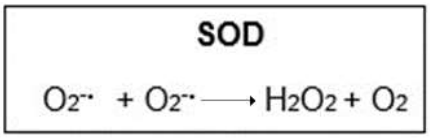
A SOD corresponde a um grupo de enzimas presente nos sistemas eucariontes com diferentes composições estruturais, como SOD-Cobre-Zinco (SOD-Cu-Zn) e

SOD-Manganês (SOD-Mn) (ENGERS; BEHLING; FRIZZO, 2011; FERREIRA; MATSUBARA, 1997).

A forma SOD-Cobre-Zinco está presente principalmente no citosol, e possui Cobre e Zinco em seu centro redox (BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997). A segunda forma de SOD está presente nas mitocôndrias celulares e tem como centro redox o Manganês (BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997). A SOD-Manganês tem sua atividade exacerbada pelo processo de dano oxidativo (BARREIROS; DAVID; DAVID, 2006).

O sistema de defesa composto pela SOD possui um importante papel antioxidante, responsável por catalisar a dismutação do $O_2^{-\cdot}$ em H_2O_2 e O_2 (Figura 6), prevenindo assim o acúmulo desse radical livre e exercendo ação protetora contra lesões ao DNA (ANDRADE JÚNIOR *et al.*, 2005; ENGERS; BEHLING; FRIZZO, 2011).

Figura 6 – Reação de Dismutação do $O_2^{-\cdot}$ em H_2O_2 e O_2.



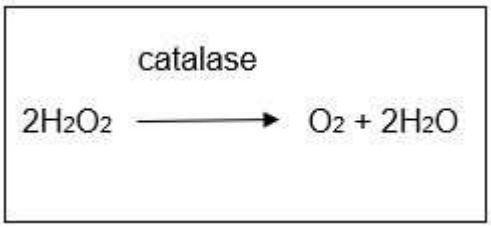
Fonte: Adaptado de ANDRADE JÚNIOR *et al*. (2005).

3.5.1.2 Catalase (CAT)

A CAT é uma enzima composta por um grupo prostético heme que pode ser encontrada em mitocôndrias e matrizes peroxissomais (ENGERS; BEHLING; FRIZZO, 2011).

Desempenha sua função antioxidante por meio da catálise da decomposição de H_2O_2 em H_2O e O_2 (Figura 7), atuando em conjunto e dividindo essa função com a GSH-Px (ANDRADE JÚNIOR *et al*., 2005; BARREIROS; DAVID; DAVID, 2006).

Figura 7 – Reação de decomposição do H_2O_2 em H_2O e O_2 pela ação da CAT.



Fonte: Adaptado de BARREIROS *et al.* (2006).

Quando em baixas concentrações de H_2O_2, este é preferencialmente eliminado do organismo pela ação da GSH-Px, mas quando em altos níveis de H_2O_2, este composto é eliminado predominantemente pela atividade antioxidante da CAT (ANDRADE JÚNIOR *et al*., 2005).

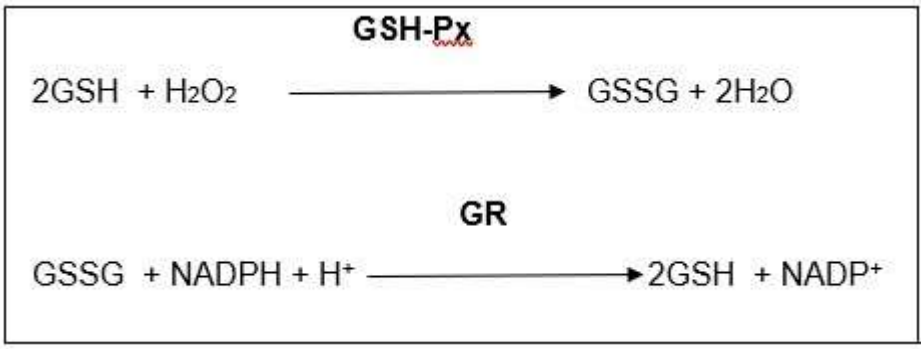
As atividades antioxidantes da CAT e da GSH-Px na decomposição do H_2O_2 em H_2O são de extrema importância, uma vez que esse composto na presença de cobre e ferro leva a geração de OH· através das reações de Fenton e Haber-Weiss, ERO altamente reativa e instável, contra a qual não existe sistema antioxidante enzimático especializado (BARBOSA *et al*., 2010).

3.5.1.3 Glutationa Peroxidase (GSH-Px) e Glutationa (GSH)

O terceiro sistema antioxidante enzimático é formado pela GSH em conjunto com as enzimas GSH-Px e glutationa redutase (GR) (BARREIROS; DAVID; DAVID, 2006). A GSH-Px está presente no citoplasma e nas mitocôndrias celulares em duas formas, a forma dependente e independente de selênio (BARBOSA *et al.*, 2010).

Assim como a CAT, esse sistema atua catalisando a dismutação do H_2O_2 em H_2O. Esse processo consiste na redução do H_2O_2 pela GSH na presença de GSH-Px, formando uma ponte dissulfeto e um composto chamado glutationa oxidada (GSSG) (Figura 8) (BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997). Posteriormente, a GSSG é reduzida sob ação da enzima GR, utilizando elétrons provenientes da nicotinamida adenina dinucleotídeo fosfato (NADPH), que é formada na via das pentoses fosfato sob ação da enzima glicose-6-fostato-desidrogenase (BARREIROS; DAVID; DAVID, 2006; FERREIRA; MATSUBARA, 1997).

Figura 8 – Catálise da dismutação do H_2O_2 em H_2O e O_2 por ação da GSH-Px.



Fonte: Adaptado de BARREIROS *et al.* (2006).

3.6 Metabolismo Energético

O termo metabolismo é empregado para descrever um conjunto de reações químicas catalisadas por enzimas com o principal objetivo de atender as necessidades estruturais e energéticas de uma célula ou de um organismo (NELSON; COX, 2014).

As reações metabólicas podem ser divididas em catabólicas e anabólicas. As reações catabólicas consistem na degradação de moléculas orgânicas, como carboidratos, lipídeos e proteínas, produzindo energia, enquanto as reações anabólicas são responsáveis pela biossíntese de moléculas orgânicas, consumindo energia (NELSON; COX, 2014).

Grande parte da energia produzida na degradação de biomoléculas durante as reações catabólicas é armazenada em ligações fosfato de alta energia do ATP (*adenosine triphosphato* – trifosfato de adenosina) ou armazenada em elétrons de alta energia do NADH, $FADH_2$ ou NADPH (SILVERTHORN, 2010). O ATP é um composto de alta energia que participa de inúmeras reações e processos metabólicos relacionados à transferência e conversão de energia celular (MCARDLE; KATCH; KATCH, 2016; NELSON; COX, 2014).

Para que a molécula de ATP seja utilizada como energia pelas células é necessário que a mesma sofra hidrólise, reação catalisada pela enzima ATPase, e seja convertida em ADP + Fósforo inorgânico (Pi) + H^+ + energia, conforme a Figura 9 (GUYTON; HALL, 2011; NELSON; COX, 2014).

Figura 9 – Hidrólise de ATP.

$$\text{ATP} + \text{H}_2\text{O} \xrightarrow{\text{ATPase}} \text{ADP} + \text{P}_i + \text{H}^+ + \text{energia}$$

Fonte: Adaptado de Silverthorn (2010).

Para que o equilíbrio celular seja mantido se fazem necessários mecanismos que controlem e adequem os processos de síntese de ATP de acordo com a necessidade energética do organismo (GUIMARAES-FERREIRA, 2014). Durante exercício físico, dependendo da intensidade e da duração, pode ser necessário uma maior geração de ATP (GIMENES; BRACHT, 2001).

A síntese de ATP pode ocorrer por meio de três principais vias metabólicas: sistema fosfogênico, glicólise e fosforilação oxidativa (SILVERTHORN, 2010).

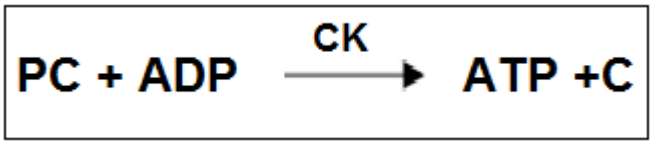
3.6.1 Sistema ATP-Pcr (ATP-fosfocreatina)

O Sistema ATP-Pcr ou sistema fosfogênico é um sistema energético constituído por fosfocreatina e ATP, o qual pode ser encontrado no estoque intramuscular (GUIMARAES-FERREIRA, 2014; GUYTON; HALL, 2011; SILVERTHORN, 2010).

Esse sistema é a fonte de ATP mais rapidamente disponível no organismo - principalmente para os músculos - e é capaz de proporcionar uma potência máxima energética muscular por aproximadamente 8 à 10 segundos, energia essa suficiente para uma corrida de 100 metros, por exemplo (GUYTON; HALL, 2011; MCARDLE; KATCH; KATCH, 2016).

Trata-se do primeiro sistema energético ativado pelo organismo para realizar a reposição de ATP. Para que a ressíntese de ATP ocorra, é necessário que a fosfocreatina (Pcr) sofra hidrólise, reação catalisada pela enzima creatinoquinase (CK), liberando um grupo fosfato para a ADP e gerando como produtos finais uma molécula de ATP e uma molécula de creatina, conforme Figura 10 (MCARDLE; KATCH; KATCH, 2016; SILVERTHORN, 2010).

Figura 10 – Hidrólise da fosfocreatina para geração de ATP.



Legenda: PC – Fosfocreatina; ADP - Adenosina Difosfato; CK – Creatinoquinase; ATP – Adenosina Trifosfato; C - Creatina.

Fonte: Adaptado de McArdle *et al.* (2016).

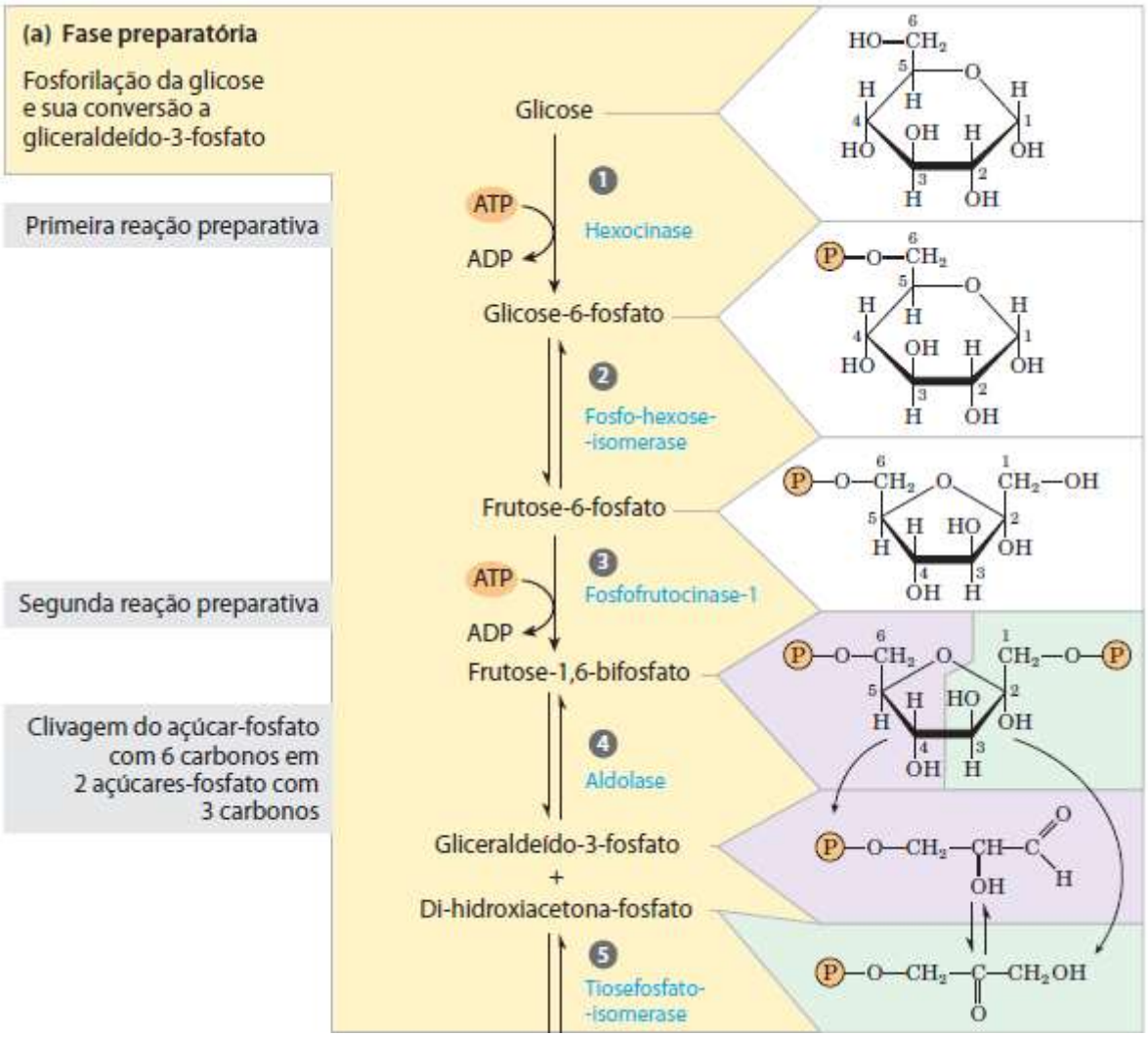
Mesmo se tratando de uma via rápida e eficaz para ressíntese de ATP, o sistema ATP-Pcr gera energia por poucos segundos (GUYTON; HALL, 2011; MCARDLE; KATCH; KATCH, 2016), fazendo com que a ativação de outros sistemas energéticos se faça necessária para garantir a continuidade da contração muscular, principalmente em casos de exercícios físicos de alta intensidade.

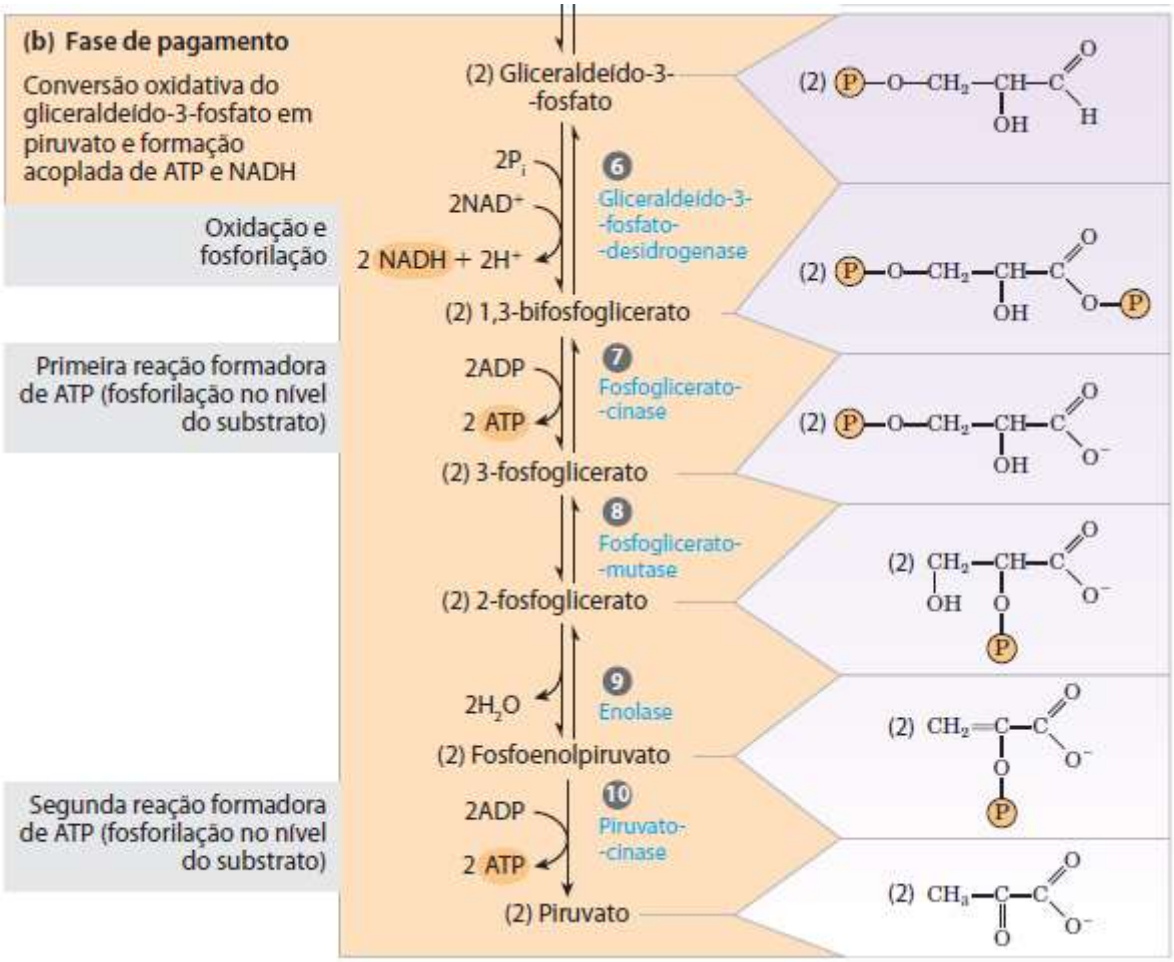
3.6.2 Glicólise

Após exauridas as reservas fosfogênicas, a via ativada é a glicólise, processo esse que consiste na quedra de uma molécula de glicose por uma série de reações catalisadas por enzimas, resultando na geração de duas moléculas de piruvato, compostas por três átomos de carbono (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014). A capacidade de produção de ATP pela glicólise é muito maior quando comparado ao sistema ATP-Pcr (MAUGHAN; GLEESON; GREENHAFF, 2000).

Trata-se de uma via anaeróbica, que acontece no citoplasma celular e que ao longo de uma sequência de dez reações enzimáticas (Figura 11), gera energia na forma de ATP e NADH (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

Figura 11 – Fases da Glicólise


(a) Fase preparatória
Fosforilação da glicose e sua conversão a gliceraldeído-3-fosfato
Primeira reação preparativa
Segunda reação preparativa
Clivagem do açúcar-fosfato com 6 carbonos em 2 açúcares-fosfato com 3 carbonos
Glicose
ATP
ADP
1
Hexocinase
Glicose-6-fosfato
2
Fosfo-hexose--isomerase
Frutose-6-fosfato
ATP
ADP
3
Fosfofrutocinase-1
Frutose-1,6-bifosfato
4
Aldolase
Gliceraldeído-3-fosfato
+
Di-hidroxiacetona-fosfato
5
Tiosefosfato--isomerase



Fonte: Adaptado de NELSON; COX, 2014.

O processo de quebra da glicose em piruvato acontece ao longo de dez etapas, divididas em duas fases, sendo as cinco primeiras denominadas de “fase preparatória” e as cinco etapas subsequentes de “fase de pagamento” (NELSON; COX, 2014).

Na fase preparatória, a 1ª etapa consiste na fosforilação da glicose em glicose-6-fosfato, por meio da ação da enzima hexoquinase (NELSON; COX, 2014). Após esse processo de fosforilação, a glicose-6-fosfato é convertida em frutose-6-fosfato pela enzima fosfo-hexose-isomerase (2ª etapa), sendo que a frutose-6-fosfato passa pelo processo de fosforilação, dando origem a molécula de frutose-1,6-bifosfato (3ª etapa) (NELSON; COX, 2014). Em ambas as reações de fosforilação, a molécula doadora dos grupos fosforil é o ATP (NELSON; COX, 2014). A 4ª etapa consiste na quebra da molécula de frutose-1,6-bifosfato em duas moléculas de três carbonos, dando origem a di-hidroxiacetona-fosfato e o gliceraldeído-3-fosfato (NELSON; COX,

2014). A enzima responsável por catalisar a 4ª etapa da fase preparatória é a enzima aldolase (NELSON; COX, 2014). Uma vez formada a di-hidroxiacetona-fosfato, a mesma sofre isomerização, gerando uma nova molécula de gliceraldeído-3-fosfato (5ª etapa), encerrando assim a fase preparatória da glicólise (NELSON; COX, 2014). Pode-se notar que ao longo da fase preparatória da glicólise, há o consumo de energia de ATP, uma vez que os grupos fosforil utilizados na fosforilação das hexoses são fornecidos pelas moléculas de ATP (NELSON; COX, 2014).

Logo após a fase preparatória, inicia-se a fase de pagamento da glicólise. É nessa fase onde são produzidas moléculas de ATP e NADH, gerando um ganho de energia, o que justifica o nome de “fase de pagamento” (NELSON; COX, 2014).

Essa fase também é constituída por cinco etapas, sendo que na etapa 6, cada molécula de gliceraldeído-3-fosfato sofre oxidação e fosforilação por fosfato inorgânico, formando duas moléculas de 1,3-bifosfoglicerato, sob ação da enzima gliceraldeído-3-fosfato-desidrogenase (NELSON; COX, 2014). São nas etapas 7, 8, 9 e 10 que ocorre a liberação de energia na forma de ATP por meio da ação de diversas enzimas que convertem as duas moléculas de 1,3-bifosfoglicerato em duas moléculas de piruvato (NELSON; COX, 2014).

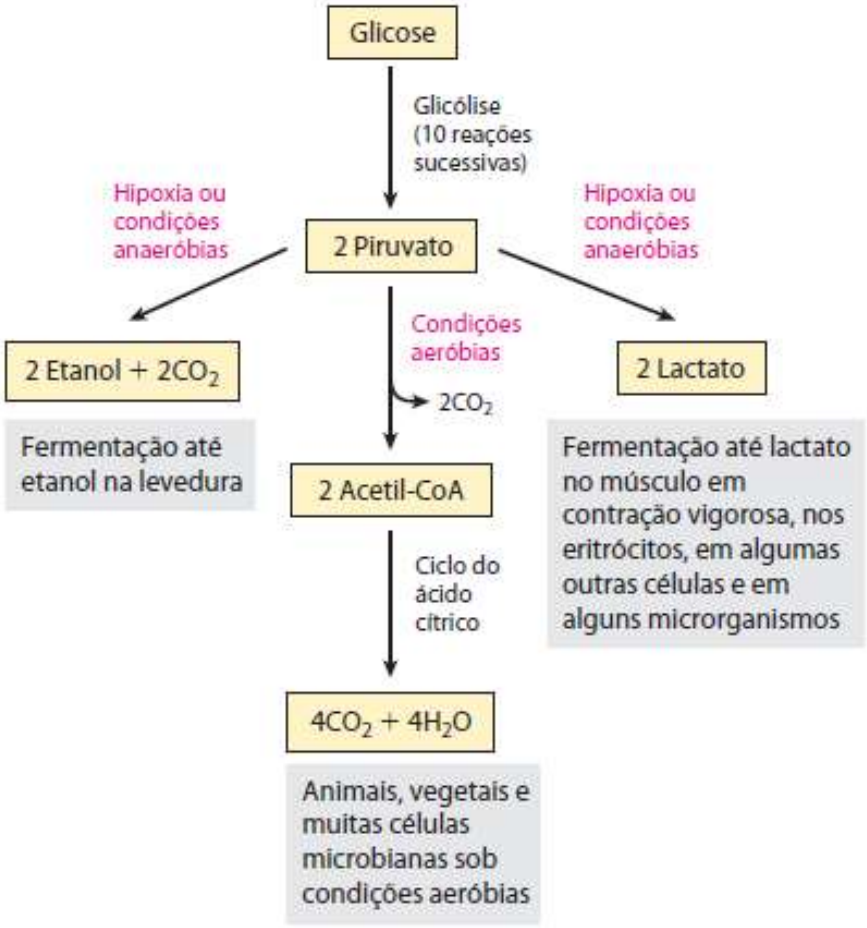
Resumidamente, a glicólise pode ser caracterizada pela conversão de uma molécula de glicose em duas moléculas de piruvato, que ao final do processo, gera um rendimento de duas moléculas de ATP e de duas moléculas de NADH por molécula de glicose utilizada (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

Embora a glicólise gere energia ao final de seu processo, trata-se apenas de uma pequena fração da energia total que pode ser disponibilizada pela molécula da glicose, uma vez que as moléculas de piruvato formadas ainda possuem potencial energético alto, podendo ser utilizadas em reações oxidativas no ciclo do ácido tricarboxílico (TCA) e na fosforilação oxidativa para formação de novas moléculas de ATP (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

3.6.3 Ciclo do Ácido Tricarboxílico (Ciclo do Ácido Cítrico ou Ciclo de Krebs)

As moléculas de piruvato geradas ao longo da glicólise podem ser catabolizadas por diversas vias metabólicas a depender das condições e necessidades do organismo, conforme Figura 12 (NELSON; COX, 2014).

Figura 12 – Destinos catabólicos do piruvato formado na glicólise.



Fonte: Adaptado de NELSON; COX, 2014.

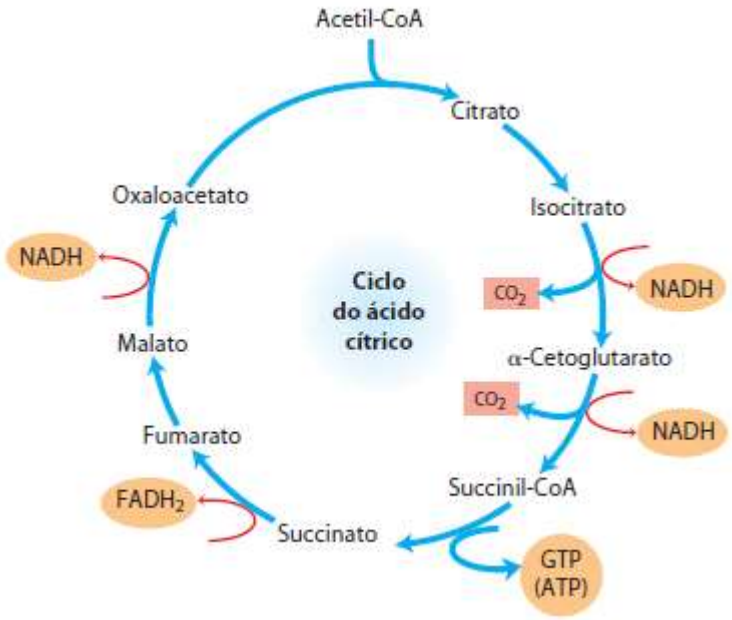
Na presença de oxigênio, essas moléculas de piruvato sofrem descarboxilação oxidativa por meio da ação do complexo mitocondrial enzimático da piruvatodesidrogenase (PDH), gerando como produto final duas moléculas de Acetil-Coenzima A (Acetil-CoA) e CO_2 (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

A Acetil-CoA é o principal material de partida para o ciclo do TCA, etapa indispensável para a geração de ATP e para o armazenamento de energia em

coenzimas reduzidas, como NADH e $FADH_2$ (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

O ciclo do TCA, diferente da glicólise, ocorre no interior das mitocôndrias e é constituído por oito etapas, conforme Figura 13 (MAUGHAN; GLEESON; GREENHAFF, 2000).

Figura 13 - Ciclo do Ácido Tricarboxílico



Fonte: Adaptado de NELSON; COX, 2014.

Na 1ª etapa do ciclo do TCA, uma molécula de Acetil-CoA e oxaloacetato sofrem condensação por meio da ação da enzima citrato sintase, gerando como produto final uma molécula de citrato (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014). O citrato produzido na etapa anterior é então convertido em seu isômero, o isocitrato, via *cis*-aconitato pela ação catalítica da enzima aconitase (2ª etapa) (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

Na 3ª etapa do Ciclo do TCA, o isocitrato sofre descarboxilação oxidativa, por meio da ação da enzima isocitrato desidrogenase, formando α-cetoglutarato, liberando uma molécula de CO_2 e outra de NADH (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014). Subsequentemente, a molécula de α-cetoglutarato é oxidada e descarboxilada formando succinil-CoA e CO_2, reação catalisada pelo complexo α-cetoglutarato desidrogenase, liberando outra molécula de

NADH (4ª etapa) (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

A etapa seguinte (5ª etapa) consiste na quebra da ligação tioéster da succinil-CoA pela enzima succinil-CoA-sintetase ou succinato tioquinase, formando succinato, liberando energia para fosforilar uma molécula de difosfato de guanidina (GDP) em trisfosfato de guanidina (GTP), sendo que essa molécula de GTP é posteriormente convertida em ATP por meio da ação da enzima nucleotídeo difosfatoquinase (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014). O succinato produzido na 5ª etapa do ciclo do TCA é então oxidado a fumarato pela flavoproteína succinato desidrogenase, ocorrendo a redução da flavina adenina dinucleotídeo (FAD) à $FADH_2$ (6ª etapa) (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

A 7ª etapa consiste na hidratação reversível do fumarato a malato, catalisada pela enzima fumarase hidratase (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014). Na 8ª e última etapa do ciclo do TCA, a enzima L-malato-desidrogenase catalisa a oxidação do malato a oxaloacetato com a redução de NAD^+ a NADH, encerrando assim o ciclo do TCA (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

Ao longo das etapas do ciclo do TCA são formados: uma molécula de ATP, três moléculas de NADH e uma de $FADH_2$, elétrons esses que posteriormente serão utilizados para produção de ATP no processo de fosforilação oxidativa, caracterizando então a principal função do ciclo do TCA: produzir átomos de hidrogênio para que os mesmos possam ser utilizados na cadeia de transporte de elétrons (MAUGHAN; GLEESON; GREENHAFF, 2000).

3.6.4 Fosforilação Oxidativa

A fosforilação oxidativa consiste na síntese de ATP por meio da transferência de elétrons do $FADH_2$ e NADH para o oxigênio molecular e ocorre exclusivamente na mitocôndria celular, uma vez que essa organela possui uma membrana externa permeável a íons, facilitando assim sua passagem para o interior da célula (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

O NADH e o $FADH_2$, por possuírem um par de elétrons, são consideradas moléculas altamente energéticas e ao transferirem esses elétrons para o oxigênio, liberam uma quantia alta de energia com capacidade de refosforilar o ADP, sintetizando então ATP (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

É por meio desse processo que organismos aeróbicos produzem energia como resultado da degradação oxidativa de carboidratos, lipídeos e aminoácidos (NELSON; COX, 2014).

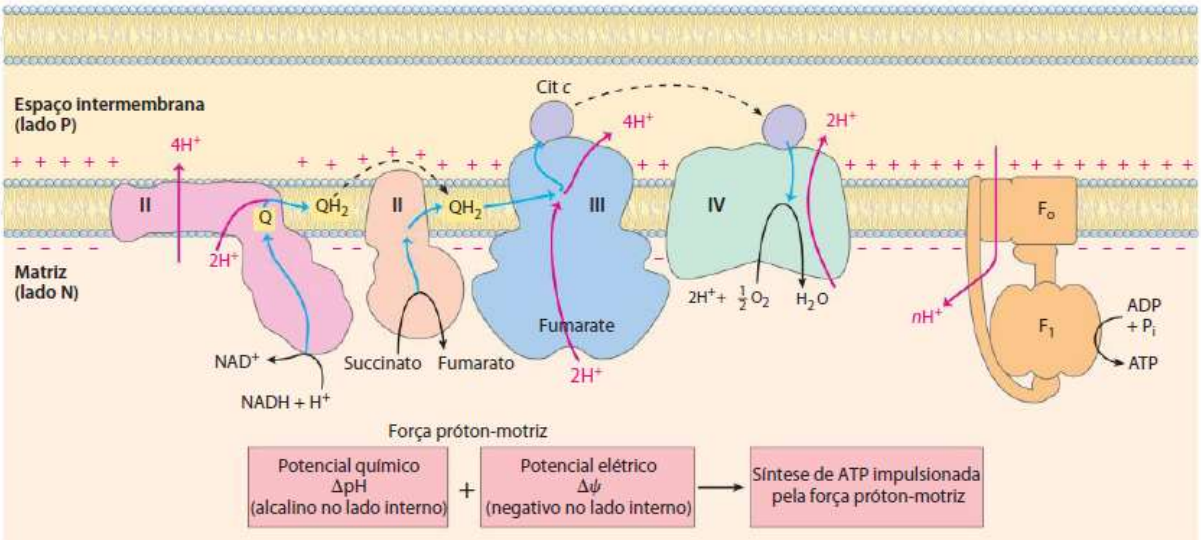
Para que essa transferência eletrônica ocorra, é necessária a ação de um conjunto de carregadores específicos, presentes na membrana mitocondrial interna, chamado de cadeia de transporte de elétrons ou cadeia respiratória mitocondrial (MAUGHAN; GLEESON; GREENHAFF, 2000; NELSON; COX, 2014).

3.6.5 Cadeia de Transporte de Elétrons ou Cadeia Respiratória Mitocondrial

A cadeia de transporte de elétrons é formada por uma série de carregadores proteicos que atuam em sequência, com potencial de aceitar ou doar um ou dois elétrons (MAUGHAN; GLEESON; GREENHAFF, 2000; MCARDLE; KATCH; KATCH, 2016; NELSON; COX, 2014).

Esses carregadores de elétrons se organizam em complexos presentes dentro da membrana mitocondrial que trabalham uns com os outros através da ubiquinona (Q) e da proteína citocromo *c*, podendo ser classificados em: Complexo I (NADH), Complexo II (Succinato), Complexo III e Complexo IV (Figura 14) (NELSON; COX, 2014).

Figura 14 – Cadeia de Transporte de Elétrons.



Fonte: Adaptado de NELSON; COX, 2014.

3.6.5.1 Complexo I

O Complexo I, também denominado NADH:ubiquinona oxidorredutase ou NADH desidrogenase, consiste no maior complexo da cadeia respiratória mitocondrial, sendo composto por 42 cadeias polipeptídicas (NELSON; COX, 2014).

Esse complexo é responsável por catalisar as seguintes reações: 1) a transferência de um íon hidreto (H^+) para a ubiquinona (Q) e de um próton para a matriz mitocondrial, (Figura 15a), e 2) a transferência de quatro prótons ($H_p{}^+$) da matriz para o espaço intramembrana (Figura 15b) (NELSON; COX, 2014).

Figura 15 – Processo de transferência de elétrons – Complexo I.

(a) $\mathrm{NADH + H^+ + Q \longrightarrow NAD^+ + QH_2}$

(b) $\mathrm{NADH + 5\,H_N^+ + Q \longrightarrow NAD^+ + QH_2 + 4\,H_P^+}$

Legenda: Q – Ubiquinona; QH_2 – Ubiquinol; $_N$ – localização do próton para o lado negativo (matriz); $_P$ - localização do próton para o lado positivo da membrana interna (espaço intermembrana).

Fonte: Adaptado de NELSON; COX, 2014.

3.6.5.2 Complexo II

O Complexo II, também pode ser nomeado como succinato desidrogenase (SDH), uma vez que é formado por essa enzima presente no ciclo do TCA, responsável pela oxidação do succinato a fumarato (NELSON; COX, 2014).

Esse complexo é relativamente menor e mais simples quando comparado com o Complexo I, sendo constituído por quatro subunidades proteicas: duas transmembrana (subunidades C e D) e duas citoplasmáticas (subunidades A e B) (NELSON; COX, 2014). As subunidades C e D possuem um grupo heme, heme b e um sítio de ligação para ubiquinona, enquanto as subunidades A e B são formadas por três centros 2Fe-2S (2 ferros – 2 enxofres), FAD ligado e um sítio de ligação ao succinato (NELSON; COX, 2014).

A SDH, ao oxidar o succinato a fumarato, promove a geração de $FADH_2$ no Complexo II, sendo que os dois elétrons do $FADH_2$ acabam sendo transferidos para a ubiquinona (Q), formando como produto final o ubiquinol (QH_2), conforme pode ser visualizado na Figura 16 (NELSON; COX, 2014)

Figura 16 – Processo de transferência de elétrons – Complexo II.

$$FADH_2 + Q \rightarrow FAD + QH_2$$

Fonte: Adaptado de NELSON; COX, 2014.

3.6.5.3 Complexo III

O Complexo III, também chamado de citocromo *c* oxidorredutase ou ubiquinona, possui como principal função a transferência de elétrons do ubiquinol (QH_2, formado nas reações do Complexo I e Complexo II), resultando na absorção de dois prótons na matriz e na liberação de quatro prótons no espaço intermembrana que são transferidos para o citocromo *c*, o qual sofre redução (NELSON; COX, 2014). De forma resumida, a ação do complexo III resulta na oxidação do ubiquinol em

ubiquinona, na redução de duas moléculas de citocromo *c* e na transferência de prótons do espaço intramembrana para a matriz mitocondrial (NELSON; COX, 2014). Uma vez reduzido, o citocromo *c* é direcionado ao Complexo IV para que seja então oxidado e transfira seu elétron, dando continuidade a cadeia respiratória mitocondrial (NELSON; COX, 2014).

3.6.5.4 Complexo IV

O Complexo IV, também chamado de citocromo oxidase, é responsável pela última etapa da cadeia respiratória mitocondrial (NELSON; COX, 2014). Sua atuação consiste na transferência de elétrons do citocromo *c*, proveniente do Complexo III, para o oxigênio molecular, gerando como produto final, H_2O (NELSON; COX, 2014).

Trata-se de um complexo mitocondrial enzimático grande, composto por 13 subunidades, sendo dois citocromos com grupos heme (heme *a* e a_3) e dois centros contendo cobre (Cu_A e Cu_B) (NELSON; COX, 2014).

A transferência de elétrons para o Complexo IV acontece quando o citocromo *c* é oxidado e doa seus elétrons para o centro Cu_A, para o grupo heme *a*, para o centro de heme a_3-Cu_B, e finalmente para o O_2, sendo convertido então a duas moléculas de H_2O (NELSON; COX, 2014). Durante esse processo, há um consumo de 4 prótons da matriz mitocondrial, criando um gradiente eletroquímico capaz de gerar um força próton-motriz impulsionadora da síntese de ATP (NELSON; COX, 2014).

3.7 Parâmetros Inflamatórios

3.7.1 Citocinas

As citocinas são proteínas produzidas por diversas células, como macrófagos, linfócitos e monócitos, como resposta do sistema imunológico a antígenos, sendo fundamentais na regulação da resposta imunológica e mediadores inflamatórios (DO PRADO *et al.*, 2009; FERNANDES *et al.*, 2011).

Existem várias citocinas, podendo elas terem ações pró-inflamatórias ou ações anti-inflamatórias (FERNANDES *et al.*, 2011; PAULUS, 1999; ROSA; BATISTA,

2005). Estudos propõem que uma resposta inflamatória anormal, com elevada expressão de citocinas pró-inflamatórias, possa estar associada ao desenvolvimento de diversas patologias, como aterosclerose, DM e obesidade (FERNANDES *et al.*, 2011; PAULUS, 1999; ROSA; BATISTA, 2005).

Dentre as principais citocinas associadas a resposta inflamatória anormal, podem-se citar o Fator de Necrose Tumoral Alfa (TNF-α), Interleucina 1β (IL-β) e Interleucina 6 (IL-6) (FERNANDES *et al.*, 2011; PAULUS, 1999; ROSA; BATISTA, 2005).

3.7.1.1 TNF-α

Estudos demonstram que o TNF-α possui ação pró-inflamatória relacionada ao desenvolvimento de diversas patologias, como neoplasias e na expressão fenotípica da obesidade, uma vez que indivíduos obesos apresentam uma expressão aumentada dessa citocina nas células adiposas (FERNANDES *et al.*, 2011).

Uma revisão bibliográfica realizada por Fernandes e colaboradores (2011), encontrou estudos que correlacionam essa expressão anormal de TNF-α com elevadas taxas metabólicas, diminuição do fluxo sanguíneo em tecidos periféricos e alteração no metabolismo lipídico e proteico de indivíduos obesos. Adicionalmente, encontraram relação entre aumento nos níveis séricos de insulina, metabolismo anormal de hormônios esteroides e intolerância ao exercício físico com concentrações elevadas de TNF-α.

3.7.1.2 IL-1β

A IL-1β é uma citocina com atividade pró-inflamatória, produzida quase que em sua totalidade, pelos monócitos e macrófagos. Primeiramente, essas células do sistema imunológico produzem a pró-IL beta, uma molécula precursora que necessita ser clivada para desempenhar seu papel biológico (FERNANDES *et al.*, 2011; PAULUS, 1999; YUZHALIN, 2011).

Diversos estudos descrevem uma associação positiva entre um aumento nas concentrações de IL-1β e o desenvolvimento de patologias, como neoplasias,

aterosclerose, infarto agudo do miocárdio (IAM) e acidentes vasculares cerebrais (AVC) (FERNANDES *et al.*, 2011; PAULUS, 1999; YUZHALIN, 2011).

Além disso, essa citocina age em conjunto com o TNF-α, estimulando nos músculos e no fígado, a produção de IL-6 (FERNANDES *et al.*, 2011; PAULUS, 1999).

3.7.1.3 IL-6

A IL-6 também é uma citocina com potencial ação pró-inflamatória, sendo que concentrações séricas elevadas são encontradas em indivíduos obesos, indivíduos com resistência à insulina, indivíduos intolerantes à glicose e/ou diabéticos (DO PRADO *et al.*, 2009; FERNANDES *et al.*, 2011).

Outra associação importante entre a obesidade e a IL-6 é que estudos já demonstraram que o tecido adiposo possui capacidade de secretar essa citocina, sendo o responsável pela liberação de 15 a 35% de IL-6 circulante (DO PRADO *et al.*, 2009; FERNANDES *et al.*, 2011).

3.8 Exercício Físico

3.8.1 Exercício Físico e Radicais Livres

O consumo de oxigênio nos músculos esqueléticos durante a prática de exercícios físicos aumenta em torno de 100 a 200 vezes quando comparamos ao consumo de oxigênio em repouso, sendo que esse consumo mais elevado promove um maior fluxo de elétrons na cadeia respiratória, e consequentemente, um aumento na síntese de EROs (MCARDLE; KATCH; KATCH, 2016; TIRAPEGUI, 2012; ZAVARIZE; SCHÖLER; BOCK, 2016).

Esse aumento na síntese de EROs acontece de acordo com a necessidade de aporte energético do indivíduo, volume de O_2 (VO_2), temperatura corporal e também, vai depender da intensidade e duração do exercício físico (TIRAPEGUI, 2012).

Entretanto, esse aumento não é totalmente prejudicial, uma vez que esse processo promove uma adaptação do sistema antioxidante endógeno (aumento nos

níveis de enzimas antioxidantes) e uma maior resistência a novos estímulos oxidativos (TIRAPEGUI, 2012).

3.8.2 Treinamento Aeróbico Intervalado de Alta Intensidade

O treinamento aeróbico intervalado de alta intensidade, referido como HIIT (do inglês: *high intensity interval training*), consiste em um exercício físico aeróbico onde são intercaladas atividades intensas submáximas, máximas ou supramáximas, com breves períodos de descanso ou atividades em intensidade baixa (PAZ; FRAGA; TENÓRIO, 2017).

Visto que atualmente, devido a rotina intensa e exaustiva, muitos indivíduos acabam não praticando atividade física devido a falta de tempo ou mesmo devido a falta de condicionamento físico e dificuldade de realização de exercícios aeróbicos intensos, o HIIT vem sendo muito estudado como uma modalidade de atividade física tempo-eficiente e capaz de promover adaptações físicas e metabólicas satisfatórias semelhantes àquelas promovidas pelo treinamento contínuo de intensidade moderada (PAZ; FRAGA; TENÓRIO, 2017; ZAVARIZE; SCHÖLER; BOCK, 2016).

Entretanto, novos estudos ainda se fazem necessários para comprovação dos efeitos do HIIT, uma vez que a literatura traz resultados conflitantes, alguns estudos apresentando resultados superiores ao treinamento contínuo moderado, e outros apresentando resultados não significativos (PAZ; FRAGA; TENÓRIO, 2017; ZAVARIZE; SCHÖLER; BOCK, 2016).

3.9 Suplementação Esportiva

De acordo com Hirschbruch e colaboradoes (2008), suplementação consiste em uma estratégica para reposição pontual de um nutriente faltante e que é de difícil obtenção somente pela dieta, com a finalidade de alcançar determinado resultado.

No caso da prática de esportes, essa suplementação pode ser realizada com o intuito de suprir uma deficiência fisiológica, bioquímica ou nutricional que venha a diminuir ou afetar negativamente o desempenho do atleta (TIRAPEGUI, 2012).

Esses métodos suplementares recebem o nome de ergogênicos e podem ser classificados em: mecânicos, psicológicos, farmacológicos, fisiológicos e nutricionais (TIRAPEGUI, 2012). Dentro da classificação dos ergogênicos nutricionais, são encontrados os carboidratos, vitaminas, aminoácidos de cadeia ramificada, creatina, carnitina, entre outros (TIRAPEGUI, 2012).

3.9.1 L-Carnitina

A L-Carnitina (3-hidroxi-4-trimetilaminobutanoato) é uma amina quaternária endógena com um papel indispensável na geração de energia celular, uma vez que é responsável pelo transporte de ácidos graxos de cadeia longa do citosol para a mitocôndria celular, promovendo a síntese de ATP por meio da sua oxidação (COELHO *et al.*, 2005; TIRAPEGUI, 2012).

É sintetizada no fígado, rins e cérebro, a partir de dois aminoácidos essenciais, a lisina e metionina, mas também pode ser absorvida de fontes exógenas, principalmente pelo consumo de alimentos de origem animal, como carne vermelha e derivados de leite (COELHO *et al.*, 2005; TIRAPEGUI, 2012).

Em determinadas situações, faz-se necessária a utilização de compostos lipídicos para produção de energia, produção essa que ocorre pela quebra das cadeias de triacilgliceróis em ácidos graxos e glicerol livres, processo chamado de lipólise (TIRAPEGUI, 2012). Na célula muscular, esses ácidos graxos de cadeias longas se unem a uma molécula de coenzima A (CoA) (TIRAPEGUI, 2012). Entretanto, essa molécula de CoA não consegue penetrar a membrana mitocondrial, e é nessa etapa que a L-Carnitina apresenta um papel fundamental (TIRAPEGUI, 2012). A mesma atua formando um complexo permeável (acil-carnitina) com a molécula de CoA, permitindo a entrada dessa molécula na membrana interna da mitocôndria (TIRAPEGUI, 2012). Posteriormente, por meio da ação de determinadas enzimas, esse complexo é desfeito e a molécula de CoA se liga a um grupo acil, formando uma molécula de acil-CoA que é transportada à matriz mitocondrial, sofrendo oxidação e dando origem ao acetil-CoA que participará do ciclo do TCA (TIRAPEGUI, 2012).

A L-Carnitina vem sendo considerada um suplemento ergogênico devido à sua participação no transporte de CoA para a mitocôndria, gerando assim um aumento do fluxo de substratos através do ciclo do TCA, o que poderia resultar em uma utilização mais eficaz do oxigênio e uma maior produção de energia para a prática de exercícios físicos (COELHO *et al.*, 2005; TIRAPEGUI, 2012).

Também vem sendo estudada como alternativa terapêutica no tratamento de dislipidemias, no tratamento de doenças renais, isquêmicas, cardiopatias, neuropatia diabética, AIDS e também como possível antioxidante (COELHO *et al.*, 2005; TIRAPEGUI, 2012).

4 METODOLOGIA

4.1 Animais

Foram utilizados ratos machos Wistar de 60 dias de idade provenientes da Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brasil. Antes do processo de experimentação, os animais foram acomodados e aclimatados por 7 dias, para adaptação em um novo ambiente. Os animais foram mantidos em salas com ciclo de 12h claro/escuro com temperatura entre 20-22°C com livre acesso à comida (ração) e água. O ciclo claro-escuro foi invertido (12h-12h) para melhor aproveitamento do período ativo dos animais para a realização dos treinos.

Os animais foram mantidos em número máximo de quatro por gaiola contendo maravalha. A frequência de troca de caixas foi realizada a cada 2 dias. Os cuidados com os animais seguiram o disposto na Lei nº11.794, de 8 de outubro de 2008, e nas demais normas aplicáveis à utilização de animais em ensino e/ou pesquisa, especialmente as Resoluções Normativas do Conselho Nacional de Controle de Experimentação Animal – CONCEA (BRASIL, 2008; MINISTÉRIO DA CIÊNCIA, TECNOLOGIA).

As condições de ambiente, iluminação, acomodação e nutrição seguiram as recomendações exigidas pelo "Guide For The Care and Use of Laboratory Animals, 2011".

Os animais foram divididos, em número igual, nos seguintes grupos:

1) Grupo Dieta Normolipídica – Sedentário (DNL-SED, n=8);
2) Grupo Dieta Hiperlipídica – Sedentário (DHL-SED, n=8);
3) Grupo Dieta Hiperlipídica – Sedentário – Suplementação de L-Carnitina (DHL-SED-C, n=8);
4) Grupo Dieta Hiperlipídica – Treinamento Intervalado de Alta Intensidade (DHL-HIIT, n=8);
5) Grupo Dieta Hiperlipídica – Treinamento Intervalado de Alta Intensidade – Suplementação de L-Carnitina (DHL-HIIT-C, n=8).

A pesquisa teve seu início após aprovação pelo Comitê de Ética em Pesquisa no Uso de Animais – CEUA, da UNIVILLE, sob o ofício de número 012/2017.

4.2 Protocolos Experimentais

4.2.1 Indução dietética da obesidade

Os animais dos grupos experimentais (DHL) foram alimentados com dieta hiperlipídica, composta por 20% das calorias provenientes de carboidratos, 20% de proteínas e 60% de lipídeos (Prag Soluções Biosciências, Jaú, São Paulo-SP), durante quatorze semanas, a fim de induzir à condição de obesidade. Os animais do grupo controle (DNL) foram tratados com dieta padrão (70% de carboidratos, 20% de proteínas e 10% de lipídeos) (Quimtia, Curitiba, Paraná, Brasil). Todos os animais receberam água *ad libidum.*

4.2.2 Suplementação com L-Carnitina

Os animais dos grupos experimentais DHL-SED-C e DHL-HIIT-C receberam a suplementação de L-Carnitina (Sigma-Aldrich) via gavagem, numa concentração de 300mg/Kg de massa corporal por dia, enquanto os animais pertencentes aos grupos DNL-SED, DHL-SED e DHL-HIIT receberam salina via gavagem uma vez ao dia.

4.2.3 Protocolo de Treinamento Intervalado Aeróbico de Alta Intensidade (HIIT)

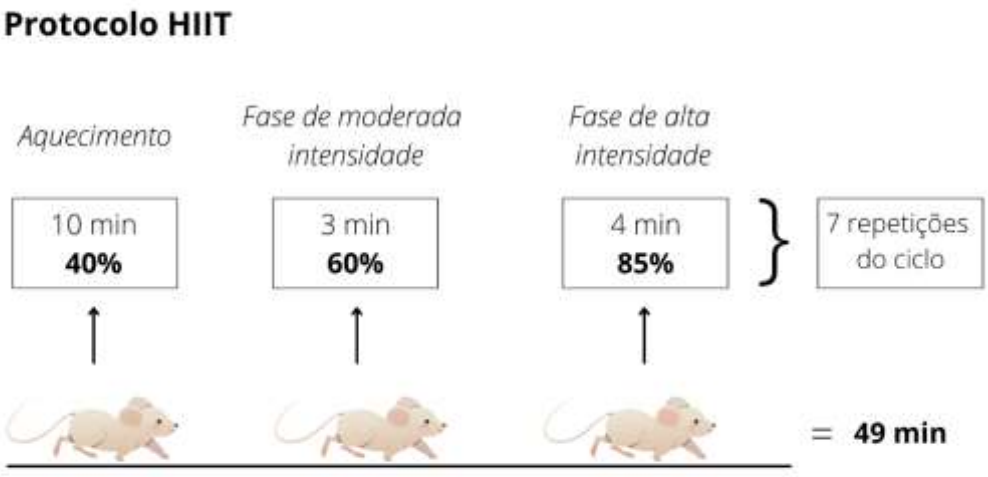
Para fins de definição da velocidade máxima dos animais e prescrição da intensidade dos treinos, inicialmente foi aplicado um Teste de Tolerância ao Esforço Máximo. Esse teste foi realizado para determinar a velocidade máxima (Vmáx) que cada animal poderia correr e essa medida serviu como parâmetro para a prescrição do protocolo HIIT.

O Teste de Tolerância ao Esforço Máximo foi realizado em três momentos: no início, após quatro semanas e na oitava semana dos protocolos de treinamento. Para a realização desse teste, foi utilizado o protoloco de Ferreira e colaboradores (2007)

e consistiu em uma corrida em esteira ergométrica (modelo KT-4000, marca Imbramed), com inclinação de 20 graus, sendo a velocidade inicial de 6m/min com acréscimo de 3m/min na velocidade a cada três minutos até a visível exaustão do animal. Uma vez identificada a Vmáx, os valores de distância e velocidade foram computados para cálculo da intensidade do treinamento.

O protocolo HIIT aconteceu com uma frequência de cinco dias na semana, 20 graus de inclinação da esteira e intensidades definidas a partir do Teste de Tolerância ao Esforço Máximo. O HIIT aconteceu da seguinte maneira: os animais corriam três minutos à intensidade de 60% seguidos de quatro minutos à 85% da Vmáx de teste (FERREIRA *et al.*, 2007). Este ciclo se repetiu sete vezes, totalizando 49 minutos de treinamento (Figura 17).

Figura 17 – Protocolo HIIT.



Fonte: O autor.

Os animais pertencentes ao grupo sedentário (SED) realizavam uma caminhada a 40% de intensidade, duas vezes na semana, a fim de manter a capacidade de deambulação dos animais para as realizações dos testes físicos posteriores.

4.3 Preparação dos tecidos

Após o término da 10ª semana de treinamento aeróbico (24 semanas de experimento), com jejum de 12 horas, ocorreu o sacrifício dos animais (48h após a última sessão de treino).

Os animais foram sacrificados por decapitação, sem anestesia, e as estruturas cerebrais (córtex cerebral, hipocampo e cerebelo), sangue, fígado, rins, tecido adiposo, coração, músculos sóleo, plantar e gastrocnêmio, foram dissecados e homogeneizados em tampão adequado de acordo com a técnica utilizada. O homogeneizado foi centrifugado a x 3.000 g, a 4°C por 15 minutos para remoção de resíduos celulares e o sobrenadante foi estocado em alíquotas e armazenado a -80°C para posterior determinação da atividade das enzimas antioxidantes (CAT, SOD e GSH-Px), conteúdo total de sulfidrilas, proteínas carboniladas, substâncias reativas ao ácido tiobarbitúrico (TBA-RS), técnicas para análises de metabolismo energético, análise dos parâmetros inflamatórios (TNF-α, IL-6 e IL-1β), dosagem de glicose, triglicerídeos, colesterol total, colesterol HDL, colesterol LDL e insulina.

4.4 Preparação dos eritrócitos e do plasma

Os eritrócitos e o plasma foram preparados a partir de amostras de sangue total obtidas de ratos. O sangue periférico foi rapidamente coletado e transferido para tubos contendo anticoagulante heparina, centrifugado a 1.000 rpm por 10 min, sendo o plasma separado e congelado para posterior determinação (LIMA *et al.,* 2017a). Os eritrócitos foram então lavados 3 vezes com solução salina gelada (0,153 mol/L cloreto de sódio). Os lisados foram preparados pela adição de 1 mL de água destilada para 100 µL de eritrócitos lavados e congelados para determinação da atividade das enzimas antioxidantes (LIMA *et al.*, 2017a).

Para determinação da atividade das enzimas antioxidantes, os eritrócitos foram congelados e descongelados três vezes e centrifugados a 13.500 rpm por 10 min. O sobrenadante foi diluído para conter aproximadamente 0,5 mg/mL de proteína (LIMA *et al.*, 2017a).

4.5 Preparação do soro

O soro foi preparado a partir de amostras de sangue total obtidas de ratos. O sangue periférico foi rapidamente coletado e transferido para tubos sem anticoagulante, centrifugado a 1.000 rpm por 10 min e o soro foi separado e utilizado para a análise de parâmetros bioquímicos (LIMA *et al.*, 2017a).

4.6 Estudos bioquímicos

4.6.1 Análise dos Parâmetros de Estresse Oxidativo

4.6.1.1 Catalase (CAT)

A atividade de CAT foi determinada pelo método de Aebi (1984). Este método baseia-se no desaparecimento do peróxido de hidrogênio (H_2O_2) em um meio de reação contendo 25 µL de amostra e 600 µL de tampão fosfato de potássio 10 mM, pH 7,0, e 20 mM H_2O_2. A absorbância foi contada a cada 10 segundos por 1 minuto e 40 segundos à 240 nm usando um espectrofotômetro Shimadzu UV-visível. Uma unidade de CAT é definida como 1 µmol de H_2O_2 consumido por minuto e a atividade específica é calculada como unidades CAT/mg de proteína.

4.6.1.2 Superóxido Dismutase (SOD)

A atividade da SOD foi determinada pelo método de auto-oxidação do pirogalol, como descrito por Marklund (1985), um processo altamente dependente de superóxido ($O_2^{\bullet -}$), que é um substrato para a SOD. Primeiramente, 15 µL de cada amostra foi adicionado à 215 µL de uma mistura contendo 50 µM de tampão Tris, 1 µM de EDTA, pH 8,2, e 30 µM de Catalase. Subsequentemente, foram adicionados 20 µL de pirogalol e a absorbância foi registrada imediatamente a cada 30 segundos durante 3 minutos à 420 nm usando um espectrofotômetro Shimadzu UV-visível. A inibição da auto-oxidação do pirogalol ocorre na presença de SOD, cuja atividade pode ser indiretamente testada espectrofotometricamente. Uma unidade de SOD é definida como a quantidade de SOD necessária para inibir 50% da auto-oxidação de pirogalol e a atividade específica é relatada como unidades de SOD/mg de proteína.

4.6.1.3 Glutationa Peroxidase (GSH-Px)

A atividade de GSH-Px foi mensurada pelo método de Wendel (1981), utilizando *tert*-butil-hidroperóxido como substrato. A decomposição do NADPH foi monitorada a 340 nm por 3 minutos e 30 segundos usando um espectrofotômetro Shimadzu UV-visível. 90 µL de cada amostra foi adicionado ao meio contendo 800 µL de tampão, 20 µL de 2,0 mM GSH, 30 µL de 0,15 U/mL GSH redutase, 10 µL de 0,4 mM de azida e 10 µL de 0,1 mM NADPH. A absorbância foi contada a cada 10 segundos por 1 minuto e 30 segundos. Posteriormente, 50 µL de 0,5 mM de tert-butilhidroperóxido foi adicionado e a absorbância foi lida por mais 2 minutos. Uma unidade de GSH-Px é definida como 1 µmol de NADPH consumido por minuto e a atividade específica é definida como unidades de GSH-Px/mg de proteína.

4.6.1.4 Dosagem de Proteínas

A determinação das proteínas foi realizada pelo método de Lowry e colaboradores (1951), utilizando-se albumina sérica bovina como padrão, a qual se baseia na complexação do cobre em meio alcalino, gerando um derivado de cor azul, que é mensurado espectrofotometricamente em 650 nm. O meio continha NaOH 0,10 M, Na_2CO_3 2%, $CuSO_4$ 1%, Tartarato de Na^+ e K^+ 2%. Resumidamente, 10 µL de homogeneizado foi adicionado a 1 mL do meio e 190 µL de água destilada. A reação foi iniciada pela adição de 100 µL de Reagente de Folin 1N e incubada durante 20 minutos à temperatura ambiente em local escuro. Os resultados foram expressos em nmol TNB/mg de proteína. Após o procedimento, os fatores de calibração parcial foram calculados.

4.6.1.5 Conteúdo Total de Sulfidrilas

O conteúdo total de sulfidrilas foi determinado de acordo com o método descrito por Aksenov e Markersbery (2001), o qual se baseia na redução do ácido ditionitrobenzóico (DTNB) por tióis, gerando um derivado amarelo (TNB) que é mensurado espectrofotometricamente em 412 nm. Primeiramente, 50 µL de homogeneizado foi adicionado a 1 mL de tampão PBS pH 7,4 contendo EDTA 1 mM. A reação foi iniciada pela adição de 30 µL de DTNB 10 mM e incubada durante 30

minutos à temperatura ambiente em local escuro. Os resultados foram expressos em nmol TNB/mg de proteína.

4.6.1.6 Substâncias reativas ao ácido tiobarbitúrico (TBA-RS)

TBA-RS foi determinada de acordo com o método descrito por Ohkawa *et al.* (1979). A metodologia para análise do TBA-RS mensura o malondialdeído (MDA), um produto da lipoperoxidação, causado principalmente por radicais OH·. Para os experimentos, os homogeneizados foram misturados com ácido tricloroacético a 10% e 0,67% de ácido tiobarbitúrico e aquecidas num banho de água fervente durante 25 min. TBA-RS foi determinada pela absorbância à 535 nm. Uma curva de calibração foi obtida utilizando 1,1,3,3-tetrametoxipropano como o precursor de MDA e cada ponto da curva foi submetido ao mesmo tratamento que o dos sobrenadantes. Os resultados foram expressos em nmol de MDA/mg de proteína.

4.6.1.7 Conteúdo Total de Proteínas Carboniladas

O conteúdo total de proteínas carboniladas foi determinado utilizando o método descrito por Reznick e Packer (1994), o qual se baseia na reação de carbonilação de proteínas com dinitrofenilhidrazina (DNPH), gerando um composto de coloração amarela, tendo sua absorbância determinada à 370 nm.

Primeiramente, 200 µL de homogeneizado foram adicionados à um tubo de ensaio contendo 400 µL de DNPH 10 mM (preparado em HCl 2 M). O tubo de ensaio foi então mantido no escuro durante 1h e agitado em vórtex a cada 15 min. Na sequência, foi adicionado 500 µL de ácido tricloroacético 20% e essa mistura foi agitada em vórtex e centrifugada a 14000 rpm durante 3 min, e o sobrenadante foi descartado. O sedimento foi lavado com 1 mL de solução de etanol:acetato de etila (proporção 1:1, v/v), agitado em vórtex e centrifugado a 14000 rpm durante 3 min. Novamente o sobrenadante foi descartado e o sedimento foi ressuspendido em 600 µL de guanidina 6 M (preparada em solução de fosfato de potássio 20 mM pH 2,3). A amostra foi agitada em vórtex e incubada à 60ºC durante 15 min em banho maria. Passados os 15 min, a amostra foi centrifugada a 14000 rpm por 3 min e a absorbância do sobranadante foi determinada em espectrofotômetro à 370 nm. Os resultados foram expressos como conteúdo de carbonilas (nmol/mg de proteína).

4.6.2 Análise dos Parâmetros do Metabolismo Energético Aeróbico e Anaeróbico

4.6.2.1 Atividade da Piruvatoquinase

Para determinação da atividade enzimática da piruvatoquinase, foi utilizado o método descrito por Leong e colaboradores (1981). O método consiste na incubação de 0,1 mM de tampão Tris-HCl pH 7,5, $MgCl_2$ 10 mM, NADH 0,16 mM, KCl 75 mM, ADP 5 mM, 7 unidades de Lactato desidrogenase, Triton X-100 0,1% (v/v) e 10 µL de sobrenadante do homogeneizado da amostra. Essa mistura foi pré-incubada por 30 min e após esse período, adicionou-se 1 mM de fosfoenolpiruvato (PEP), iniciando-se a reação. Os resultados foram expressos em µmol de piruvato formado por minuto por mg de proteína.

4.6.2.2 Citrato Sintase

A determinação da atividade máxima da enzima citrato sintase foi realizada pelo método descrito por Alp e colaboradores (1976), por meio da quantificação do complexo CoA+ácido 5,5´ditio-bis 2 nitrobenzóico (DTNB). As estruturas cerebrais e musculares foram homogeneizadas em tampão fosfato 50 mM PBS + 1 mM EDTA, pH 7,4, e posteriormente, centrifugados a 12000 rpm por 15 minutos numa temperatura de 4ºC. À cubeta de quartzo, foram adicionados 800 µL de tampão, 100 µL de DTNB 1 Mm, 40 µL de Acetil-CoA 2,5 mM, 10 µL de Triton 10% e 10 µL do homogeneizado. Após a realização dessa mistura, a mesma foi mantida em repouso por dois minutos à temperatura ambiente, e após passado esse tempo, foram adicionados 50 µL de oxaloacetato 4 mM, dando início a reação e a leitura da absorbância foi realizada em 412 nm durante 3 minutos. Os resultados foram expressos como nmol de substrato consumido por minuto, sendo corrigidos pela quantidade de proteína citosólica utilizada no ensaio.

4.6.2.3 Atividades do Complexo II e Succinato Desidrogenase

As atividades do Complexo II e da enzima Succinato Desidrogenase (SDH) foram realizadas nos homogeneizados de músculos plantar, gastrocnêmio e sóleo e também das estruturas cerebrais, utilizando o método de Fischer e colaboradores

(1985). Esse método baseia-se no acompanhamento da diminuição na absorbância da amostra, devido à redução do 2,6-dicloindofenol (DCIP) a 600 nm, na presença de fenazina metossulfato (PMS). Ao homogeneizado, foram adicionados 40 mM de fosfato de potássio pH 7,4, succinato 16 mM e DCIP 8 µm, sendo essa mistura pré-incubada a 30ºC por 20 minutos. Em seguida, foram adicionados 4 mM de azida sódica, 7 µM de rotenona e a reação foi iniciada pela adição de 40 µM de DCIP e a leitura da absorbância foi realizada por 5 minutos para determinação da atividade do Complexo II. A atividade da SDH foi determinada pelo mesmo meio de incubação com a adição de 1 mM de PMS e a leitura da absorbância foi monitorada por 5 minutos.

4.6.2.4 Atividade da Citocromo *C* Oxidase

A determinação da atividade enzimática da citocromo *C* oxidase foi realizada pelo método descrito por Rustin e colaboradores (1994). A atividade enzimática foi determinada observando o decréscimo na absorbância da amostra devido à oxidação do citocromo *C* previamente reduzido, em um comprimento de onda de 550 nm. À cubeta, foram adicionados 10 mM de fosfato de potássio pH 7,0, n-dodecil-β-D-maltosídeo 0,6 nM e o homogeneizado da amostra, sendo a reação iniciada após a adição de 0,7 µg de citocromo *C* reduzido. A atividade enzimática foi mensurada durante 10 min em espectrofotômetro em uma temperatura de 25ºC.

4.6.3 Análise dos Parâmetros Inflamatórios (TNF-α, IL-1β e IL-6)

A determinação dos níveis das citocinas TNF-α, IL-1β e IL-6 foi realizada em amostras de soro coletadas no momento do sacrifício dos animais, utilizando seus respectivos kits ELISA, de acordo com as orientações do fabricante Sigma-Aldrich.

4.6.4 Análise dos Parâmetros Bioquímicos

4.6.4.1 Dosagem de Glicose, Triglicerídeos, Colesterol Total, Colesterol HDL e Colesterol LDL

A dosagem de glicose, triglicerídeos, colesterol total e colesterol HDL foi realizada em amostras de soro coletadas no momento do sacrifício dos animais,

utilizando kits específicos da marca Labtest. A absorbância foi determinada usando um espectrofotômetro UV-vísivel da marca Shimadzu, de acordo com as orientações do fabricante. Os valores de colesterol LDL foram determinados por meio da fórmula de Friedewald.

4.6.4.2 Dosagem de Insulina

A dosagem de insulina foi realizada em amostras de soro coletadas no momento do sacrifício dos animais, utilizando de equipamento automatizado Advia Centaur CP Immunoassay System da empresa Siemens Healthineers.

4.7 Análise estatística

A análise estatística foi realizada utilizando o software R versão 4.0.3 com auxílio dos pacotes Car e Desctools. Foi aplicado um modelo de regressão linear múltipla, seguido de Análise de Variância (ANOVA) para dados não balanceados, a fim de verificar se havia diferenças nas variáveis de desfecho. A suposição de normalidade dos resíduos do modelo foi verificada por meio do teste de normalidade de Shapiro-Wilk. A homocedasticidade foi avaliada por meio de gráficos e teste de Levene. Para identificar diferenças entre os grupos, utilizou-se o teste de Duncan para comparações múltiplas.

Para a análise estatística do peso corporal e do tecido adiposo dos ratos, foi aplicado ANOVA com medidas repetidas para analisar a relação entre as variáveis independentes e a variável desfecho ao longo do período de análise. Para verificar a esfericidade foi aplicado o teste de Mauchly e a correção de Greenhouse-Gueisser, quando necessário. Para os testes de comparações múltiplas, foi aplicado o teste *t* com ajuste de Bonferroni. Para analisar a associação entre as variáveis independentes e o peso do tecido adiposo na última semana, foi aplicado ANOVA de via única. Na comparação múltipla, foi utilizado o teste de Duncan.

Valores de $p<0,05$ foram considerados significativos. Os resultados foram expressos como média ± DP para oito experimentos independentes (animais) realizados em duplicata.

5 INTERDISCIPLINARIDADE

A Organização Mundial de Saúde (OMS) estima que até o ano de 2025, 2,3 bilhões de adultos se encontrarão acima do peso, sendo 700 milhões de indivíduos com obesidade (WHO, 2020). No Brasil, houve um aumento de 72% na prevalência da obesidade nos últimos 13 anos, subindo de 11,8% em 2006 para 20,3% em 2019, segundo dados da Associação Brasileira para Estudo da Obesidade e Síndrome Metabólica (ABESO) (ABESO, 2022).

Já é de conhecimento público que a obesidade é uma doença multifatorial, decorrente da interação de fatores genéticos, metabólicos, sociais, comportamentais e culturais, sendo que na maioria dos casos, existe um desequilíbrio entre a ingestão calórica e o gasto energético (TAVARES; NUNES; SANTOS, 2010).

Dentre os fatores sociais, econômicos e culturais destacam-se: aumento da concentração das populações no meio urbano, redução do gasto energético no trabalho e na rotina diária, automação das atividades, maior oferta e crescente industrialização dos alimentos que podem estar relacionadas à falta de equilíbrio entre a produção e consumo, entre o trabalho e o lazer, promovendo quadros de obesidade associados ao sedentarismo (TAVARES; NUNES; SANTOS, 2010).

Esse desequilíbrio no padrão de consumo alimentício, como dito anteriormente, vem acompanhado de um maior consumo de produtos industrializados, provocando um maior uso de embalagens e matérias primas, acarretando em problemas para o meio ambiente, como aumento na geração de lixo, aumento no consumo de plásticos e papel para confecção de embalagens, aumento na emissão de poluentes gerados pelos processos industriais, aumento no consumo de energia elétrica, entre outros.

Segundo a Organização Pan-Americana de Saúde, o sedentarismo constitui um importante fator de risco para o desenvolvimento de doenças crônico-degenerativas não transmissíveis, como hipertensão arterial sistêmica (HAS), DM e câncer (OPAS, 2003). Dessa maneira, a obesidade e o sedentarismo, isolados ou em conjunto, possuem papel importante no planejamento da saúde pública, uma vez que são considerados fatores de risco desencadeadores de diversas patologias, as quais se tornam dispendiosas ao sistema público de saúde (DA COSTA; VASCONCELOS; DA FONSECA, 2014).

Uma vez que a obesidade está relacionada a um aumento no consumo energético e no sedentarismo, muitas vezes em decorrência da falta de tempo para realização de atividades físicas, se torna interessante estudar a associação entre treinamento aeróbico intervalo de alta intensidade (HIIT) e a suplementação com L-Carnitina, pois há a possibilidade de um tratamento mais efetivo, resultando na redução de custos com a obesidade e suas comorbidades, seja pela redução no uso de medicamentos, redução de procedimentos hospitalares, redução no tempo de internação ou pela redução da geração de resíduos hospitalares e laboratoriais, e de forma geral, otimizando os gastos com saúde e com o meio ambiente.

6 RESULTADOS E DISCUSSÕES

6.1 Artigo:

EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING AND L-CARNITINE SUPPLEMENTATION ON OXIDATIVE STRESS AND ENERGY METABOLISM PARAMETERS IN THE PLANTAR AND SOLEUS MUSCLES OF OBESE RATS

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ABSTRACT

The aim of this study was to evaluate the protective effects of high-intensity interval training (HIIT) and L-Carnitine supplementation on oxidative stress and energy metabolism parameters in the plantar and soleus muscles of obese rats. Animals were divided into five groups: Normal Diet + Untrained (ND-UNT), High-Fat Diet + Untrained (HFD-UNT), High-Fat Diet + Untrained + Carnitine (HFD-UNT-C), High-Fat Diet + High-intensity Interval Training (HFD-HIIT) and High-Fat Diet + High-intensity Interval Training + Carnitine (HFD-HIIT-C). To induce obesity, animals in the HFD groups were fed with a HFD for 14 weeks, while animals in the ND groups were treated with a standard diet. Animals in the HFD-UNT-C and HFD-HIIT-C groups received L-Carnitine by gavage as soon as the HIIT protocol was started; the HIIT protocol was performed 5 days a week, while animals in the UNT group walked at 40% intensity, twice a week. After the end of the 10th week of training, animals were sacrificed by decapitation and the plantar and soleus muscle were separated and homogenized in appropriate buffer. The antioxidant activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), levels of thiobarbituric acid reactive substances (TBARS), total sulfhydryl and protein carbonyl content and pyruvate kinase, citrate synthase, succinate dehydrogenase (SDH), complex II and cytochrome *c* oxidase activities were determined. Results showed that the HFD promoted oxidative stress and alterations in mitochondrial function. The HIIT protocol, when used alone and, sometimes, when associated with L-Carnitine, prevented these alterations in the plantar and soleus muscles of obese rats.

Keywords: Obesity; Oxidative stress; Energy Metabolism; L-Carnitine; High-intensity Interval Training.

1 INTRODUCTION

According to the World Health Organization (WHO), the term obesity is used to describe an excessive accumulation of body fat harmful to the health of the individual[1]. Studies have been able to associate obesity with the development of several complications, including: cardiovascular (arterial hypertension, coronary insufficiency, atherosclerosis), respiratory, skeletal muscle, and dermatological complications, disorders related to the endocrine-metabolic system (diabetes mellitus and dyslipidemia) and also to the appearance of certain neoplasms[2]. With regard to skeletal muscle disorder, accumulating evidence suggests that obesity and excessive intake of saturated fatty acids can exacerbate sarcopenia[3–5].

Sarcopenic obesity, whose prevalence is increasing throughout the world, is a combination of reduced muscle mass and increased body fat in older adults, and represents a serious public health concern[6]. Skeletal muscle loss and accumulation of intramuscular fat are associated with a variety of pathologies due to a combination of factors, including oxidative stress, inflammatory cytokines, mitochondrial dysfunction, insulin resistance, and inactivity[3].

Associations between obesity and oxidative stress, and changes in cellular energy metabolism have been reported. According to França *et al*. (2013), obese patients have biological changes that make the organism more susceptible to oxidative damage[7]. These individuals have an imbalance between the amounts of fat, body weight, lipoproteins and lipids, an imbalance that promotes increased metabolic needs and oxygen consumption and, consequently, an increased production of reactive oxygen species (ROS), such as superoxide and peroxide hydrogen[7].

In addition to the increase in the production of free radicals, studies suggest that obesity is associated with a decrease in the activity of antioxidant enzymes. In 2002, Olusi measured superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzyme activities in 250 obese individuals and concluded that obese patients have reduced erythrocyte antioxidant enzyme activity, which may generate progressive damage to cells and represent an important risk factor for the development of several pathologies in obesity[8]. With regard to alterations in energy metabolism, studies have correlated obesity with changes in mitochondrial respiratory chain function and with deleterious effects on carbohydrate metabolism.

The daily practice of physical exercises has already been proven to be one of the best ways to promote physical, mental and cognitive health in human beings, regardless of age and gender[9]. The association between physical exercise and the generation of free radicals was initially studied in the 1970s, through the analysis of lipid peroxidation biomarkers in skeletal muscle tissue[10,11]. In 1983, Quintanilha and Packer showed that aerobic physical training provoked an increase in the production of antioxidant agents and in the expression of antioxidant enzymes, thus benefiting the antioxidant defenses of the cardiovascular and skeletal muscular systems [12].

The high-intensity interval training (HIIT) protocol is characterized by the intercalation of intense submaximal, maximum or supramaximal activities with periods of rest or low-intensity activities[13]. Some studies, such as those of Gibala et al. and Terada and collaborators, have found associations between HIIT protocols and improvements in the oxidative capacity of muscle and in mitochondrial biogenesis[14,15].

L-Carnitine (3-hydroxy-4-N-trimethylamino-butyrate) is a quaternary amine with a fundamental function in the generation of energy by the cell, since it acts on the transfer reactions of free fatty acids from the long chain of the cytosol to mitochondria, facilitating oxidation and the generation of adenosine triphosphate (ATP)[16]. Increasing the flow of substrates through the Krebs Cycle could result in more effective production and use of oxygen, in addition to improving the ability to perform physical tasks[16]. L-Carnitine is frequently used as a supporting treatment for dyslipidemia, since it acts as an important cofactor in the oxidation of long-chain fatty acids, increasing the use of triglycerides for energy supply, and is also used by active individuals as a support in the reduction of body fat[16]. Additionally, since this substance is produced in the body under normal conditions and with good tolerability, L-Carnitine supplementation has been studied for its possible antioxidant effects, both in healthy individuals and those with special needs, such as patients with ischemic diseases and diabetic neuropathy[16].

Considering that obesity is related to oxidative stress and alterations in energy metabolism, and that studies indicate that the HIIT protocol and supplementation with L-Carnitine promote an improvement in muscle oxidative capacity, this study aimed to evaluate the protective effects of HIIT and L-Carnitine supplementation on oxidative stress and energy metabolism parameters in the plantar and soleus muscles of obese rats.

2 MATERIALS AND METHODS

2.1 Animals and Reagents

Sixty-day-old male Wistar rats from the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil, were used in the experiments. Before the experiments, animals were accommodated and acclimatized for 7 days to adapt to their new environment. Animals were kept in rooms with a 12h light/dark cycle with the temperature maintained between 20-22ºC and free access to food and water. The 12h light/dark cycle was inverted for better use of the animals' active period for training. The animals were kept in cages with a maximum number of four per cage; box exchange was performed every 2 days. Animal care was carried out in accordance with Law Nº. 11794 (October 8, 2008), and other regulations applicable to the use of animals in teaching and/or research, especially the Normative Resolutions of the National Council for the Control of Animal Experimentation – CONCEA [17,18]. Room lighting, accommodation and nutrition used followed the recommendations of the Guide for the Care and Use of Laboratory Animals[19].

For the *in vivo* experiments, animals were divided, in equal numbers, into the following groups:

1) Normal Diet + Untrained (ND-UNT, n = 8);
2) High-Fat Diet +Untrained (HFD-UNT, n = 8);
3) High-Fat Diet + Untrained + L-Carnitine (HFD-UNT-C, n = 8);
4) High-Fat Diet + High-intensity Interval Training (HFD-HIIT, n = 8);
5) High-Fat Diet + High-intensity Interval Training + L-Carnitine (HFD-HIIT-C, n = 8).

The experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 012/2017. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2 Experimental Protocols

2.2.1 Dietary induction of obesity

The animals in the experimental HFD groups were fed with a high-fat diet, composed of 20% of calories from carbohydrates, 20% of proteins and 60% lipids (Prag Soluções Biosciences, Jaú, São Paulo-SP), for fourteen weeks, in order to induce the condition of obesity. The animals in the control group (ND) were treated with a standard diet (70% carbohydrates, 20% proteins and 10% lipids) (Quimtia, Curitiba, Paraná, Brazil). All animals received water *ad libidum*.

2.2.2 L-Carnitine supplementation

The animals from the experimental groups HFD-UNT-C and HFD-HIIT-C received L-Carnitine supplementation by gavage, in a concentration of 300 mg/ kg of body mass per day, while animals from the experimental groups ND-UNT, HFD-UNT and HFD-HIIT, received saline by gavage once a day.

2.2.3 High-Intensity Interval Training Protocol

The maximum effort tolerance test was performed to find the maximum speed that each rat could run. This data served as a parameter for the prescription of the training speeds of the HIIT protocol.

The maximum effort test was performed at three times: at the beginning, after four weeks and during the eighth week of the training protocol. Following the protocol of Ferreira et al, the test consisted of a running on a treadmill (model KT-4000, IMBRAMED), with an inclination of 20 degrees, with an initial speed of 6 m/min and an increase of 3 m/min every three minutes, until exhaustion of the animal (visible fatigue)[20]. Once the maximum speed was found, distance and speed were computed to calculate training intensity.

The HIIT training protocol was carried out on five days a week, using a 20° inclination of the treadmill, with intensities defined using the exercise tolerance test. HIIT was performed for three minutes at 60% intensity, followed by four minutes at 85% of the maximum test speed[20]. This cycle was repeated seven times, totaling 49 minutes of training (Fig.1).

The animals of the untrained group (UNT) performed a 40% intensity walk, twice a week, in order to maintain the animals' ability to walk for subsequent physical tests.

2.2.4 Tissue preparation

After the end of the 10th week of training (24 weeks of experiment), the animals were sacrificed (48 hours after the last training session) by decapitation, without anesthesia, since the use of anesthetics can interfere with the determination of oxidative parameters[21,22], and the plantar and soleus muscle were dissected and homogenized in suitable buffer, according to the technique used. The homogenate was centrifuged at x 3,000 g at 4°C for 15 min to remove cellular debris and the supernatant was stored in aliquots at -80°C for the analyses of parameters of oxidative stress and energy metabolism.

2.3 Biochemical studies

2.3.1 Catalase Assay (CAT)

CAT activity was determined by the method of Aebi (1984)[23]. This method is based on the disappearance of hydrogen peroxide (H_2O_2) in a reaction medium composed of 25µL of sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. The absorbance was counted every 10 seconds for 1 minute and 40 seconds at 240 nm using a spectrophotometer. One CAT corresponds to 1 µmol of H_2O_2 consumed per minute and the specific activity was calculated as CAT units/mg protein.

2.3.2 Superoxide Dismutase Assay (SOD)

The activity of SOD was assayed by the method described by Marklund[24], using a process highly dependent on superoxide ($O2^{\bullet -}$), which is a substrate for SOD. Sample (15 µL) was added to 215 µL of a mixture containing 50 µM Tris buffer, 1 µM EDTA, pH 8.2, and 30 µM CAT. Subsequently, 20 µL of pyrogallol were added and the absorbance was measured every 30 seconds for 3 minutes at 420 nm using a UV-vis Shimadzu spectrophotometer. Inhibition of the auto-oxidation of pyrogallol occurs in the presence of SOD, the activity can be tested indirectly spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity was reported as SOD units/mg protein.

2.3.3 Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel (1981)[25], using *tert*-butylhydroperoxide as substrate. The decomposition of NADPH was controlled in a spectrophotometer at 340 nm for 3 minutes and 30 seconds. 90 µL of each sample were added to the medium containing 800 µL of buffer, 20 µL of 2.0 mM GSH, 30 µL of 0.15 U/mL GSH reductase, 10 µL of 0.4 mM azide, and 10 µL of 0.1 mM NADPH. The absorbance was counted every 10 seconds for 1 minute and 30 seconds. Subsequently, 50 µL of 0.5 mM *tert*-butylhydroperoxide were added and the absorbance was read for a further 2 minutes. One GSH-Px unit is characterized as 1 µmol of NADPH consumed per minute and the specific activity was defined as GSH-Px units/mg of protein.

2.3.4 Total Sulfhydryl Content

The total sulfhydryl content was measured following the method of Aksenov & Markersbery (2001)[26], based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, yielding a yellow derivative (TNB), which is evaluated spectrophotometrically at 412 nm. For the assay, 50 µL of homogenate were added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction started with the addition of 30 µL of 10 mM DTNB and incubated for 30 minutes at room temperature in the dark. Analyses of a blank (DTNB absorbance) were also performed. The results were presented as nmol TNB/mg protein.

2.3.5 Thiobarbituric acid reactive substances (TBA-RS)

TBA-RS were defined according to the method of Ohkawa et al. (1979)[27]. The methodology for the study of TBA-RS measures malondialdehyde (MDA), resulting from lipoperoxidation, provided predominantly by hydroxyl free radicals. At first, the soleus and plantar muscle, in 1.15% KCl, were mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBA-RS were determined by the absorbance at 535 nm. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was exposed to the same treatment as that of the supernatants. TBA-RS content was presented in nanomoles of MDA formed per milligram of protein.

2.3.6 Protein carbonyl content

Protein Carbonyl content was tested using the methodology detailed by Reznick & Packer (1994)[28], based on the reaction of protein carbonyls with dinitrophenylhydrazine, in order to form dinitrophenylhydrazone, a yellow compound that is measured spectrophotometrically at 370 nm. Briefly, 200 µL of soleus and plantar muscle homogenate were added to plastic tubes containing 400 µL of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). Samples were kept in the dark for 1 h and vortexed every 15 min. Afterwards, 500 µL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 14 000 rpm for 3 min and the resulting supernatant was excluded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed and centrifuged at 14 000 rpm for 3 min. The supernatant was discarded and the pellet re-suspended in 600 µL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), pre-vortexed and incubated at 60ºC for 15 min. Thus, samples were centrifuged at 14 000 rpm for 3 min and the supernatant was used to measure absorbance at 370 nm (UV) in a quartz cuvette. Results were described as nmol of carbonyl content /mg protein.

2.3.7 Protein determination

Protein was measured by the Lowry et al. (1951)[29] method, using serum bovine albumin as standard.

2.3.8 Pyruvate kinase activity (PK)

Pyruvate kinase activity was assayed essentially as described by Leong et al[30]. The incubation medium consisted of 0.1 M Tris–HCl buffer, pH 7.5, 10.0 mM $MgCl_2$, 0.16 mM NADH, 75.0 mM KCl, 5.0 mM ADP, 7.0 units of L-lactate dehydrogenase, 0.1% (v/v) Triton X-100, and 10.0 µL of the mitochondria-free supernatant in a final volume of 0.5 mL. Unless otherwise stated, the reaction was started after 30 min of pre-incubation by the addition of 1.0 mM phosphoenolpyruvate (PEP). All assays were performed in duplicate at 25ºC. Results were expressed as µmol of pyruvate formed per min per mg of protein.

2.3.9 Citrate synthase activity (CS)

The activity of citrate synthase was assessed using spectrophotometry, as described by Alp et al[31]. Homogenized plantar and soleus muscle samples (10µL) were added to cuvettes with 800 µL buffer (1M Tris-HCL, pH 8), 100 µL 1mM DTNB, 40 µL 2.5 mM acetyl-CoA and 10 µL 10% Triton. The reaction was started by the addition of 50 µL of oxaloacetate (4 mM) and changes in absorbance were observed during 3 minutes at 412 nm.

2.3.10 SDH and complex II activities (CII)

The activities of succinate:phenazine oxyreductase (soluble SDH) and complex II (succinate: DCIP oxyredutase) were measured in soleus and plantar muscle homogenates by following the decrease in absorbance due to the reduction of 2,6-dichloroindophenol (DCIP) at 600 nm with 700 nm as the reference wavelength (ε=19.1 mM^{-1} cm^{-1}) in the presence of phenazine methasulphate (PMS), according to Fischer et al (1985)[32]. The reaction mixture, consisting of 40.0 mM potassium phosphate, pH 7.4, 16.0 mM succinate and 8 μM DCIP, was preincubated with 40-80 μg homogenate protein at 30°C for 20 min. Subsequently, for complex II activity, 4.0 mM sodium azide and 7 μM rotenone were added and the reaction was initiated by the addition of 40 μM DCIP and monitored for 5 min. The activity of SDH was accessed in the same incubation medium by the addition of 1.0 mM PMS and monitored for 5 min.

2.3.11 Cytochrome *C* Oxidase (COX) activity

The activity of cytochrome *c* oxidase was measured according to Rustin et al[33]. Enzymatic activity was measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm with 580 nm as the reference wavelength (ε = 19.1 mM^{-1}x cm^{-1}). The reaction buffer contained 10.0 mM potassium phosphate, pH 7.0, 0.6 mM *n*-dodecyl-β-D-maltoside, 2-4 μg homogenate protein and the reaction was initiated by the addition of 0.7 μg reduced cytochrome *c*. The activity of cytochrome c oxidase was measured at 25°C for 10 min.

2.4 Statistical Analysis

Statistical treatment was performed using the R software version 4.0.3 with the aid of the Car and Desctools packages[34–36]. A multiple linear regression model was applied, followed by Analysis of Variance (ANOVA) for unbalanced data, in order to verify whether there were differences in the outcome variables. The normality assumption of the model residuals was verified using the Shapiro-Wilk normality test. Homoscedasticity was assessed using graphs and Levene's test. To identify differences between groups, Duncan's test was used for multiple comparisons.

For the statistical analysis of body weight and adipose tissue of rats, ANOVA with repeated measures was applied to analyze the relationship between the independent variables and the outcome variable over the period of analysis. To verify the sphericity, the Mauchly test was applied and the Greenhouse-Gueisser correction was applied, if necessary. For the multiple comparison tests, the *t* test with Bonferroni adjustment was applied. To analyze the association between the independent variables and the weight of adipose tissue in the final week, a single-way ANOVA was applied. In the multiple comparison, the Duncan test was used.

Values of $p<0.05$ were considered significant. Results are expressed as means ± SD for eight independent experiments (animals) performed in duplicate.

3 RESULTS

3.1 Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats

We initially verified the effects of high fat-diet, high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats, with the aim of validating our obesity model. Fig.2A shows that there were no significant differences on rats´ starting weight between the experimental groups, and shows that in the 14th week, HFD increased the body weight in HFD-UNT, HFD-UNT-C and HFD-HIIT-C groups, when compared to ND-UNT group. Fig.2A also shows that HIIT protocol, isolated or in association with L-Carnitine supplementation, in the 24th week, was able to revert the increase in this parameter.

With regard to adipose tissue, Fig.2B shows that HFD increased this parameter in HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C, when compared to ND-UNT

groups, and that HIIT protocol, isolated or associated with L-Carnitine supplementation, decreased this parameter, when compared to HFD-UNT-C group.

3.2 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on oxidative stress parameters in the plantar and soleus muscles of obese rats

Subsequently, we verified the effects of the HIIT protocol and L-Carnitine supplementation on TBA-RS, total sulfhydryl content, protein carbonyl content and on the activities of antioxidant enzymes in the plantar and soleus muscle of obese rats. Table 1 shows that the HIIT protocol and the association between L-Carnitine supplementation and the HIIT protocol significantly reduced TBA-RS levels in the plantar muscle, when compared with ND-UNT group. With regard to the TBA-RS levels in the soleus muscle, Table 1 shows that the HFD enhanced this parameter, and that L-Carnitine supplementation alone, or the HIIT protocol alone, partially reversed this increase, while L-Carnitine supplementation associated with the HIIT protocol reversed this increase.

Table 1 also shows that, in the plantar muscle, the association between L-Carnitine supplementation and the HIIT protocol significantly increased total sulfhydryl content, when compared with the HFD-UNT group. Table 1 shows that, in the soleus muscle, the HIIT protocol, isolated and associated to L-Carnitine supplementation, was able to increase this parameter, when compared with the ND-UNT, HFD-UNT and HFD-UNT-C groups. Table 1 also shows that there were no significant differences between experimental groups in carbonyl protein content in either of the muscles.

With regard to antioxidant enzyme activities, Fig.3A shows that the HFD enhanced CAT activity in the plantar muscle, when compared to the ND-UNT group. The HIIT protocol in isolation and when associated with L-Carnitine potentiated this increase. On the other hand, Fig.3A shows that, in the soleus muscle, HFD alone and with L-Carnitine supplementation reduced CAT activity, when compared to the ND-UNT group, while the protocol HIIT alone and the association between the HIIT protocol and L-Carnitine supplementation were able to reverse this decrease, when compared to the HFD-UNT and HFD-UNT-C groups.

As regards SOD activity, Fig.3B shows that HFD alone or when associated with L-Carnitine supplementation reduced this enzyme's activity in the plantar and soleus muscles, when compared to the ND-UNT group. Additionally, the HIIT protocol, when used alone or associated with L-Carnitine supplementation, was able to reverse the alteration in this enzyme activity in both muscles.

Fig.3C demonstrates that HFD decreased GSH-Px activity in the plantar and soleus muscles, when compared to the ND-UNT group. In the plantar muscle, the HIIT protocol when used alone was able to partially reverse this alteration and the association of the HIIT protocol and L-Carnitine supplementation reversed and increased this enzyme's activity, when compared to the ND-UNT group. In the soleus muscle, the HIIT protocol, when used alone and when associated with L-Carnitine supplementation, was unable to totally reverse the alteration in GSH-Px activity, when compared to ND-UNT.

3.3 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on energy metabolism parameters in the plantar and soleus muscles of obese rats

Subsequently, the effects of HIIT and L-Carnitine supplementation on energy metabolism parameters were also analyzed in the plantar and soleus muscles of obese rats. As can be seen in Fig.4A, the HIIT protocol, when used alone and together with L-Carnitine supplementation, increased pyruvate kinase (PK) activity in the plantar muscle, when compared to the ND-UNT, HFD-UNT and HFD-UNT-C groups. In the soleus muscle, Fig.4A shows that the HFD decreased PK activity, while the HIIT protocol reversed the alteration in this enzyme's activity, and the association between the HIIT protocol and L-Carnitine supplementation reversed this alteration and increased this enzyme's activity, when compared to the other groups.

With regard to citrate synthase (CS) activity, Fig.4B shows that the association between the HIIT protocol and L-Carnitine supplementation enhanced this parameter in the plantar and soleus muscles, when compared to the other groups.

Fig.4C shows that, in the plantar muscle, the HIIT protocol, when used alone and together with L-Carnitine supplementation, promoted an increase in the activity of complex II enzyme, when compared to the other experimental groups. Conversely, in

the soleus muscle, Fig.4C shows that the HFD reduced this parameter and shows that the HIIT protocol, when used alone or in association with L-Carnitine supplementation, was able to reverse and increase the activity of complex II enzyme when compared to the ND-UNT, HFD-UNT and HFD-UNT-C groups.

With regard to SDH activity, Fig.4D shows that, in the plantar muscle, the HFD decreased this enzyme´s activity, when compared to the ND-UNT group, while L-Carnitine supplementation when used alone, the HIIT protocol alone and the association between the HIIT protocol and L-Carnitine supplementation, reversed and increased SDH activity, when compared to the ND-UNT and HFD-UNT groups. In the soleus muscle, Fig.4D shows that SDH activity was increased by the HIIT protocol, when used alone and associated with L-Carnitine supplementation, when compared to the ND-UNT, HFD-UNT and HFD-UNT-C groups.

Fig.4E shows that the HFD by itself reduced cytochrome *c* oxidase (COX) activity in the plantar muscle, when compared to the ND-UNT group, and that L-Carnitine supplementation partially reversed this effect. The HIIT protocol when used alone, and in association with L-Carnitine supplementation, reversed and increased this parameter when compared to the ND-UNT, HFD-UNT and HFD-UNT-C groups. In the soleus muscle, there were no significant differences between the experimental groups in cytochrome *c* oxidase activity.

4 DISCUSSION

In the present study, we investigated whether a HIIT protocol and L-Carnitine supplementation could prevent or protect against oxidative stress and energy metabolism dysfunction in the plantar and soleus muscles of obese rats. First of all, our results showed that HFD increased body weight and promoted adipose tissue gain in the experimental groups, validating our methodology. Our results also showed that HFD promoted alterations in the activities of antioxidant enzymes, in energy metabolism parameters, damaged proteins, and incurred lipoperoxidation in both muscles. Furthermore, we showed that the HIIT protocol, when used alone and, sometimes, when associated with L-Carnitine supplementation, was able to reverse and even improve these alterations.

With regard to antioxidant enzyme activities, our results show that the HFD increased CAT activity in the plantar muscle and reduced this activity in the soleus muscle, and decreased SOD and GSH-Px activities in both muscles. The decrease in SOD activity may suggest that the HFD induced an increase in the production of ROS, such as the superoxide radical, and as a consequence, a depletion in the activity of this antioxidant enzyme. Additionally, increased superoxide radical production may enhance the production of hydrogen peroxide, which could explain the saturation of CAT activity in the soleus muscle and GSH-Px activity in both muscles. Furthermore, the increase in CAT activity in the plantar muscle may have occurred to compensate the enzymatic reduction of GSH-Px. According to Travacio and Llesuy (1996), the increase in the antioxidant activity of an enzyme may be due to a response to oxidative stress, in order to reduce or prevent damage caused by free radicals[37].

The HIIT protocol, when used alone and in association with L-Carnitine supplementation, enhanced and potentiated CAT activity and SOD activity in the plantar muscle. The HIIT protocol, when used alone, partially reversed the alteration in GSH-Px activity, while the HIIT protocol associated with L-Carnitine supplementation increased this enzyme's activity. In the soleus muscle, the HIIT protocol alone and its association with L-Carnitine were able to reverse the alterations in CAT and SOD activities. In contrast, the HIIT protocol, isolated and in association with L-Carnitine supplementation, partially reversed the reduction in GSH-Px activity.

Several studies suggest an increase in the production of free radicals after exercise of high or exhausting intensities, largely due to the greater need for oxygen supply and consumption, promoting an accumulation of ROS[38,39]. However, studies also show that physical exercise is capable of generating an adaptive response to this stress, through the activation of pathways to regulate enzymatic expression, with the main objective of promoting balance between oxidant and antioxidant agents at the cellular level[39]. This response may corroborate and justify the increases in CAT, SOD and GSH-Px activities in the plantar muscle and the reversal of the reduction in CAT and SOD activities in the soleus muscle observed in the HIIT protocol experimental groups. Li *et al*. (2015) studied the effects of exercise training on several oxidative stress parameters in diet-induced obesity mice[40]. They found an increase in

superoxide anion in the skeletal muscle, which was partially reversed by exercise training.

Subsequently, we evaluated TBA-RS levels, total sulfhydryl and carbonyl protein contents. TBA-RS levels are a lipid peroxidation parameter and are used as an indication of damage to plasma membranes[39,41,42]. Since lipid peroxidation induces changes in lipid structure, permeability and the cellular transport of membranes, excess lipid oxidation can result in cell death[39,41,42]. On the other hand, the determination of the total sulfhydryl and carbonyl protein contents serves as a parameter indicative of protein damage. Changes in protein structures can promote an increase in their fragmentation and aggregation, in addition to making them more susceptible to degradation by proteasomes[41,42].

Our results show that the HFD did not alter TBA-RS levels; in contrast, the HIIT protocol, when used alone or associated with L-Carnitine supplementation, was able to significantly reduce this parameter in the plantar muscle. In the soleus muscle, the HFD enhanced TBA-RS levels, promoting lipoperoxidation; L-Carnitine supplementation alone or the HIIT protocol alone partially reversed this effect, while L-Carnitine associated with the HIIT protocol totally reversed this increase. These data suggest that HIIT protocol and L-Carnitine have a protective role against lipoperoxidation in both muscles. In 2015, Li *et al*., when evaluating the effect of physical exercise using a treadmill running protocol (60 min, 5 times a week, for 8 weeks), identified an increase in TBA-RS levels in the soleus muscle of male rats fed on a HFD; they found that this increase was partially reversed by exercise, corroborating the findings of our study[40].

With regard to total sulfhydryl content, our data show that the HFD did not alter this parameter in the plantar muscle, but that the association between the HIIT protocol and L-Carnitine supplementation significantly increased this parameter. In the soleus muscle, the HIIT protocol, when used alone or in association with L-Carnitine supplementation, was able to increase this parameter. These results suggest that the HIIT protocol and L-Carnitine supplementation exert protective effects against protein damage by increasing the total sulfhydryl content in both muscles of obese rats.

Although our data did not show significant changes in the total carbonyl protein content, a study carried out in 2013 identified a reduction in this parameter in the

adipose tissue of male mice fed with a HFD and submitted to physical exercise (8 weeks, 5 times a week), demonstrating that physical exercise has a protective effect on protein damage[43].

With regard to the energy metabolism of the skeletal muscle, the HFD, when use alone or in association with L-Carnitine supplementation, decreased the PK activity in the soleus muscle. In the plantar muscle, HFD *per se* did not alter this parameter. The PK enzyme is responsible for the oxidation of phospoenolpyruvate to ATP and pyruvate in glycolysis, and this decrease can impair the aerobic and anaerobic pathways of energy metabolism[44,45]. In addition, the HIIT protocol, in isolation or association with L-Carnitine, was able to increase PK activity in the plantar muscle and reverse the reduction observed in the soleus muscle.

HFD did not alter the CS activity in the muscles, but the HIIT protocol, when used alone or associated with L-Carnitine, was able to increase this enzyme's activity, improving aerobic metabolism. With regard to SDH activity, HFD did not alter this parameter in the soleus muscle; in contrast, in the plantar muscle, HFD decreased this enzyme's activity. Complex II was not altered by the HFD in the plantar muscle, but was decreased by the HFD in the soleus muscle. In the plantar muscle, L-Carnitine supplementation alone and the HIIT protocol alone, as well their association, reversed and increased the activity of the SDH enzyme. In the soleus muscle, the HIIT protocol, when used alone and when associated with L-Carnitine supplementation, increased SDH activity. Furthermore, in both the plantar and soleus muscles, the HIIT protocol, alone and associated with L-Carnitine supplementation, increased and reversed, and increased the activity of complex II, respectively.

Regarding COX activity, the HFD decreased the activity of this enzyme in the plantar muscle, while in the soleus muscle, there were no significantly differences between the experimental groups. L-Carnitine supplementation partially reversed this parameter in the plantar muscle, and the HIIT protocol, when used alone and associated with L-Carnitine, reversed and increased this COX activity, which suggests that mitochondrial energy efficiency can be improved by exercise[14].

Brunetta (2020) investigated the effects of the HFD (given for 28 days) on mitochondrial function and glycemic homeostasis, and found 40% and 50% decreases in CS, complex II and complex IV activities, respectively, in the soleus muscle of obese

mice[46]. In contrast, Yokota *et al*. (2009) investigated the effects of the HFD in the skeletal muscles of diabetic and obese mice, but did not find significant differences in the activities of complex II and IV[45]. However, several studies have reported that mitochondrial function was impaired in skeletal muscles of HFD-fed mice and rats; since the mitochondria are a target for DNA oxidative damage and this can result in a decrease in the electron transport chain, further corroborating our findings[45].

Taken together, our results provide insights into the relative contribution of the HFD to oxidative stress and energy metabolism dysfunction in the skeletal muscle. Furthermore, the HIIT protocol and L-Carnitine supplementation may represent approaches to prevent and, sometimes, reverse this damage.

Conflict of interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

Acknowledgements

This work was supported by grants from Fundo de Apoio à Pesquisa da Universidade da Região de Joinville, Fundo de Apoio à Pesquisa de Santa Catarina (FAPESC), Fundação Universidade Regional de Blumenau (FURB), Uniedu/Pipe/Article 170 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

We thank the statistics teacher, Dra. Elisa Henning (Udesc/Joinville), for statistical analysis.

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Table 1 - Thiobarbituric acid reactive substances (TBA-RS), Total Sulfhydryl Content and Protein carbonyl content in the plantar and soleus muscles of rats in the different experimental groups.

	Structure	ND-UNT	HFD-UNT	HFD-UNT-C	HFD-HIIT	HFD-HIIT-C
TBA-RS (nmol TBA-RS/min. mg protein)	*Plantar muscle*	2.63 ± 0.53	2.58 ± 0.66	2.57 ± 0.59	1.96 ± 0.31 [a]	1.91 ± 0.26 [a]
	Soleus muscle	3.70 ± 0.59	6.31 ± 0.69 [b]	5.04 ± 0.64 [b,c]	4.3 ± 0.63 [c,d]	3.53 ± 0.58 [c,e,f]
Total Sulfhydryl Content (nmol TBN/mg protein)	*Plantar muscle*	115.65 ± 23.50	110.1 ± 22.45	125.11 ± 10.27	126.67 ± 15.96	134,84 ± 10.13 [g]
	Soleus muscle	73.87 ± 4.38	69.24 ± 2.65	68.46 ± 4.34 [h]	81.76 ± 5.40 [i, j]	80.77 ± 5.65 [i, j]
Protein carbonyl content (nmol carbonyl/mg protein)	*Plantar muscle*	4.62 ± 0.46	4.95 ± 0.50	4.86 ± 0.57	4.60 ± 0.58	4.42 ± 0.51
	Soleus muscle	5.14 ± 0.35	5.38 ± 0.18	5.26 ± 0.44	5.13 ± 0.52	5.04 ± 0.34

Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval

Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* ND-UNT, HFD-UNT and HFD-UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.001 *vs* HFD-UNT; [d], p<0.05 *vs* HFD-UNT-C; [e], p<0.001 *vs* HFD-UNT-C; [f], p<0.05 *vs* HFD-HIIT; [g], p<0.05 *vs* HFD-UNT; [h], p<0.05 *vs* ND-UNT; [i], p<0.01 *vs* ND-UNT; [j], p<0.001 *vs* HFD-UNT and HFD-UNT-C.

Fig.1. HIIT Protocol

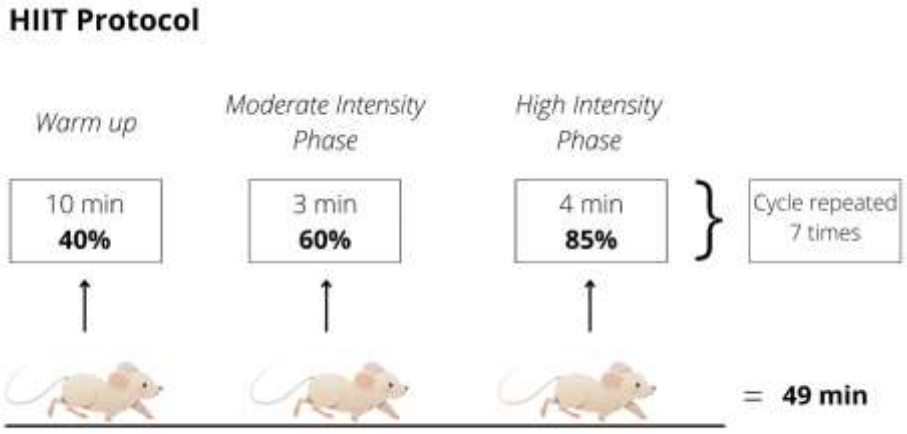


Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols.

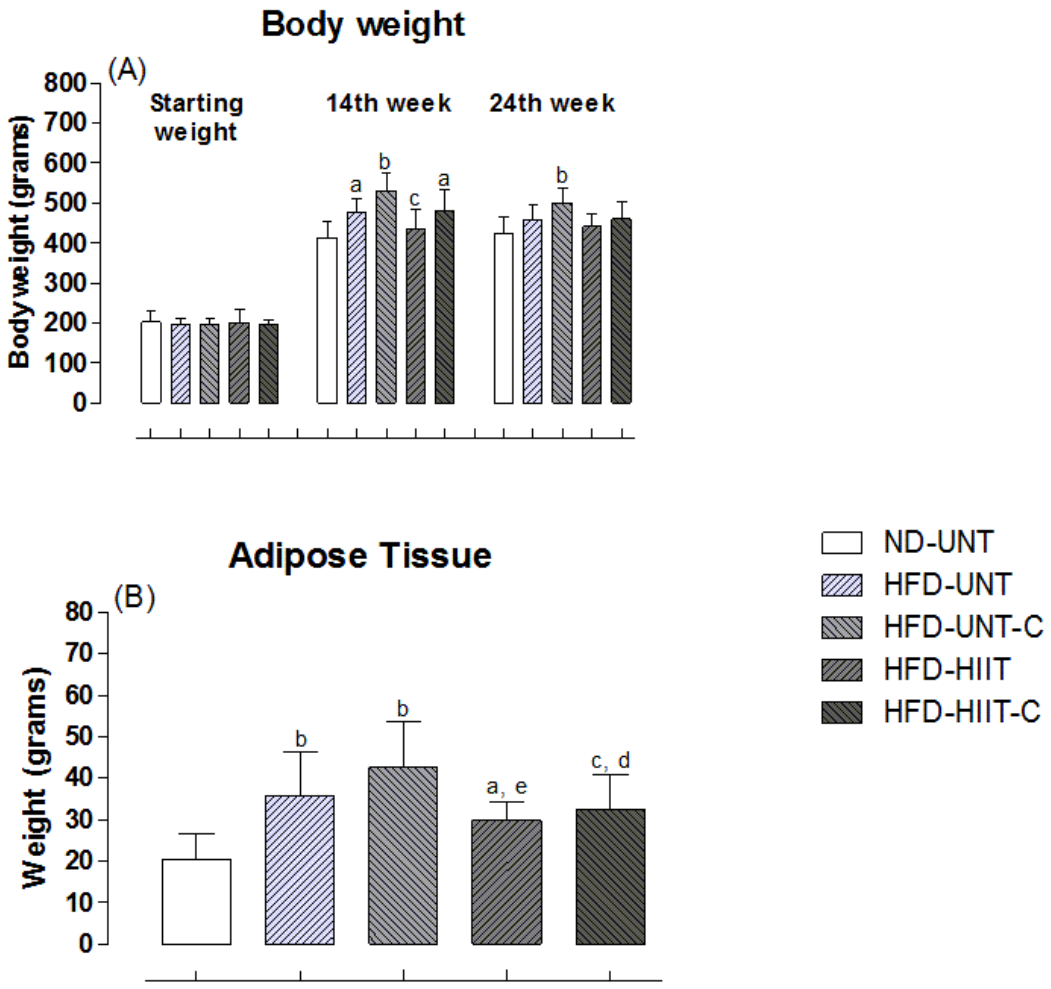


Fig. 3. Effects of high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the plantar and soleus muscle of obese rats.

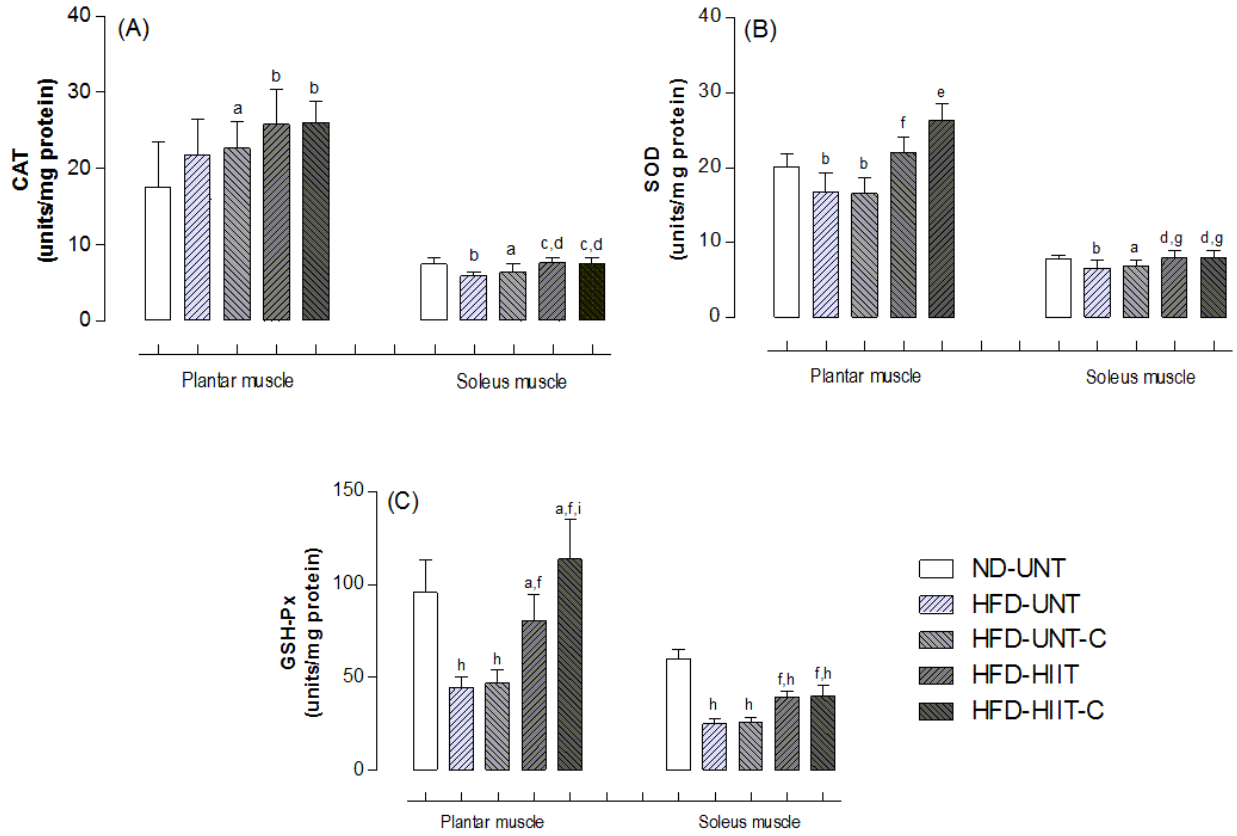
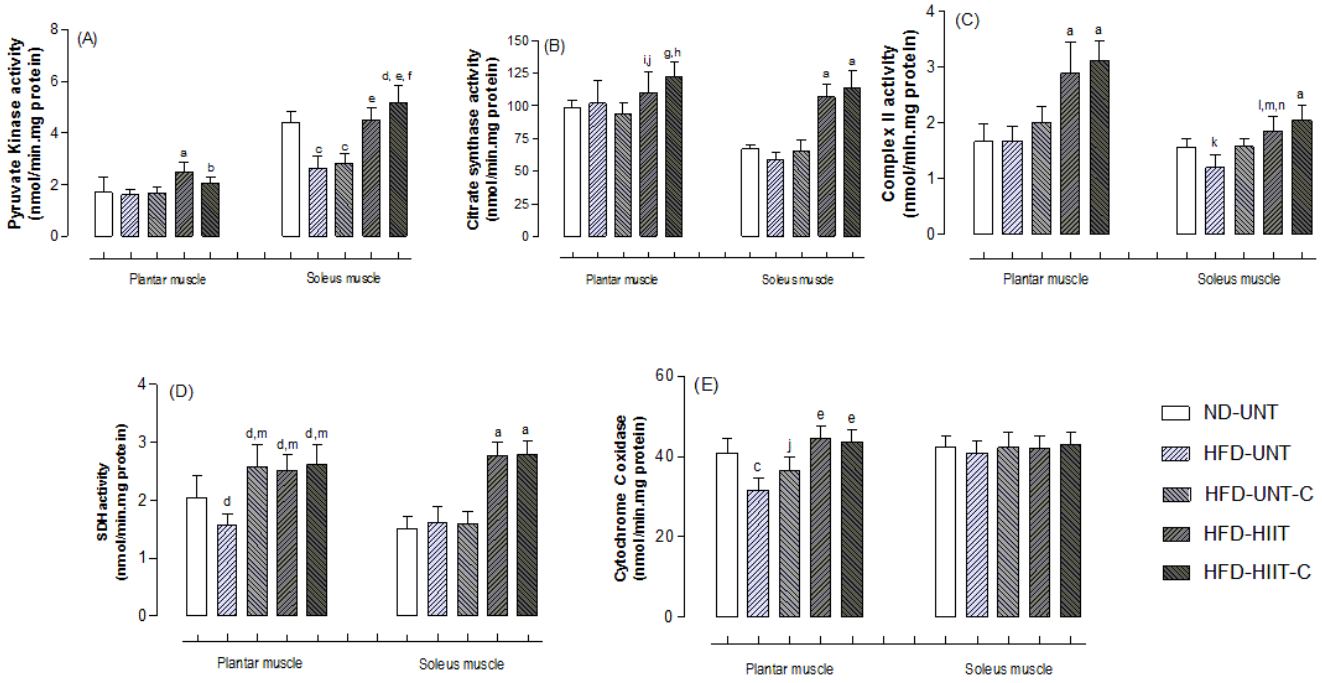


Fig. 4. Effects of high-intensity interval training protocol and L-Carnitine supplementation on energy metabolism parameters in the plantar and soleus muscle of obese rats.



Legends to figures

Fig.1. HIIT Protocol.

Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* ND-UNT; [b], $p<0.001$ *vs* ND-UNT; [c], $p<0.05$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-UNT; [e], $p<0.01$ *vs* HFD-UNT-C.

Fig.3. Enzyme activities of catalase (CAT) (A), superoxide dismutase (SOD) (B) and Glutathione peroxidase (GSH-Px) (C) in the plantar and soleus muscles of rats submitted to different experimental protocols. Data are presented as means ± SD for

8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* ND-UNT; [b], p<0.01 *vs* ND-UNT; [c], p<0.001 *vs* HFD-UNT; [d], p<0.05 *vs* HFD-UNT-C; [e], p<0.001 *vs* ND-UNT, HFD-UNT, HFD-UNT-C and HFD-HIIT; [f], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [g], p<0.01 *vs* HFD-UNT; [h], p<0.001 *vs* ND-UNT; [i], p<0.001 *vs* HFD-HIIT.

Fig.4. Enzyme activities of (A) pyruvate kinase, (B) citrate synthase, (C) complex II, (D) succinate dehydrogenase (SDH) and (E) cytochrome *c* oxidase in the plantar and soleus muscles of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* ND-UNT, HFD-UNT and HFD-UNT-C; [b], p<0.05 *vs* HFD-UNT and HFD-UNT-C; [c], p<0.001 *vs* ND-UNT; [d], p<0.01 *vs* ND-UNT; [e], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [f], p<0.05 *vs* HFD-HIIT; [g], p<0.01 *vs* ND-UNT and HFD-UNT; [h], p<0.001 *vs* HFD-UNT-C; [i], p<0.01 *vs* HFD-UNT-C; [j], p<0.05 *vs* ND-UNT and HFD-UNT; [k], p<0.01 *vs* ND-UNT and HFD-UNT-C; [l], p<0.05 *vs* ND-UNT; [m], p<0.001 *vs* HFD-UNT; [n], p<0.05 *vs* HFD-UNT-C.

6.2 Artigo:

PROTECTIVE EFFECTS OF HIIT AND L-CARNITINE SUPPLEMENTATION AGAINST OBESITY-INDUCED OXIDATIVE STRESS AND BIOCHEMICAL ALTERATIONS IN THE BLOOD OF RATS

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ABSTRACT

This study evaluated the effects of High-intensity Interval Training (HIIT) and L-Carnitine supplementation on oxidative stress, inflammatory and biochemical parameters in the blood of obese rats. Animals were divided into five groups: Normal Diet-Untrained (ND-UNT), High-Fat Diet-Untrained (HFD-UNT), High-Fat Diet-Untrained + Carnitine (HFD-UNT-C), High-Fat Diet + HIIT (HFD-HIIT) and High-Fat Diet + HIIT + Carnitine (HFD-HIIT-C). To induce obesity, animals in the HFD groups

were fed on a high-fat diet for 14 weeks, while animals in the ND groups were fed on a standard diet. Animals in the HFD-UNT-C and HFD-HIIT-C groups received L-Carnitine by gavage as soon as the HIIT protocol was started (frequency of 5 days a week) and animals in the UNT group walked at 40% intensity (twice a week). After the end of the 10th week of training, animals were sacrificed by decapitation and their blood was collected and prepared according to the technique to be used. The antioxidant activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), levels of thiobarbituric acid reactive substances (TBARS), total sulfhydryl and protein carbonyl content, interleukin-1β, interleukin-6, tumor necrosis factor α, glucose, insulin, triglycerides, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c) and total cholesterol were determined. The HFD promoted oxidative stress and alterations in biochemical parameters, such as lipoperoxidation, protein damage, and in the activities of antioxidant enzymes, and increased LDL-c and insulin levels; the HIIT protocol, on its own and sometimes when associated with L-Carnitine, prevented these alterations.

Keywords: Obesity; Oxidative stress; Blood; L-Carnitine; High-intensity Aerobic Training.

1 INTRODUCTION

Obesity, considered a global public health problem, is a polygenic and multifactorial disease, characterized by an excess of body fat and is identified as a risk factor for the development of cardiometabolic disorders, diabetes, dyslipidemia, atherosclerosis, cancer, respiratory disease and other inflammatory diseases[1,2]. Several studies have shown that obese patients present higher levels of circulating cytokines, promoting an inflammatory state, that may also be related to insulin resistance, hyperlipidemia and metabolic syndrome[3].

Furthermore, obesity has been associated with oxidative stress by several researchers. According to França *et al*. (2013), obese patients present some biological alterations that make them more susceptible to oxidative damage, probably due to an imbalance between the amounts of fat, body weight, lipoproteins and lipids, an imbalance that promotes an increase in metabolic need and oxygen consumption and, consequently, an increased production of reactive oxygen species (ROS), such as superoxide and peroxides of hydrogen[4].

The association between obesity, inflammation and oxidative stress is mediated by different physicochemical pathways, such as an increase in blood glucose levels,

an increase in the generation and storage of lipids, stimulation in fatty acid oxidation and also an increase in proinflammatory cytokines, promoting oxidative stress and metabolic disorders[2].

Among the various forms of obesity prevention and treatment, great importance has been given to the regular practice of physical activity, due to its beneficial results and low cost[1,5,6]. According to Aparicio *et al*. (2016), high-intensity interval training (HIIT) may be more beneficial than moderate intensity exercise to improve body composition and metabolic syndrome alterations[5]. In addition, L-Carnitine has been studied as a supplement option in these cases and can be used as a supporting treatment for dyslipidemia, due to its potential antioxidant effects, and it ability to participate in the transfer reactions of free fatty acids, improving their oxidation[7].

Considering that obesity is related to oxidative stress, inflammatory and biochemical alterations, and that studies indicate that the HIIT protocol and L-Carnitine supplementation can promote benefits in obese patients, this study evaluated the protective effects of HIIT and L-Carnitine supplementation on oxidative stress, inflammatory and biochemical parameters in the blood of obese rats.

2 MATERIALS AND METHODS

2.1 Animals and Reagents

Sixty-day-old male Wistar rats from the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil, were used in the experiments. Before the experiments, animals were accommodated and acclimatized for 7 days to adapt to their new environment. Animals were kept in rooms with a 12h light/dark cycle with the temperature maintained between 20-22ºC and free access to food and water. The 12h light/dark cycle was inverted for better use of the animals' active period for training. The animals were kept in cages with a maximum number of four per cage; box exchange was performed every 2 days. Animal care was carried out in accordance with Law Nº. 11794 (October 8, 2008), and other regulations applicable to the use of animals in teaching and/or research, especially the Normative Resolutions of the National Council for the Control of Animal Experimentation – CONCEA[8,9]. Room

lighting, accommodation and nutrition used followed the recommendations of the Guide for the Care and Use of Laboratory Animals[10].

For the *in vivo* experiments, animals were divided, in equal numbers, into the following groups:

6) Normal Diet-Untrained (ND-UNT, n = 8);
7) High-Fat Diet-Untrained (HFD-UNT, n = 8);
8) High-Fat Diet - Untrained + L-Carnitine (HFD-UNT-C, n = 8);
9) High-Fat Diet + High-intensity Interval Training (HFD-HIIT, n = 8);
10) High-Fat Diet + High-intensity Interval Training + L-Carnitine (HFD-HIIT-C, n = 8).

The experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 012/2017. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2 Experimental Protocols

2.2.1 Dietary induction of obesity

The animals in the experimental groups (HFD) were fed with a high-fat diet, composed of 20% of calories from carbohydrates, 20% of proteins and 60% of lipids (Prag Soluções Biosciences, Jaú, São Paulo-SP), for fourteen weeks, in order to induce the condition of obesity. The animals in the control group (ND) were treated with a standard diet (70% carbohydrates, 20% proteins and 10% lipids) (Quimtia, Curitiba, Paraná, Brazil). All animals received water *ad libitum*.

2.2.2 L-Carnitine supplementation

The animals from the experimental groups HFD-UNT-C and HFD-HIIT-C received L-Carnitine supplementation by gavage, in a concentration of 300 mg/ kg of body mass per day, while animals from the experimental groups ND-UNT, HFD-UNT and HFD-HIIT, received saline by gavage once a day.

2.2.3 High Intensity Interval Training Protocol

A maximum effort tolerance test was applied in order to define the animals' maximum speed and then prescribe the training intensity. This data served as a parameter for the prescription of the training speeds of the HIIT protocol.

The maximum effort test was performed in three moments: at the beginning, after four weeks and in the eighth week of the training protocols. Following the protocol of Ferreira et al, the test consisted of running on a treadmill (model KT-4000, IMBRAMED), with an inclination of 20 degrees, with an initial speed of 6 m/min that increased by 3 m/min every three minutes, until exhaustion of the animal (visible fatigue)[11]. Once the maximum speed was found, distance and speed were computed to calculate training intensity.

The HIIT protocol was administered with a frequency of five days a week, 20° inclination of the treadmill and intensities defined from the exercise tolerance test. HIIT was applied in three minutes at 60% intensity followed by four minutes at 85% of the maximum test speed[11]. This cycle was repeated seven times, totaling 49 minutes of training (Fig.1).

The animals of the untrained group (UNT) performed a 40% intensity walk for 10 minutes, twice a week, in order to maintain the animals' ability to walk for subsequent physical tests.

2.2.4 Preparation of samples

After the end of the 10th week of training (24 weeks of experiment), the animals were fasted for 12 hours, before being sacrificed (48 hours after the last training session) by decapitation, without anesthesia, since the use of anesthetics can interfere with the determination of oxidative parameters[12,13], and peripheral whole blood was collected and processed, according to the technique to be used.

For erythrocyte separation, peripheral blood was collected and transferred to heparinized tubes, which were centrifuged at 1,000 rpm; the plasma was then removed by aspiration and maintained frozen at −80 °C until assay. Erythrocytes were washed three times with cold saline solution (0.153 mol/L sodium chloride) and lysates were prepared by the addition of 1 mL of distilled water to 100 µL washed erythrocytes and maintained frozen at −80 °C until determination of the activities of antioxidant enzyme [14]. For the determination of antioxidant enzyme activity, erythrocyte lysates were frozen

and thawed three times, and centrifuged at 13,500 rpm for 10 min. The supernatant was diluted in order to contain approximately 0.5 mg/mL of protein[14].

Serum was prepared from blood samples obtained from rats. The peripheral blood was rapidly collected and transferred to tubes without anticoagulant and centrifuged at 1,000 rpm for 10 min; the serum was then separated and used for the measurement of glucose, insulin, triglycerides, total cholesterol, HDL-cholesterol (HDL-c), LDL-c, TNF-α, IL-6 and IL-1β[14].

2.3 Biochemical studies

2.3.1 Analysis of oxidative stress parameters

2.3.1.1 Catalase assay (CAT)

CAT activity was determined by the method of Aebi[15], using a UV-vis Shimadzu spectrophotometer. The method used is based on the disappearance of hydrogen peroxide (H_2O_2) at 240 nm in a reaction medium containing 25µL of sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. The absorbance was measured every 10 seconds for 1 minute and 40 seconds at 240 nm using a spectrophotometer. One CAT unit is defined as 1 µmol of H_2O_2 consumed per minute and the specific activity was calculated as CAT units/mg protein.

2.3.1.2 Superoxide dismutase assay (SOD)

The activity of SOD was assayed by the method described by Marklund[16], using a process highly dependent on superoxide ($O_2^{\bullet-}$), which is a substrate for SOD. Sample (15 µL) was added to 215 µL of a mixture containing 50 µM Tris buffer, 1 µM EDTA, pH 8.2, and 30 µM CAT. Subsequently, 20 µL of pyrogallol were added and the absorbance was measured every 30 seconds for 3 minutes at 420 nm using a UV-vis Shimadzu spectrophotometer. Inhibition of the auto-oxidation of pyrogallol occurs in the presence of SOD, the activity of which can be tested indirectly spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity was reported as SOD units/mg protein.

2.3.1.3 Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel[17] using *tert*-butyl hydroperoxide as substrate. The decomposition of NADPH was monitored in a spectrophotometer at 340 nm for 3 minutes and 30 seconds using a UV-vis Shimadzu spectrophotometer. The medium contained 90 µL of each sample, 800 µL of buffer, 20 µL of 2.0 mM GSH, 30 µL of 0.15 U/mL GSH reductase, 10 µL of 0.4 mM azide and 10 µL of 0.1 mM NADPH. The absorbance was determined every 10 seconds for 1 minute and 30 seconds. Afterwards, 50 µL of 0.5 mM *tert*-butylhydroperoxide were added and the absorbance was read for another 2 minutes. One GSH-Px unit is defined as 1 µmol of NADPH consumed per minute and the specific activity was reported as GSH-Px units/mg of protein.

2.3.1.4 Total sulfhydryl content

The total sulfhydryl content was determined, according to the method described by Aksenov and Markersbery[18], which is based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, generating a yellow derivative (TNB) that is measured spectrophotometrically at 412 nm. For the assay, 50 µL of plasma were added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction was started with the addition of 30 µL of 10 mM DTNB and incubated for 30 minutes at room temperature in the dark. Analyses of a blank (DTNB absorbance) were also performed. The results were expressed as nmol TNB/mg protein.

2.3.1.5 Thiobarbituric acid reactive substances (TBA-RS)

TBA-RS were determined according to the method described by Ohkawa *et al*.[19]. The TBA-RS methodology measures malondialdehyde (MDA), a product of lipoperoxidation, generated mainly by $OH^{\cdot}$ radicals. TBA-RS were determined by the absorbance at 535 nm. At first, plasma in 1.15% KCl was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid, and heated in a boiling water bath for 60 min. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was exposed to the same treatment as that of the supernatants. The results were expressed in nmol of MDA/mg protein.

2.3.1.6 Protein carbonyl content

Protein carbonyl content was assayed by a method described by Reznick & Packer (1994)[20], based on the reaction of protein carbonyls with dinitrophenylhydrazine to form dinitrophenylhydrazone, a yellow compound that is measured spectrophotometrically at 370 nm. Initially, 200 µL of plasma were added to plastic tubes containing 400 µL of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). Samples were kept in the dark for 1 h and vortexed every 15 min. Subsequently, 500 µL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 14,000 rpm for 3 min and the resulting supernatant was discarded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed and centrifuged at 14,000 rpm for 3 min. The supernatant was discarded and the pellet re-suspended in 600 µL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), pre-vortexed and incubated at 60ºC for 15 min. Afterwards, samples were centrifuged at 14,000 rpm for 3 min and the supernatant was used to measure absorbance in a quartz cuvette. Results were reported as carbonyl content (nmol/mg protein).

2.3.1.7 Protein determination

Protein was measured by the Lowry et al. (1951)[21] method, using serum bovine albumin as standard.

2.3.2 Analysis of inflammatory parameters (TNF-α, IL-1β e IL-6)

Levels of TNF-α, IL-1β and IL-6 cytokines were determined in serum samples collected at the time of animal sacrifice, using suitable commercial ELISA kits (Sigma-Aldrich), according to the manufacturer's instructions.

2.3.3 Analysis of biochemical parameters

2.3.3.1 Determination of glucose, triglycerides, total cholesterol, HDL-c, LDL-c and insulin

The measurements of glucose, triglycerides, total cholesterol and HDL-c were performed in serum samples collected at the time of animal sacrifice, using specific kits from the Labtest brand. Absorbance was determined using a Shimadzu UV-visible

spectrophotometer, according to the manufacturer's guidelines. LDL-c levels were determined using Friedewald´s formula.

The measurement of insulin was performed in serum samples collected at the time of animal sacrifice, using the Advia Centaur CP Immunoassay System (Siemens Healthineers).

2.4 Statistical analysis

Statistical analysis was performed using the R software version 4.0.3 with the aid of the Car and Desctools packages[22–24].

A multiple linear regression model was applied, followed by Analysis of Variance (ANOVA) for unbalanced data, in order to verify whether there were differences in the outcome variables. The normality assumption of the model residuals was verified using the Shapiro-Wilk normality test. Homoscedasticity was assessed using graphs and Levene's test. To identify differences between groups, Duncan's test was used for multiple comparisons.

For the statistical analysis of body weight and adipose tissue of rats, ANOVA with repeated measures was applied to analyze the relationship between the independent variables and the outcome variable over the period of analysis. To verify the sphericity, the Mauchly test was applied and the Greenhouse-Gueisser correction was applied, if necessary. For the multiple comparison tests, the *t* test with Bonferroni adjustment was applied. To analyze the association between the independent variables and the weight of adipose tissue in the final week, a single-way ANOVA was applied. In the multiple comparison, the Duncan´s test was used.

Values of $p<0.05$ were considered significant. Results are expressed as means ± SD for eight independent experiments (animals) performed in duplicate.

3 RESULTS

3.1 Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats

We initially verified the effects of high fat-diet, high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the body weight and adipose tissue

of rats, with the aim of validating our obesity model. Fig.2A shows that there were no significant differences on rats´ starting weight between the experimental groups, and shows that in the 14th week, HFD increased the body weight in HFD-UNT, HFD-UNT-C and HFD-HIIT-C groups, when compared to ND-UNT group. Fig.2A also shows that HIIT protocol, isolated or in association with L-Carnitine supplementation, in the 24th week, was able to reverse the increase in this parameter.

With regard to adipose tissue, Fig.2B shows that HFD increased this parameter in HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C, when compared to ND-UNT groups, and that HIIT protocol, isolated or associated with L-Carnitine supplementation, decreased this parameter, when compared to HFD-UNT-C group.

3.2 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on oxidative stress parameters in the blood of obese rats

Subsequently, we verified the effects of the HIIT protocol and L-Carnitine supplementation on TBA-RS, total sulfhydryl content, protein carbonyl content and on the activities of antioxidant enzymes in the plasma and erythrocytes of obese rats.

Fig. 3A shows that the HFD significantly increased TBA-RS levels in the plasma ($p<0.001$), when compared with ND-UNT group, while L-Carnitine supplementation, on its own, partially reversed this increase. The HIIT protocol, alone and when associated with L-Carnitine supplementation, reversed this alteration.

With regard to the total sulfhydryl content, Fig. 3B shows that the HFD significantly decreased these parameter levels in the plasma ($p<0.01$), when compared with the ND-UNT group; furthermore, the HIIT protocol, when used alone or in association with L-Carnitine, was able to reverse this alteration.

In addition, Fig. 3C shows that, HFD increased the protein carbonyl content ($p<0.001$) in the plasma, when compared with the ND-UNT group. L-Carnitine supplementation, when used alone, partially reduced this increase, and the HIIT protocol, in isolation or in association with L-Carnitine, reversed this alteration.

With regard to antioxidant enzymes activities, Fig.4A shows that the HFD reduced CAT activity in the erythrocytes ($p<0.001$), when compared with the ND-UNT group. L-Carnitine supplementation and the HIIT protocol (in isolation and in association with L-Carnitine) were able to reverse and increase this parameter.

Fig.4B shows that L-Carnitine on its own and the HIIT protocol, alone or in association with L-Carnitine, enhanced SOD activity in the erythrocytes ($p<0.05$), when compared with ND-UNT group.

As can be seen in Fig.4C, HFD decreased GSH-Px activity in the erythrocytes of obese rats ($p<0.001$), while neither L-Carnitine supplementation nor the HIIT protocol, alone or when associated with L-Carnitine, were able to reverse this alteration. The HIIT protocol isolated partially reversed this alteration, when compared with ND-UNT.

3.3 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on inflammatory parameters in the blood of obese rats

Subsequently, the effects of the HIIT protocol and L-Carnitine supplementation on inflammatory parameters in the serum of obese rats were analyzed by measuring interleukin 6 (IL-6), interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α). As can be seen in Table 1, there were no significant differences in IL-6 and IL-1β concentrations between the experimental groups. With regard to TNF-α, results were inconclusive, making statistical analysis impossible.

3.4 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on biochemical parameters in the blood of obese rats

Finally, this study evaluated the effects of the HIIT protocol and L-Carnitine supplementation on biochemical parameters, such as glucose, insulin, triglycerides, HDL-c, LDL-c and total cholesterol levels, in the serum of obese rats.

Fig.5A shows that HFD did not alter glucose; however, when we analyzed the other experimental groups, the HIIT protocol, when used alone and in association with L-Carnitine supplementation, reduced blood glucose, when compared with the ND-UNT, HFD-UNT and HFD-UNT-C groups.

The HIIT protocol decreased total cholesterol levels (Fig.5B; $p<0.01$), when compared with the ND-UNT, HFD-UNT and HFD-UNT-C groups. With regard to the HDL-c levels, Fig.5C also shows that HFD, associated with L-Carnitine supplementation, reduced this parameter ($p<0.01$), when compared to the ND-UNT group, and that the HIIT protocol in association with L-Carnitine was able to partially reverse this alteration in the serum of obese rats.

Fig.5D shows that the HFD, on its own and when associated with L-Carnitine, significantly enhanced LDL-c levels in the serum of obese rats ($p<0.01$ and $p<0.05$, respectively), when compared to the ND-UNT group. In contrast, the HIIT protocol, when used alone and in association with L-Carnitine supplementation, reversed this alteration. Fig.5E also shows that neither HFD, the HIIT protocol nor L-Carnitine supplementation, in isolation or in association, were able to alter triglyceride levels in the serum of obese rats.

Regarding insulin levels, Fig.5F shows that the administration of HFD alone and in association with L-Carnitine supplementation increased this parameter ($p<0.05$), when compared with the ND-UNT group; the HIIT protocol was able to reverse the alteration in this parameter.

4 DISCUSSION

The present study contributes valuable information regarding the effects of the HIIT protocol, when used alone or in combination with L-Carnitine supplementation, on oxidative stress, biochemical and inflammatory parameters in the blood of obese rats. We found that these blood parameters are sensitive to the positive effects of exercise training and L-Carnitine supplementation, which protected animals against obesity-induced oxidative stress and biochemical disorders. First of all, our results showed that HFD increased body weight and promoted adipose tissue gain in the experimental groups, validating our methodology. Furthermore, we showed that the HIIT protocol, when used alone or in association with L-Carnitine, improved the function of the antioxidant enzyme system, thereby decreasing lipoperoxidation and protein damage. In addition, this treatment (exercise and/or L-Carnitine supplementation) decreased HFD-induced alterations in glucose, total cholesterol, LDL-cholesterol and insulin, therefore protecting against metabolic syndrome.

Since oxidative stress can cause damage to lipids, protein and DNA, TBA-RS is an important parameter that is indicative of lipid peroxidation and plasma membrane damage[25–27]. Furthermore, the determination of total sulfhydryl and carbonyl protein contents serves as an indication of increased protein fragmentation and aggregation, and thereby protein damage, as a result of changes in protein structures, which could make proteins more susceptible to degradation by proteasomes[25–27].

Our results show that HFD increased TBA-RS and protein carbonyl content levels, and decreased the total sulfhydryl content; the HIIT protocol, when used alone or in association with L-Carnitine supplementation, was able to reverse these alterations in the plasma of obese rats. These data suggest that the HFD promoted lipoperoxidation and protein damage, while the HIIT protocol and L-Carnitine supplementation provided protective effects against lipids and protein damage by reversing the alterations in these parameters in the blood of obese rats.

In 2018, Lima *et al.* evaluated the effects of moderate-intensity continuous training (MICT) and HIIT protocols on the alterations in oxidative stress parameters caused by HFD. Authors reported that a HFD increased TBA-RS levels and protein carbonyl content and decreased total sulfhydryl content in the plasma of obese rats[2]. They also identified that the HIIT protocol prevented the increase in TBA-RS levels and totally reversed the increase in protein carbonyl content, but did not correct the alteration in total sulfhydryl content[2]. Also corroborating the findings of our investigation, a study carried out in 2015 identified an increase in TBA-RS levels in the blood of male rats fed on a high-fat diet, which were partially reversed in animals submitted to physical exercise (treadmill running protocol for 60 min, 5 times a week, for 8 weeks)[28].

With regard to antioxidant enzyme activities, our results show that the HFD reduced CAT and GSH-Px activities, but did not alter SOD activity. The HIIT protocol, in isolation or associated with L-Carnitine supplementation, was able to prevent the alteration in CAT activity, but not in GSH-Px activity. We suggest that a longer exercise time, associated with L-Carnitine supplementation, could increase GSH-Px activity, thereby reversing the decrease caused by HFD. With regard to SOD activity, our results show that both L-Carnitine and the HIIT protocol, alone or in association with L-Carnitine, enhanced this enzyme's activity in the erythrocytes of obese rats.

The decreases in CAT and GSH-Px activities suggest that the HFD promoted an increase in the production of ROS, such as hydrogen peroxide, and a consequent depletion in the activity of these antioxidant enzymes. In contrast, the significant increase in SOD activity, in response to HIIT, may suggest that this protocol enhanced the production of the superoxide radical, since several studies show that high or exhausting intensities of exercise may lead to an increase in the production of ROS,

due to the greater need for oxygen consumption and supply, which may corroborate our findings[25,29].

However, several studies demonstrate that chronic and moderate intensity physical exercises promote a reduction in ROS production, a decrease in oxidative stress, or an increase in antioxidant enzymes and repair processes, a fact that may justify the increase in CAT and GSH-Px activities found in the blood of HIIT protocol experimental groups[30].

A study carried out by Lima *et al*. (2018) also identified HFD-induced decreases in CAT and GSH-Px activities that were prevented by a HIIT protocol[2]. In addition, they also reported that the HIIT protocol increased the activity of SOD, probably due to a greater removal of superoxide radical and the formation of hydrogen peroxide, in agreement with our data[2].

In turn, the effects of the HIIT protocol and L-Carnitine supplementation on inflammatory parameters in the serum of obese rats were also analyzed. Our data did not show any significant differences between experimental groups with regard to serum concentrations of IL-6 and IL-1β. Results for TNF-α were inconclusive, which made statistical analysis of the data impossible. Although our data did not identify significant changes in inflammatory parameters, several studies indicate that circulating level of cytokines and acute phase proteins associated with inflammation are elevated in obese patients[3]. It is also known that the excess of adipose tissue, found in obesity, accelerates the production of adipokines, such as IL-1β, IL-6 and TNF-α, with a huge impact on body functions, such as metabolism and lipid and body homeostasis, insulin resistance, and energy balance, etc[3]. We speculate that a longer period of lipid intake would cause inflammation with changes in these parameters in rats. Additionally, skeletal muscle has been associated with the synthesis and release of cytokines during the process of muscle contraction, contributing to the balance of pro and anti-inflammatory cytokines. As a consequence, physical exercise may be an alternative to reduce pro-inflammatory cytokines in the plasma[31].

Finally, this study evaluated the effects of the HIIT protocol and L-Carnitine supplementation on biochemical parameters, such as glucose, insulin, triglycerides, HDL-c, LDL-c and total cholesterol levels, in the serum of obese rats. Initially, we evaluated blood glucose levels in the serum of obese rats. Our data show that HFD

alone was not able to alter this parameter. However, when we analyzed the other experimental groups, we found that the HIIT protocol, alone and in association with L-Carnitine supplementation, was able to reduce blood glucose. With regard to the insulin levels in the serum of rats, HFD increased this parameter and HIIT protocol reversed the alteration in this parameter. Our results show that chronic ingestion of a HFD causes insulin resistance and suggest that prolonged periods of ingestion would probably cause hyperglycemia.

With regard to the lipid profile, our study shows that the HIIT protocol decreased total cholesterol levels, while HFD decreased the HDL-c levels, which was reversed by the HIIT protocol, in association with L-Carnitine, in the serum of obese rats. With regard to the LDL-c levels, our data show that the HFD alone and when associated with L-Carnitine significantly enhanced this parameter and that the HIIT protocol, in isolation and in association with L-Carnitine supplementation, was able to reverse this alteration. Our data show no significant differences in triglyceride levels between groups.

In a study carried out by Yokota *et al*. (2009), mice were fed on normal diet or high fat diet (HFD) for 8 weeks[32]. Animals from the HFD group presented significantly increased fasting blood glucose, plasma insulin and triglyceride levels, but no difference was found in total cholesterol[32]. Zambon *et al*. (2009) investigated the effects of two different types of swimming exercise on adiposity and lipid profile in rats with exogenous obesity[33]. Their results showed that the high fat diet increased serum concentrations of triglycerides, total cholesterol and high-density lipoprotein (HDL). In addition, Wang *et al*. (2017) demonstrated that rats submitted to the HIIT protocol 5 days/week for 8 weeks presented significant decreases in triglycerides, and total cholesterol and LDL-c levels, when compared to sedentary groups, but no differences in HDL-c between groups[1]. Aparicio *et al.* (2015) investigated whether interval aerobic training, combined with strength-endurance exercise, improves metabolic markers, when used together with caloric restriction in Zucker rats. Authors found a decrease in fasting glucose, serum insulin, total cholesterol, LDL and HDL-c in the exercise groups, compared to the sedentary groups, corroborating our data showing that the HIIT protocol is an important alternative for preventing changes in the glycemic and lipid profile caused by HFD[5].

In summary, our findings show that the HFD alters antioxidant defenses and biochemical parameters in the blood of rats, inducing lipoperoxidation, oxidative damage to proteins, alterations in antioxidant enzyme activities and modulating glucose, insulin, HDL-c, LDL-c and total cholesterol. In turn, we demonstrated that the HIIT protocol, when used alone and in association with L-Carnitine, is an important tool for combating the damage caused by obesity, since this training protocol was able to reverse most of the deleterious effects of HFD ingestion in the blood of obese rats, thereby, protecting against metabolic syndrome.

Conflict of interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

Acknowledgements

This work was supported by grants from Fundo de Apoio à Pesquisa da Universidade da Região de Joinville, Fundo de Apoio à Pesquisa de Santa Catarina (FAPESC) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

We thank statistics teacher, Dra. Elisa Henning (Udesc/Joinville), for statistical analysis.

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Table 1 – Serum concentrations of interleukin 6 (IL-6), interleukin 1-β (IL-1β) and tumor necrosis factor α (TNF-α) in obese rats.

IL-6 (Interleukin 6) (pg/mL)	ND-UNT	HFD-UNT	HFD-UNT-C	HFD-HIIT	HFD-HIIT-C
	0.12 ± 0.02	0.14 ± 0.06	0.10 ± 0.01	0.11 ± 0.03	0.11 ± 0.03
IL-1β (Interleukin 1β) (pg/mL)	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.02
Tumor Necrosis Factor α (TNF-α) (pg/mL)	*	*	*	*	*

Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine.

Fig.1. HIIT Protocol

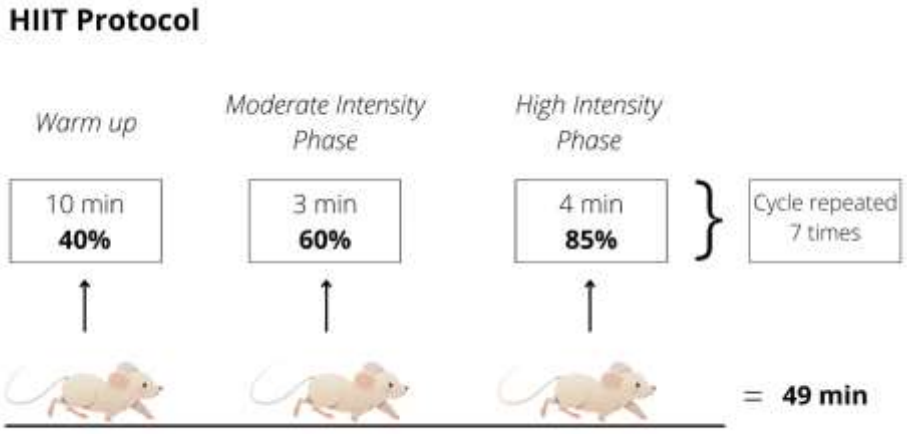
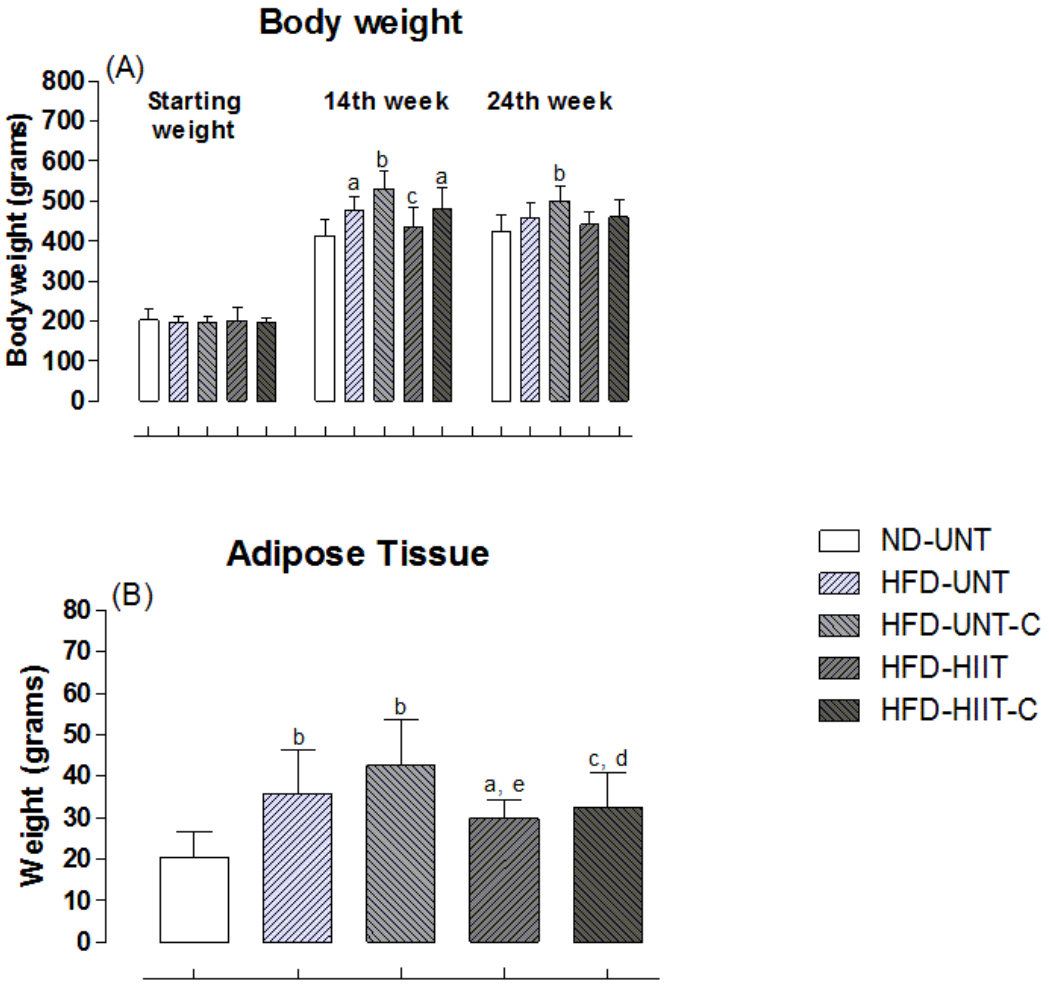
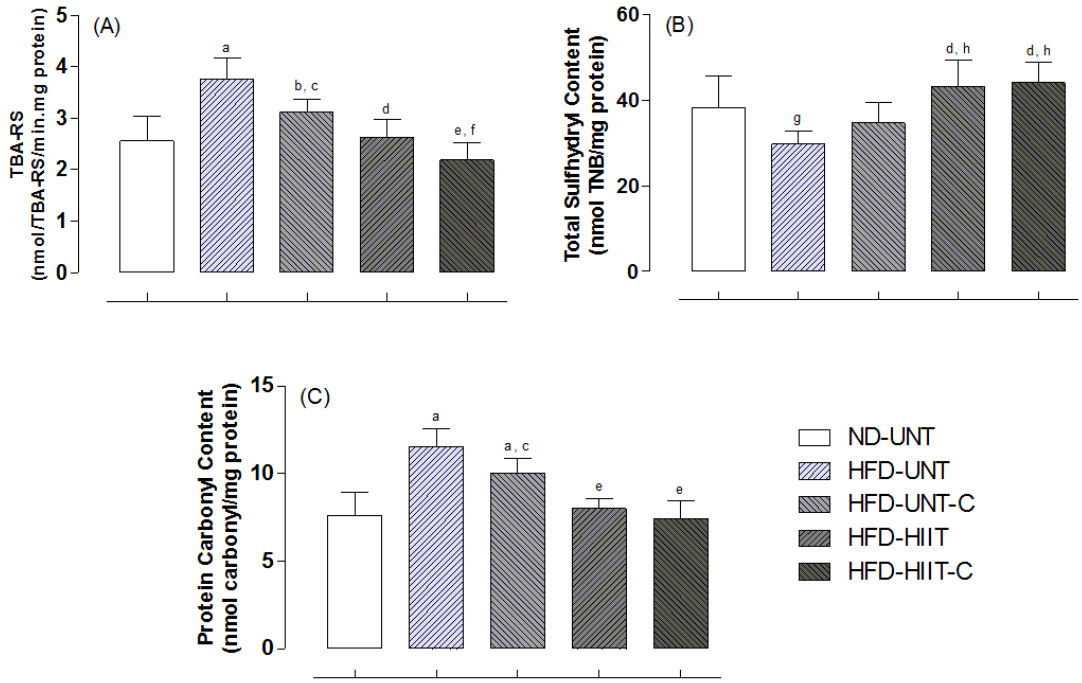


Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols.



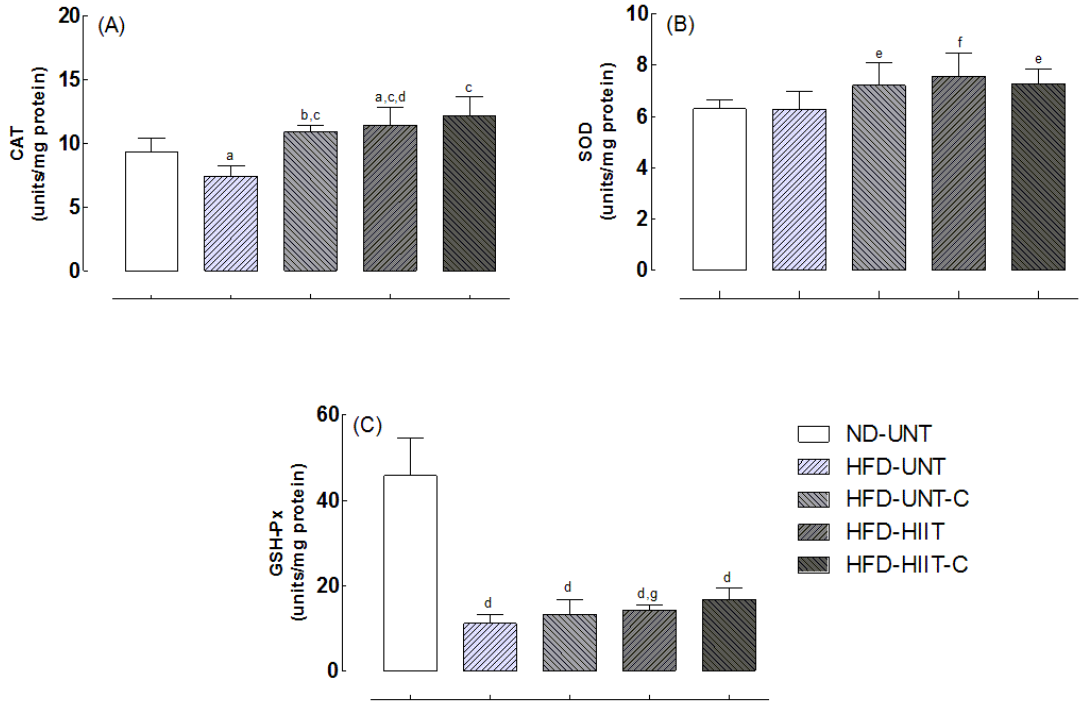
Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* ND-UNT; [b], $p<0.001$ *vs* ND-UNT; [c], $p<0.05$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-UNT; [e], $p<0.01$ *vs* HFD-UNT-C.

Fig.3. Oxidative stress biomarkers in the plasma of obese rats submitted to experimental protocols.



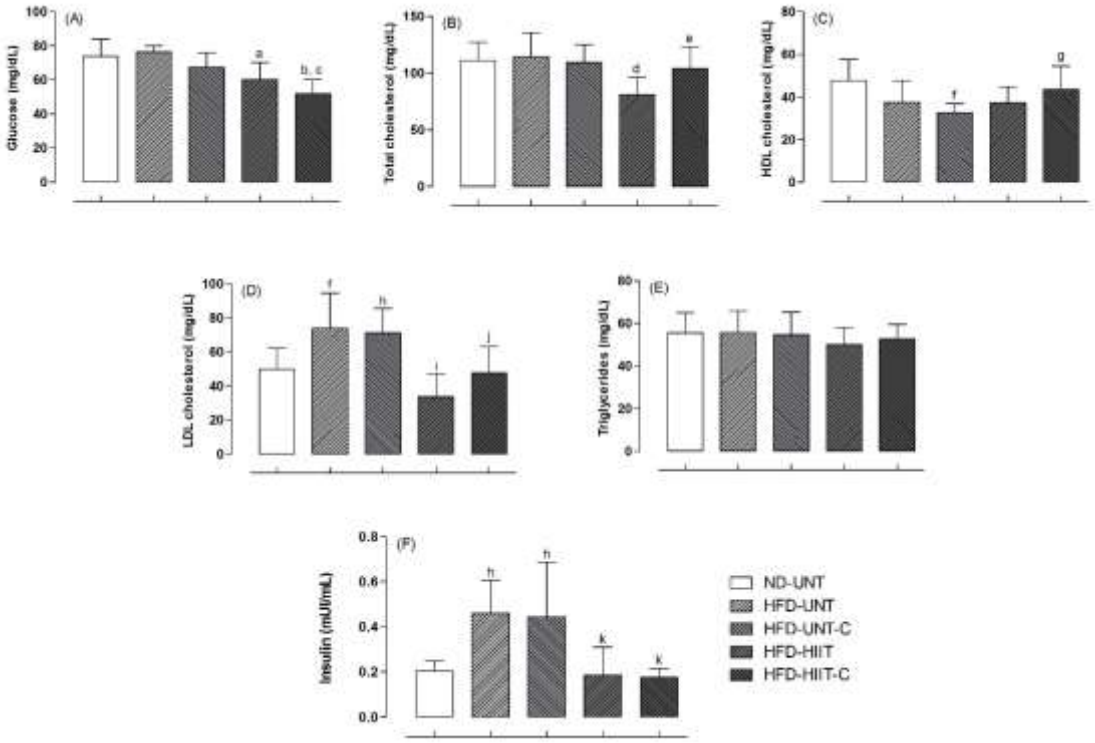
Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.001$ *vs* ND-UNT; [b], $p<0.05$ *vs* ND-UNT; [c], $p<0.01$ *vs* HFD-UNT; [d], $p<0.001$ *vs* HFD-UNT; [e], $p<0.001$ *vs* HFD-UNT and HFD-UNT-C; [f], $p<0.01$ *vs* HFD-HIIT; [g], $p<0.01$ *vs* ND-UNT; [h], $p<0.01$ *vs* HFD-UNT-C.

Fig.4. Effects of the high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the blood of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.001$ *vs* ND-UNT; [b], $p<0.05$ *vs* ND-UNT; [c], $p<0.001$ *vs* HFD-UNT; [d], $p<0.001$ *vs* ND-UNT; [e], $p<0.05$ *vs* ND-UNT and HFD-UNT; [f], $p<0.01$ *vs* ND-UNT and HFD-UNT; [g], $p<0.05$ *vs* HFD-UNT.

Fig.5. Effects of high-intensity interval training protocol and L-Carnitine supplementation on glucose, lipid profile and insulin parameters in the serum of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.01 *vs* ND-UNT and HFD-UNT; [b], p<0.01 *vs* ND-UNT and HFD-UNT-C; [c], p<0.001 *vs* HFD-UNT; [d], p<0.01 *vs* ND-UNT, HFD-UNT and HFD-UNT-C; [e], p<0.05 *vs* HFD-HIIT; [f], p<0.01 *vs* ND-UNT; [g], p<0.01 *vs* HFD-UNT-C; [h], p<0.05 *vs* ND-UNT; [i], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [j], p<0.01 *vs* HFD-UNT and HFD-UNT-C; [k], p<0.05 *vs* HFD-UNT.

Legends to figures

Table 1. Serum interleukin 6 (IL-6), interleukin 1-β (IL-1β) and tumor necrosis factor α (TNF-α) concentrations in obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine.

Fig.1. HIIT Protocol.

Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* ND-UNT; [b], $p<0.001$ *vs* ND-UNT; [c], $p<0.05$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-UNT; [e], $p<0.01$ *vs* HFD-UNT-C.

Fig.3. Oxidative stress biomarkers in the plasma of obese rats submitted to experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.001$ *vs* ND-UNT; [b], $p<0.05$ *vs* ND-UNT; [c], $p<0.01$ *vs* HFD-UNT; [d], $p<0.001$ *vs* HFD-UNT; [e], $p<0.001$ *vs* HFD-UNT and HFD-UNT-C; [f], $p<0.01$ *vs* HFD-HIIT; [g], $p<0.01$ *vs* ND-UNT; [h], $p<0.01$ *vs* HFD-UNT-C.

Fig.4. Effects of high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the blood of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.001$ *vs* ND-UNT; [b], $p<0.05$ *vs* ND-UNT; [c], $p<0.001$ *vs* HFD-UNT; [d], $p<0.001$ *vs* ND-UNT; [e], $p<0.05$ *vs* ND-UNT and HFD-UNT; [f], $p<0.01$ *vs* ND-UNT and HFD-UNT; [g], $p<0.05$ *vs* HFD-UNT.

Fig.5. Effects of the high-intensity interval training protocol and L-Carnitine supplementation on glucose, lipid profile and insulin parameters in the serum of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-

UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.01 *vs* ND-UNT and HFD-UNT; [b], p<0.01 *vs* ND-UNT and HFD-UNT-C; [c], p<0.001 *vs* HFD-UNT; [d], p<0.01 *vs* ND-UNT, HFD-UNT and HFD-UNT-C; [e], p<0.05 *vs* HFD-HIIT; [f], p<0.01 *vs* ND-UNT; [g], p<0.01 *vs* HFD-UNT-C; [h], p<0.05 *vs* ND-UNT; [i], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [j], p<0.01 *vs* HFD-UNT and HFD-UNT-C; [k], p<0.05 *vs* HFD-UNT.

6.3 Artigo:

PROTECTIVE EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING AND L-CARNITINE SUPPLEMENTATION AGAINST OXIDATIVE STRESS IN THE HEART, LIVER AND KIDNEYS OF OBESE RATS

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ABSTRACT

This study evaluated the protective effects of high-intensity interval training (HIIT) and L-Carnitine supplementation against oxidative stress parameters in the heart, liver and kidneys of obese rats. Animals were divided into five groups: Normal Diet-Untrained (ND-UNT), High-Fat Diet-Untrained (HFD-UNT), High-Fat Diet-Untrained + L-Carnitine (HFD-UNT-C), High-Fat Diet + HIIT (HFD-HIIT) and High-Fat Diet + HIIT + L-Carnitine (HFD-HIIT-C). To induce obesity, animals in the HFD groups were fed on a high-fat diet for 14 weeks, while animals in the ND groups received a standard diet. Animals in the HFD-UNT-C and HFD-HIIT-C groups received L-Carnitine by gavage as soon as the HIIT protocol started. The HIIT protocol was administered with a frequency of 5 days per week, while animals in the UNT group walked at 40% intensity, twice a week. After the end of the 10th week of training, animals were sacrificed by decapitation and organs were separated for subsequent measurement of the antioxidant activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), levels of thiobarbituric acid reactive substances (TBA-RS), total sulfhydryl content and protein carbonyl content. Results showed that the HFD promoted oxidative stress, causing lipoperoxidation, protein damage and alterations in the activities of antioxidant enzymes in all organs. Co-administration of the HIIT protocol, by itself and, sometimes, when associated with L-Carnitine, prevented these alterations.

Keywords: Obesity; Oxidative stress; L-Carnitine; Heart; Liver; Kidney; High-Intensity Aerobic Training.

1 INTRODUTION

Obesity is considered a disease of pandemic proportions, in view of its increasing prevalence worldwide. The disease is also multifactorial, being caused by a close relationship between factors that contribute to its development, such as genetic susceptibility, associated with environmental and social factors[1]. An excessive accumulation of body fat characterizes obesity, where this fat accumulation results from an imbalance in energy, in which the intake of energy exceeds expenditure. This results in the conversion of excess energy into triacylglycerols and free fatty acids that, when stored in the adipose tissue, expand, thereby increasing body fat and, consequently, the body mass index (BMI), one of the parameters used for the diagnosis of obesity[2,3].

Classified as an independent risk factor for cardiovascular and chronic kidney disease, obesity is one of the leading causes of diseases such as dyslipidemia, type 2 diabetes, and hypertension[4,5]. Since the liver is involved in maintaining lipid homeostasis and energy balance, obesity is also closely related to a higher propensity to develop fatty liver and progress to complications such as nonalcoholic steatohepatitis (NASH), cirrhosis, and liver cancer[6].

The association between obesity and oxidative stress has been suggested to be key to the development of metabolic disorders. According to Rani *et al.* (2016), the damage caused by obesity at the cellular level comes from oxidative stress, which results in the excessive production of free radicals and reactive oxygen species (ROS)[7]. The resulting oxidative imbalance increases the propensity for the development of a number of heart diseases, including cardiac hypertrophy, heart failure, ischemia-reperfusion injury and diabetic cardiomyopathy[8]. Studies have shown that obese patients are more susceptible to greater oxidative damage, due to the depletion of the antioxidant defense system, including the enzymatic and non-enzymatic systems[9].

In order to minimize deleterious effects at the cellular and systemic level, complementary substances, such as L-carnitine, have been proposed for use in the

adjuvant treatment of obesity and cardiovascular diseases, due to their potential ability to reduce the oxidative stress generated by these conditions[10]. Accordingly, studies have reported that L-carnitine, which is an ammonium compound, could be an importantly in post-workout muscular recovery[11]. In addition, L-carnitine has also been demonstrated to aid in preserving the cell membrane and DNA from the damage caused by oxidative stress, band is therefore considered to be an essential nutrient that is found in some foods, and especially in red meat[11]. Furthermore, this compound has been shown to exert antioxidant and protective activities against ROS[11]. The protective effects of L-carnitine in the liver, kidneys and cardiovascular system have been demonstrated in several studies. This therapeutic mechanism is observed especially when any toxicity reaches the liver cells, and levels of carnitine decrease[12]. When this occurs, the transport of fatty acids is disrupted due to a metabolic imbalance and numerous physiological functions are disrupted, supporting the notion that supplementation with exogenous L-carnitine could be used to combat such toxicity[12].

In addition to oral supplementation strategies with substances that can help modulate metabolism, physical exercise could contribute to such modulation. High intensity interval training (HIIT) is one of the most popular exercises, since it promotes high energy expenditure in less time, and is seen as easy to include in everyday activities and as an alternative to moderate intensity continuous training (MICT)[13]. Among the benefits of HIIT, its capacity to reduce cardiovascular risk is the most important, followed by improvements in microvascular endothelial function[14]. Furthermore, this type of training is recognized to provide protection against ROS[14]. HIIT has been shown to be closely associated with blood pressure reduction and vasodilating effects, when compared with less intense exercise[15]. Additionally, protection of kidney function has also been correlated with HIIT protocol practice[15], together with numerous benefits observed in studies with rats, compared with sedentary groups of rats[15].

As obesity and oxidative stress cause the functional impairment of several organs and systems and studies indicate benefits of HIIT protocol administration and L-Carnitine supplementation in obese patients, this study aimed to evaluate the protective effects of HIIT and L-Carnitine supplementation against oxidative stress parameters in the heart, liver and kidneys of obese rats.

2 MATERIALS AND METHODS

2.1 Animals and Reagents

Sixty-day-old male Wistar rats from the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil, were used in the experiments. Before the experiments, animals were accommodated and acclimatized for 7 days to adapt to their new environment. Animals were kept in rooms with a 12h light/dark cycle, with the temperature maintained between 20-22ºC and free access to food and water. The 12h light/dark cycle was inverted for better use of the animals' active period for training. The animals were kept in cages with a maximum number of four per cage; box exchange was performed every 2 days. Animal care was carried out in accordance with Law Nº. 11794 (October 8, 2008), and other regulations applicable to the use of animals in teaching and/or research, especially the Normative Resolutions of the National Council for the Control of Animal Experimentation – CONCEA[16,17]. Room lighting, accommodation and nutrition used followed the recommendations of the Guide for the Care and Use of Laboratory Animals[18].

For the *in vivo* experiments, animals were divided, in equal numbers, into the following groups:

11) Normal Diet-Untrained (ND-UNT, n = 8);
12) High-Fat Diet-Untrained (HFD-UNT, n = 8);
13) High-Fat Diet - Untrained + L-Carnitine (HFD-UNT-C, n = 8);
14) High-Fat Diet + HIIT (HFD-HIIT, n = 8);
15) High-Fat Diet + HIIT + L-Carnitine (HFD-HIIT-C, n = 8).

The experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 012/2017. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2 Experimental Protocols

2.2.1 Dietary induction of obesity

The animals in the experimental groups (HFD) were fed with a high-fat diet, composed of 20% of calories from carbohydrates, 20% of proteins and 60% of lipids (Prag Soluções Biosciences, Jaú, São Paulo-SP), for fourteen weeks, in order to induce the condition of obesity. The animals in the control group (ND) were treated with a standard diet (70% carbohydrates, 20% proteins and 10% lipids) (Quimtia, Curitiba, Paraná, Brazil). All animals received water *ad libidum*.

2.2.2 L-Carnitine supplementation

The animals in the experimental groups, HFD-UNT-C and HFD-HIIT-C, received L-carnitine supplementation by gavage, at a concentration of 300 mg/ kg of body mass *per* day, while animals from the experimental groups ND-UNT, HFD-UNT and HFD-HIIT, received saline by gavage once a day.

2.2.3 High-Intensity Interval Training protocol

A maximum effort tolerance test was applied in order to define the animals' maximum running speed. These data served as the parameter for the prescription of the training speeds of the HIIT protocol. The maximum effort test was performed at three time points; at the beginning, after four weeks and in the eighth week of the training protocols. Following the protocol of Ferreira *et al*. (2007), the test consisted of running on a treadmill (model KT-4000, IMBRAMED), with an inclination of 20 degrees, with an initial speed of 6 m/min with an increase of 3 m/min every three minutes, until the exhaustion of the animal (visible fatigue)[19]. Once the maximum speed was found, distance and speed were computed to calculate training intensity.

The HIIT protocol was administered five days a week, with a 20° inclination of the treadmill and intensities that were defined from the exercise tolerance test[19]. HIIT was achieved with three minutes at 60% maximum intensity followed by four minutes at 85% of the maximum test speed[19]. This cycle was repeated seven times, totaling 49 minutes of training (Fig.1).

The animals of the untrained group (UNT) performed a 40% intensity walk, twice a week, in order to maintain the animals' ability to walk for subsequent physical tests.

2.2.4 Sample preparation

After the end of the 10th week of training (24 weeks of experiment), the animals were fasted for 12 hours before sacrificing (at 48 hours after the last training session) by decapitation, without anesthesia. Anesthetics were not used as these can interfere with the determination of oxidative parameters, and the heart, liver and kidney were separated and prepared, according to the technique used[20,21].

2.3 Biochemical studies

2.3.1 Analysis of oxidative stress parameters

2.3.1.1 Catalase assay (CAT)

CAT activity was determined by the method of Aebi[22]. The method used is based on the disappearance of hydrogen peroxide (H_2O_2) at 240 nm in a reaction medium containing 25µL of sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. The absorbance was counted every 10 seconds for 1 minute and 40 seconds, at 240 nm using a spectrophotometer (UV-vis Shimadzu spectrophotometer). One CAT unit is defined as 1 µmol of H_2O_2 consumed per minute and the specific activity was calculated as CAT units/mg protein.

2.3.1.2 Superoxide dismutase (SOD) assay

The activity of SOD was assayed by the method described by Marklund[23], using a process highly dependent on superoxide ($O_2^{\bullet -}$), which is a substrate for SOD. The sample (15 µL) was added to 215 µL of a mixture containing 50 µM Tris buffer, 1 µM EDTA, pH 8.2, and 30 µM CAT. Subsequently, 20 µL of pyrogallol were added and the absorbance was measured every 30 seconds for 3 minutes at 420 nm using a UV-vis Shimadzu spectrophotometer. Inhibition of the auto-oxidation of pyrogallol occurs in the presence of SOD, the activity of which can be tested indirectly spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity was reported as SOD units/mg protein.

2.3.1.3 Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel[24], using *tert*-butyl hydroperoxide as substrate. The decomposition of NADPH was monitored in a

spectrophotometer at 340 nm for 3 minutes and 30 seconds using a UV-vis Shimadzu spectrophotometer. The medium contained 90 µL of each sample, 800 µL of buffer, 20 µL of 2.0 mM GSH, 30 µL of 0.15 U/mL GSH reductase, 10 µL of 0.4 mM azide and 10 µL of 0.1 mM NADPH. The absorbance was counted every 10 seconds for 1 minute and 30 seconds. Subsequently, 50 µL of 0.5 mM *tert*-butylhydroperoxide was added and the absorbance was read for 2 more minutes. One GSH-Px unit is defined as 1 µmol of NADPH consumed per minute and the specific activity was reported as GSH-Px units/mg of protein.

2.3.1.4 Total sulfhydryl content

The total sulfhydryl content was determined, according to the method described by Aksenov and Markersbery[25], which is based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, generating a yellow derivative (TNB) that is measured spectrophotometrically at 412 nm. For the assay, 50 µL of homogenized sample were added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction was started by the addition of 30 µL of 10 mM DTNB and incubated for 30 minutes at room temperature in the dark. Analyses of a blank (DTNB absorbance) were also performed. The results were expressed as nmol TNB/mg protein.

2.3.1.5 Thiobarbituric acid reactive substances (TBA-RS) measurement

TBA-RS were determined according to the method described by Ohkawa *et al*.[26]. The TBA-RS methodology measures malondialdehyde (MDA), a product of lipoperoxidation, generated mainly by $OH^{\cdot}$ radicals. TBA-RS were determined by the absorbance at 535 nm. At first, sample homogenized in 1.15% KCl was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid, and heated in a boiling water bath for 60 min. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was exposed to the same treatment as that of the supernatants. The results were expressed in nmol of MDA/mg protein.

2.3.1.6 Protein carbonyl content

Protein carbonyl content was assayed by a method described by Reznick & Packer (1994)[27], based on the reaction of protein carbonyls with dinitrophenylhydrazine to form dinitrophenylhydrazone, a yellow compound that is measured spectrophotometrically at 370 nm. Initially, 200 µL of sample homogenized were added to plastic tubes containing 400 µL of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). Samples were kept in the dark for 1 h and vortexed every 15 min. Subsequently, 500 µL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 14 000 rpm for 3 min and the resulting supernatant was excluded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed and centrifuged at 14 000 rpm for 3 min. The supernatant was discarded and the pellet re-suspended in 600 µL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), pre-vortexed and incubated at 60ºC for 15 min. Thus, samples were centrifuged at 14 000 rpm for 3 min and the supernatant was used to measure absorbance in a quartz cuvette. Results were reported as carbonyl content (nmol/mg protein).

2.4 Statistical analysis

Statistical analysis was performed using the R software version 4.0.3 with the aid of the Car and Desctools packages[28–30]. A multiple linear regression model was applied, followed by Analysis of Variance (ANOVA) for unbalanced data, in order to verify whether there were differences in the outcome variables. The normality assumption of the model residuals was verified using the Shapiro-Wilk normality test. Homoscedasticity was assessed using graphs and Levene's test. To identify differences between groups, the Duncan's test was used for multiple comparisons.

For the statistical analysis of body weight and the adipose tissue of rats, ANOVA with repeated measures was applied to analyze the relationship between the independent variables and the outcome variable over the period of analysis. To verify the sphericity, the Mauchly test was applied and the Greenhouse-Gueisser correction was applied, if necessary. For the multiple comparison tests, the *t* test with Bonferroni adjustment was applied. To analyze the association between the independent variables and the weight of adipose tissue in the final week, a single-way ANOVA was applied. For multiple comparisons, the Duncan´s test was used.

Values of $p<0.05$ were considered significant. Results are expressed as means ± SD for eight independent experiments (animals) performed in duplicate.

3 RESULTS

3.1 Effects of the high fat-diet, the high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats

We initially verified the effects of the high fat-diet, the high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats, with the aim to validate our obesity model. Fig.2A shows that there were no significant differences between the experimental groups, for the starting weight of the rats and shows that, in the 14th week, the HFD increased body weight in the HFD-UNT, HFD-UNT-C and HFD-HIIT-C groups, when compared to the ND-UNT group. Fig.2A also shows that the HIIT protocol, when used alone or in association with L-carnitine supplementation, was able to reverse the increase in this parameter by the 24th week.

With regard to adipose tissue, Fig.2B shows that the HFD increased this parameter in the HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C groups, when compared to the ND-UNT groups, and that the HIIT protocol, when used alone or in association with L-Carnitine supplementation, decreased this parameter, when compared to the HFD-UNT-C group.

3.2 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on parameters of oxidative stress in the heart, liver and kidneys of obese rats

We initially verified the effects of the high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on TBA-RS, total sulfhydryl content and protein carbonyl content in the heart, liver and kidneys of obese rats. Table 1 shows that neither the HFD, the HIIT protocol nor L-Carnitine supplementation altered TBA-RS levels in the heart and kidneys of obese rats. However, Table 1 shows that the HFD increased this parameter in the liver in the HFD-UNT and HFD-UNT-C groups,

when compared with the ND-UNT group, and that the HIIT protocol, when administered alone and in association with L-Carnitine, reversed this alteration.

In addition, Table 1 shows that the HFD decreased the total sulfhydryl content in the heart and kidneys of the HFD-UNT and HFD-UNT-C groups, when compared to the ND-UNT group. The HIIT protocol, when used alone or in association with L-Carnitine, was able to reverse this alteration. In contrast, in the liver, the HFD alone did not alter this parameter, but the HIIT protocol, alone or in association with L-Carnitine supplementation, significantly increased total sulfhydryl content, when compared to the ND-UNT, HFD-UNT and HFD-UNT-C groups.

Table 1 also shows that there were no significant differences between experimental groups in the carbonyl protein content in the heart and liver of obese rats, while in the kidneys, the HFD increased the carbonyl protein content in the HFD-UNT and HFD-UNT-C groups, when compared to the ND-UNT group. The HIIT protocol, when used alone and in association with L-Carnitine supplementation, reversed this increase.

3.3 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on the enzymatic antioxidant system in the heart, liver and kidneys of obese rats

We also investigated the effects of the high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the heart, liver and kidneys of obese rats. Fig.3A shows that the HFD enhanced CAT activity in the heart of the HFD-UNT and HFD-UNT-C groups, when compared to the ND-UNT group, and that the HIIT protocol, when used alone and in association with L-Carnitine supplementation, reversed this increase. With regard to the liver, Fig.3A shows that the HFD increased CAT activity in the HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C groups, when compared to the ND-UNT group. On the other hand, there were no significant differences between experimental groups for CAT activity in the kidney (Fig.3A).

For SOD activity, Fig.3B shows that there were no significant differences in SOD activities between the experimental groups in the liver of obese rats, while in the kidneys, the HFD increased SOD activity in the HFD-UNT and HFD-UNT-C groups,

when compared to the ND-UNT group. The HIIT protocol, when administered alone or in association with L-Carnitine supplementation, reversed this increase. With regard to the heart, Fig.3B shows that the HFD alone did not alter this parameter, but the HIIT protocol, when used alone or associated with L-Carnitine, increased SOD activity in the heart, when compared to the ND-UNT group.

As can be seen in Fig.3C, the HFD decreased the GSH-Px activities in the liver and kidneys of the HFD-UNT and HFD-UNT-C groups, when compared to the ND-UNT group. The HIIT protocol, when used alone and when associated with L-Carnitine supplementation, did not reverse this alteration in the liver and partially reversed this decrease in the kidney, when compared to ND-UNT, HFD-UNT and HFD-UNT-C. On the other hand, Fig.3C shows that the HFD increased GSH-Px activity in the heart of the HFD-UNT and HFD-UNT-C groups, when compared to the ND-UNT group. The HIIT protocol, when administered on its own or in association with L-Carnitine, reversed this increase.

4 DISCUSSION

In the present study, we investigated whether a HIIT protocol and L-Carnitine supplementation could prevent or protect against oxidative stress in the heart, liver and kidneys of obese rats. Firstly, our results confirmed that the HFD increased body weight and promoted adipose tissue gain in the experimental groups, validating our methodology. Our results also showed that the HFD promoted alterations in the activities of antioxidant enzymes in the heart, liver and kidneys, damaged proteins in the heart and kidneys, and incurred lipoperoxidation in the liver of obese rats. Furthermore, we showed that the HIIT protocol, when used alone and, sometimes, when associated with L-Carnitine supplementation, was able to reverse and even improve some of these alterations.

The imbalance between antioxidant defense systems and ROS results in oxidative stress that makes cellular structures and different molecules more susceptible to the deleterious effects of free radicals[31]. Obese individuals are more vulnerable to oxidative injury, as HFD alters oxygen metabolism, contributing to oxidative reactions in double-bonded fatty acid molecules and consequent lipid peroxidation. This causes the breakdown of cell membrane lipids into low molecular

weight fragments, such as hydrocarbons, epoxides and ketones, in addition to generating malondialdehyde (MDA), considered the most important byproduct of lipoperoxidation, which can be measured to quantitate the extent of oxidative damage in various organs[31,32].

According to our results, the HFD alone did not alter TBA-RS levels in the heart and kidneys of the animals; this may be due to the exposure time to the HFD, which may not have been long enough to promote the oxidative stress that would significantly affect this parameter. In contrast, in the liver, the HFD increased TBA-RS levels in the HFD-UNT and HFD-UNT-C groups, and the HIIT protocol, when used alone and when associated with L-Carnitine supplementation, reversed this change. A study carried out by Vieira-Souza *et al*. (2021) investigated the effects of short-term HIIT on oxidative stress markers and muscle damage in rats and found reduced levels of TBA-RS in the liver tissue of animals that underwent HIIT, suggesting a protective effect against the oxidative damage caused by obesity, and corroborating our findings[33].

The protective effect of L-Carnitine against lipid peroxidation, found in our study, may be explained by its fatty acid oxidation-promoting action, which may reduce the availability of fatty acids for lipid peroxidation[34]. According to our results, L-Carnitine supplementation is more effective when associated with HIIT, suggesting that a higher energy demand resulted in a better efficiency of L-Carnitine in the transport of fatty acids and consequent oxidation. Several studies have reported on the protective role of L-Carnitine in cardiac cells, where supplementation has been shown to improve cardiac energy homeostasis, thereby mitigating oxidative stress and hypoxic cell damage and, consequently, reducing cell death[35]. Furthermore, in addition to the antioxidant activity of L-Carnitine, supplementation with this amino acid can increase ATP production, enhancing the cellular synthesis of antioxidant enzymes and protecting these enzymes from further peroxidative damage[36].

In addition to causing lipid damage, oxidative stress can cause protein damage, favoring aggregation of proteins susceptible to proteolytic degradation[37]. Protein damage can be assessed by measuring total sulfhydryl content and protein carbonyl content. With regard to these parameters, our data show that, in the heart and kidneys, the HFD decreased total sulfhydryl content, while the HIIT protocol, when used alone and in association with L-Carnitine, prevented this alteration. In contrast, in the liver,

the HFD did not alter this parameter, but the HIIT protocol, alone or in association with L-Carnitine supplementation, significantly increased total sulfhydryl content. These data suggest that the HFD promoted protein damage in the heart and kidneys of obese rats, and that the HIIT protocol exerts a protective effect against this damage.

With regard to the carbonyl protein content, our data show that there were no significant differences between experimental groups in the heart and liver of obese rats. In contrast, in the kidneys, the HFD significantly enhanced this parameter, promoting protein damage; importantly, the HIIT protocol, when used alone and associated with L-Carnitine supplementation, reversed this alteration. Noeman *et al.* (2011), however, reported increased levels of carbonyl protein content in the heart, liver and kidney tissues of obese rats fed on a HFD, contributing to cellular damage[38]. The differences between our results and the study carried out by Noeman *et al.* (2011) may be linked to a shorter time of exposure to the HFD, as the animals in the experimental groups (HFD) of our study were fed with a high-fat diet for fourteen weeks, while the aforementioned study administered the HFD for sixteen weeks[38].

The antioxidant enzymes, SOD, CAT and GSH-Px, are the primary defense against the ROS that are generated during exercise[39]. Mitochondrial metabolism is also affected in obesity, favoring the production of ROS and therefore increasing oxidative stress[40]. The CAT enzyme is present in abundance in peroxisomes and neutralizes H_2O_2, acting as a catalyst in the reduction reaction of hydrogen peroxide to water and molecular oxygen[41]. Its increase may be related to an attempt to minimize H_2O_2 levels and the formation of hydroxyl radicals caused by obesity, which attack structures such as proteins, lipids, and DNA that are susceptible to damage[41].

In the present study, the HFD increased CAT activity in the heart of obese rats and HIIT, alone or in association with L-Carnitine, was able to reverse this increase. In the liver, the HFD increased CAT activity in the HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C groups, indicating that neither HIIT nor L-Carnitine were able to reverse this change, possibly due to high production of ROS. On the other hand, in the kidney, there were no significant differences between experimental groups for CAT activity. Conversely, Noeman *et al.* (2011), found a decrease in CAT activity in the liver of rats fed on a HFD and no significant differences in this activity in the heart and liver of experimental groups[38]. Yuan *et al*. investigated the effects of HIIT and MICT on

oxidative stress parameters in liver tissue and observed significant increases in the activities of the SOD, CAT and GSH-Px enzymes with the HIIT and MICT protocols, suggesting a protective effect against oxidative imbalance[6]. According to Travacio and Llesuy (1996), the increase in the antioxidant activity of an enzyme may be due to a response to oxidative stress, in order to reduce or prevent damage caused by free radicals[42].

Another important component in the primary defense against oxidative stress is the superoxide dismutase enzyme (SOD), which is a metalloenzyme[43]. This antioxidant enzyme is responsible for neutralizing the free radical excess and can be useful in many areas of study[43]. Many studies have shown that the antioxidant capacity of SOD may aid in preventing cardiovascular damage, neurodegenerative diseases or disorders in metabolic functions[43]. SOD reduces superoxide anion, a vasoconstrictor, thereby favoring blood flow.

Our data demonstrated that, in obese hearts of rats, the HFD alone did not alter SOD activity, but animals trained with the HIIT protocol, with or without L-Carnitine supplementation, presented increased SOD activity levels. In the kidney, the HFD increased SOD activity, and the HIIT protocol, alone and associated with L-Carnitine supplementation, was able to reverse this alteration. In contrast to the kidney and heart, there were no differences between the different groups in the liver cells. The increase in SOD activity may suggest that the HFD induced an increase in the production of ROS, such as the superoxide radical, and as a consequence, an increase in the activity of this antioxidant enzyme. Additionally, this finding suggests that the protocol and the supplementation were effective for reversing the damage caused by the imbalance between ROS and the antioxidant defense system. This fact corroborates and justifies the changes that several studies show in metabolism during exercise and during the process of high-fat diet ingestion[43].

Glutathione peroxidase is another intracellular enzyme responsible for the antioxidant defense system and can be found in mitochondria or in the cytosol as well[44]. This enzyme can be used to evaluate the role of mitochondria in metabolic protection[44]. When overexpressed, GSH-Px improves reperfusion in the cardiovascular system, helps in kidney diseases by preventing the remodeling of the glomerulus and has many other functions; as such, more studies are needed to

understand the mechanism of action of this enzyme[44]. Our results show that GSH-Px activity in the heart was increased by the HFD, and that the HIIT protocol, when used alone or associated with L-Carnitine supplementation, reversed this increase. In addition, the HFD decreased GSH-Px activity in the liver and kidneys of obese rats, and the HIIT protocol, alone or in association with L-Carnitine, did not reverse this alteration in the liver and partially reversed it in the kidney. We suggest that a longer period of training is needed to increase GSH-Px expression or activity. In a study comparing rats fed on HFD and a control group, Noeman and collaborators (2011) found decreases in GSH-Px activity in the heart, liver and kidneys of obese rats, corroborating our data[38]. With regard to L-Carnitine supplementation, there are reports that this molecule exerts its antioxidant effects by increasing glutathione levels, inducing the transcription of genes involved in its biosynthesis, thereby increasing and maintaining its bioavailability[45].

Taken together, our results provide insights into the relative contribution of obesity to oxidative stress in the heart, liver and kidneys. Furthermore, the HIIT protocol and L-Carnitine supplementation may represent approaches to prevent and, sometimes, reverse this damage, thereby protecting against obesity-associated pathologies.

Conflict of interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

Acknowledgements

This work was supported by grants from Fundo de Apoio à Pesquisa da Universidade da Região de Joinville, Fundo de Apoio à Pesquisa de Santa Catarina (FAPESC) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

We thank the statistician, Dra. Elisa Henning (Udesc/Joinville), for statistical analysis.

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Table 1 - Oxidative stress biomarkers in the heart, liver and kidney of obese rats in the different experimental groups.

	Organ	ND-UNT	HFD-UNT	HFD-UNT-C	HFD-HIIT	HFD-HIIT-C
TBA-RS (nmol TBA-RS/min. mg protein)	*Heart*	2.20 ± 0.38	2.26 ± 0.41	2.39 ± 0.49	2.10 ± 0.44	2.11 ± 0.34
	Liver	3.79 ± 0.56	5.26 ± 0.49[b]	5.07 ± 0.53[b]	3.61 ± 0.39[a]	3.61 ±0.39[a]
	Kidney	2.40 ± 0.26	2.77 ± 0.72	2.66 ± 0.59	2.50 ± 0.28	2.37 ± 0.34
Total sulfhydryl content (nmol TBN/mg protein)	*Heart*	39.94 ± 3.50	15.77 ± 2.19[d]	23.21 ± 4.3[c,d]	42.36 ± 5.02[a]	42.53 ± 4.76[a]
	Liver	65.79 ± 4.49	63.87 ± 3.16	64.37 ± 4.99	84.70 ± 7.39[e]	85.87 ± 7.25 [e]
	Kidney	35.90 ± 5.80	30.54 ± 3.81[g]	29.77 ± 2.04[g]	32.93 ± 3.52	41.43 ± 3.59[f,g]
Protein carbonyl content (nmol carbonyl/mg protein)	*Heart*	6.90 ± 0.24	7.30 ± 0.58	7.03 ± 0.42	6.80 ± 0.46	6.90 ± 0.59
	Liver	6.99 ± 0.30	7.13 ± 0.47	7.01 ± 0.28	6.71 ± 0.44	6.79 ± 0.43
	Kidney	7.00 ± 0.73	12.89 ± 0.61[b]	11.71 ± 1.39[h,b]	7.07 ± 0.75[a]	6.13 ± 0.63[a]

Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.01 *vs* HFD-UNT; [d], p<0.001 *vs* ND-UNT; [e], p<0.001 *vs* ND-UNT, HFD-UNT and HFD-C-UNT; [f], p<0.001 *vs* HFD-UNT, HFD-UNT-C and HFD-HIIT; [g], p<0.05 *vs* ND-UNT; [h], p<0.05 *vs* HFD-UNT.

Fig.1. HIIT Protocol

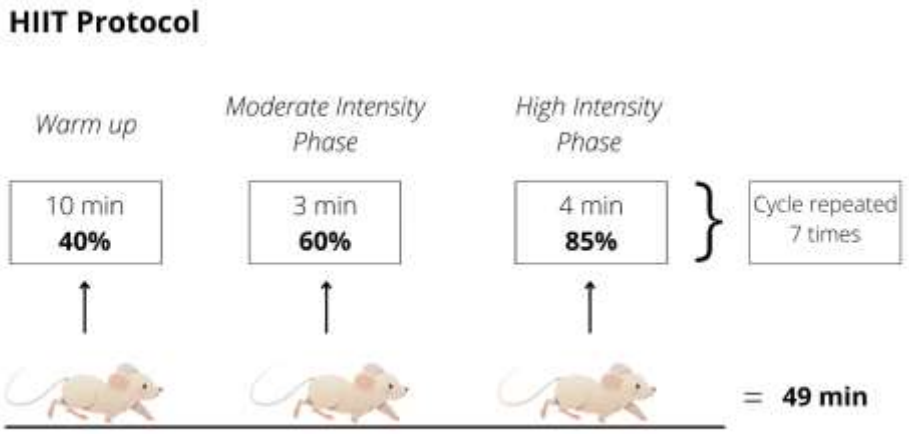
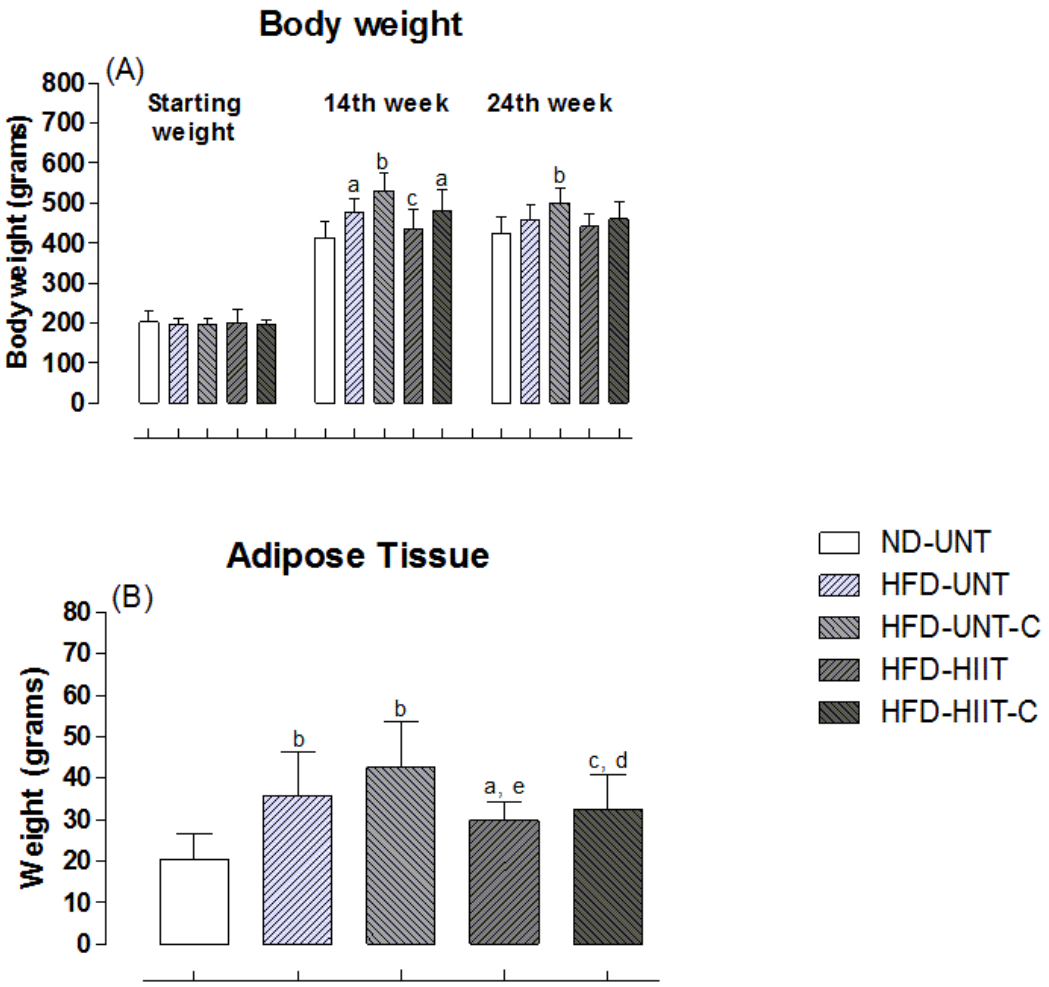
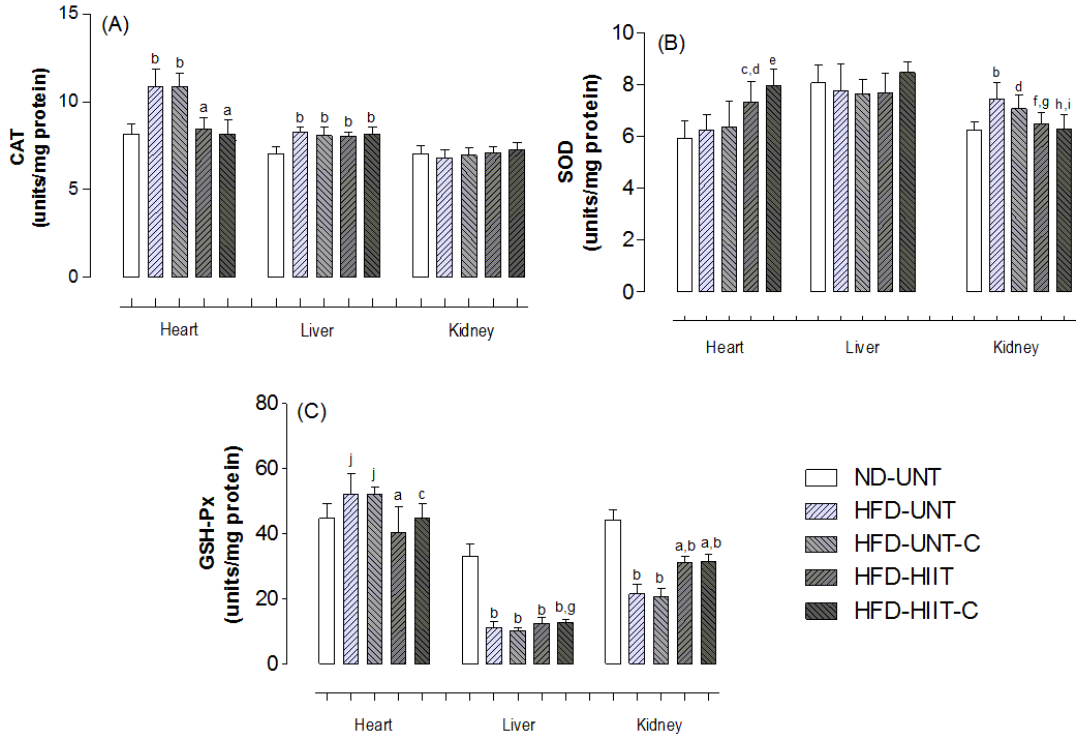


Fig.2. Effects of the high fat-diet, the high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* ND-UNT; [b], $p<0.001$ *vs* ND-UNT; [c], $p<0.05$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-UNT; [e], $p<0.01$ *vs* HFD-UNT-C.

Fig.3. Effects of the high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the heart, liver and kidneys of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.05 *vs* HFD-UNT and HFD-UNT-C; [d], p<0.01 *vs* ND-UNT; [e], p<0.001 *vs* ND-UNT, HFD-UNT and HFD-C-UNT; [f], p<0.01 *vs* HFD-UNT; [g], p<0.05 *vs* HFD-UNT-C; [h], p<0.001 *vs* HFD-UNT; [i], p<0.01 *vs* HFD-UNT-C; [j], p<0.05 *vs* ND-UNT.

Legends to figures

Table 1. Oxidative stress biomarkers in the heart, liver and kidney of obese rats in the different experimental groups. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-Intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-Intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* HFD-UNT and HFD-

UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.01 *vs* HFD-UNT; [d], p<0.001 *vs* ND-UNT; [e], p<0.001 *vs* ND-UNT, HFD-UNT and HFD-C-UNT; [f], p<0.001 *vs* HFD-UNT, HFD-UNT-C and HFD-HIIT; [g], p<0.05 *vs* ND-UNT; [h], p<0.05 *vs* HFD-UNT.

Fig.1. HIIT Protocol.

Fig.2. Effects of the high fat-diet, the high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-Intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-Intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* ND-UNT; [b], p<0.001 *vs* ND-UNT; [c], p<0.05 *vs* HFD-UNT-C; [d], p<0.01 *vs* ND-UNT; [e], p<0.01 *vs* HFD-UNT-C.

Fig.3. Effects of the high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the heart, liver and kidneys of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-Intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-Intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.05 *vs* HFD-UNT and HFD-UNT-C; [d], p<0.01 *vs* ND-UNT; [e], p<0.001 *vs* ND-UNT, HFD-UNT and HFD-C-UNT; [f], p<0.01 *vs* HFD-UNT; [g], p<0.05 *vs* HFD-UNT-C; [h], p<0.001 *vs* HFD-UNT; [i], p<0.01 *vs* HFD-UNT-C; [j], p<0.05 *vs* ND-UNT.

6.4 Artigo:

EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING AND L-CARNITINE SUPPLEMENTATION ON OXIDATIVE STRESS AND ENERGY METABOLISM PARAMETERS IN THE BRAIN OF OBESE RATS

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ABSTRACT

The aim of this study was to evaluate the protective effects of high-intensity interval training (HIIT) and L-Carnitine supplementation on oxidative stress and energy metabolism parameters in the brain of obese rats. Animals were divided into five groups: Normal Diet + Untrained (ND-UNT), High-Fat Diet + Untrained (HFD-UNT), High-Fat Diet + Untrained + Carnitine (HFD-UNT-C), High-Fat Diet + High-intensity Interval Training (HFD-HIIT) and High-Fat Diet + High-intensity Interval Training + Carnitine (HFD-HIIT-C). To induce obesity, animals in the HFD groups were fed with a HFD for 14 weeks, while animals in the ND groups were treated with a standard diet. Animals in the HFD-UNT-C and HFD-HIIT-C groups received L-Carnitine by gavage as soon as the HIIT protocol was started; the HIIT protocol was performed 5 days a week, while animals in the UNT group walked at 40% intensity, twice a week. After the end of the 10th week of training, animals were sacrificed by decapitation and the cerebral cortex, cerebellum and hippocampus were separated and homogenized in appropriate buffer. The antioxidant activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), levels of thiobarbituric acid reactive substances (TBARS), total sulfhydryl content and pyruvate kinase, citrate synthase, succinate dehydrogenase (SDH), complex II and cytochrome *c* oxidase activities were determined. Results showed that the HFD promoted oxidative stress and alterations in mitochondrial function. The HIIT protocol, when used alone and, sometimes, when associated with L-Carnitine, prevented some of these alterations in the brain of obese rats.

Keywords: Obesity; Oxidative stress; Energy Metabolism; L-Carnitine; High-intensity Interval Training; Brain.

1 INTRODUCTION

According to the World Health Organization, obesity is considered a worldwide epidemic, a public health problem, known for its ability to increase morbidity and mortality[1,2]. It is characterized by an imbalance in energy intake and expenditure, resulting in large amount of adipose tissue, being a risk factor for many diseases, such as hypercholesterolemia, hyperlipidemia, hyperglycemia and cardio-cerebrovascular disorders[1,3].

With regard to cerebrovascular disorders, several studies show that high fat-diet (HFD) and obesity may be related to an increase in cases of ischemic brain injury, in the incidence of Alzheimer´s disease, in development of vascular cognitive impairment,

in addition to deficits in memory, learning and in the capacity of executive functions, once HFD and obesity can interfere with hippocampus structure and functions[1,3,4].

It is well known that the excessive accumulation of body fat contributes to making people more susceptible and more vulnerable to develop diseases triggered by oxidative stress, energy metabolism disfunction and inflammation[5].

Oxidative stress can be described as an imbalance in body´s ability to neutralize free radicals, especially reactive oxygen species (ROS), due to an excessive production of free radicals or a depletion in antioxidant defense systems, and when induced by obesity, has been related to cause neuronal brain inflammation and cognitive impairments, promoting neuronal injury and death in the brain[1,4,6]. In 2019, Park *et al*. found higher levels of oxidative stress parameters, such as 4-hydroxy-2-nonenal (HNE) in the hippocampus of HFD-fed animals, when compared to animals fed with normal diet, consistent with lipid peroxidation[1].

With regard to energy metabolism disfunction, several studies have pointed obesity with changes in mitochondrial respiratory chain function and with deleterious effects on carbohydrate metabolism[5].

Physical exercise seems to be an ally as a strategy for treatment of obesity. As more people report not having enough time to exercise, high intensity interval training (HIIT) has become a popular exercise, since it results in a higher energy expenditure in less time, when compared to moderate intensity training (MICT)[7]. Furthermore, it has been suggested that HIIT can improve mitochondrial biogenesis, improve antioxidant defenses, protecting against ROS, playing an important role against energy metabolism disfunction and oxidative stress-induced by obesity[8,9].

Besides physical exercise, other strategies have been proposed in the adjuvant treatment of obesity in order to reduce deleterious effects caused by oxidative stress[10]. L-Carnitine (3-hydroxy-4-N-trimethylamino-butyrate) is a quaternary amine that plays a fundamental role in the generation of energy by the cell, since it is responsible for the free fatty acids oxidation in mitochondria, facilitating the generation of adenosine triphosphate (ATP)[11].

Furthermore, studies also suggest that L-Carnitine improves the ability to perform physical tasks and muscular recover post-workout, helps preserving cell membrane and DNA from the damage caused by oxidative stress, reason why L-Carnitine have been studied as a possible antioxidant[10,11].

Considering that obesity is related to oxidative stress and alterations in energy metabolism in the brain, and that studies indicate that the HIIT protocol and supplementation with L-Carnitine promote an improvement in oxidative capacity and energy metabolism function, the present work aimed to evaluate the protective effects of HIIT and L-Carnitine supplementation on oxidative stress and energy metabolism parameters in the cerebral cortex, cerebellum and hippocampus of obese rats.

2 MATERIALS AND METHODS

2.1 Animals and Reagents

Sixty-day-old male Wistar rats from the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil, were used in the experiments. Before the experiments, animals were accommodated and acclimatized for 7 days to adapt to their new environment. Animals were kept in rooms with a 12h light/dark cycle with the temperature maintained between 20-22ºC and free access to food and water. The 12h light/dark cycle was inverted for better use of the animals' active period for training. The animals were kept in cages with a maximum number of four per cage; box exchange was performed every 2 days. Animal care was carried out in accordance with Law Nº. 11794 (October 8, 2008), and other regulations applicable to the use of animals in teaching and/or research, especially the Normative Resolutions of the National Council for the Control of Animal Experimentation – CONCEA [12,13]. Room lighting, accommodation and nutrition used followed the recommendations of the Guide for the Care and Use of Laboratory Animals[14].

For the *in vivo* experiments, animals were divided, in equal numbers, into the following groups:

16) Normal Diet + Untrained (ND-UNT, n = 8);
17) High-Fat Diet +Untrained (HFD-UNT, n = 8);
18) High-Fat Diet + Untrained + L-Carnitine (HFD-UNT-C, n = 8);
19) High-Fat Diet + High-intensity Interval Training (HFD-HIIT, n = 8);
20) High-Fat Diet + High-intensity Interval Training + L-Carnitine (HFD-HIIT-C, n = 8).

The experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 012/2017. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2 Experimental Protocols

2.2.1 Dietary induction of obesity

The animals in the experimental HFD groups were fed with a high-fat diet, composed of 20% of calories from carbohydrates, 20% of proteins and 60% lipids (Prag Soluções Biosciences, Jaú, São Paulo-SP), for fourteen weeks, in order to induce the condition of obesity. The animals in the control group (ND) were treated with a standard diet (70% carbohydrates, 20% proteins and 10% lipids) (Quimtia, Curitiba, Paraná, Brazil). All animals received water *ad libidum*.

2.2.2 L-Carnitine supplementation

The animals from the experimental groups HFD-UNT-C and HFD-HIIT-C received L-Carnitine supplementation by gavage, in a concentration of 300 mg/ kg of body mass per day, while animals from the experimental groups ND-UNT, HFD-UNT and HFD-HIIT, received saline by gavage once a day.

2.2.3 High-Intensity Interval Training Protocol

A maximum effort tolerance test was applied in order to define the animals' maximum speed and then prescribe the training intensity. The maximum effort tolerance test was performed to find the maximum speed that each rat could run. This data served as a parameter for the prescription of the training speeds of the HIIT protocol.

The maximum effort test was performed at three times: at the beginning, after four weeks and during the eighth week of the training protocol. Following the protocol of Ferreira *et al*. (2007), the test consisted of a running on a treadmill (model KT-4000, IMBRAMED), with an inclination of 20 degrees, with an initial speed of 6 m/min and an increase of 3 m/min every three minutes, until exhaustion of the animal (visible

fatigue)[15]. Once the maximum speed was found, distance and speed were computed to calculate training intensity.

The HIIT training protocol was carried out on five days a week, using a 20° inclination of the treadmill, with intensities defined using the exercise tolerance test. HIIT was performed for three minutes at 60% intensity, followed by four minutes at 85% of the maximum test speed[15]. This cycle was repeated seven times, totaling 49 minutes of training (Fig.1).

The animals of the untrained group (UNT) performed a 40% intensity walk, twice a week, in order to maintain the animals' ability to walk for subsequent physical tests.

2.2.4 Tissue preparation

After the end of the 10th week of training (24 weeks of experiment), the animals were sacrificed (48 hours after the last training session) by decapitation, without anesthesia, since the use of anesthetics can interfere with the determination of oxidative parameters[16,17], and the cerebral cortex, cerebellum and hippocampus were dissected and homogenized in suitable buffer, according to the technique used. The homogenate was centrifuged at x 3,000 g at 4°C for 15 min to remove cellular debris and the supernatant was stored in aliquots at -80°C for the analyses of parameters of oxidative stress and energy metabolism.

2.3 Biochemical studies

2.3.1 Catalase Assay (CAT)

CAT activity was determined by the method of Aebi (1984)[18]. This method is based on the disappearance of hydrogen peroxide (H_2O_2) in a reaction medium composed of 25µL of sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. The absorbance was counted every 10 seconds for 1 minute and 40 seconds at 240 nm using a UV-vis Shimadzu spectrophotometer. One CAT corresponds to 1 µmol of H_2O_2 consumed per minute and the specific activity was calculated as CAT units/mg protein.

2.3.2 Superoxide Dismutase Assay (SOD)

The activity of SOD was assayed by the method described by Marklund (1985)[19], using a process highly dependent on superoxide ($O2^{\bullet-}$), which is a substrate for SOD. Sample (15 µL) was added to 215 µL of a mixture containing 50 µM Tris buffer, 1 µM EDTA, pH 8.2, and 30 µM CAT. Subsequently, 20 µL of pyrogallol were added and the absorbance was measured every 30 seconds for 3 minutes at 420 nm using a UV-vis Shimadzu spectrophotometer. Inhibition of the auto-oxidation of pyrogallol occurs in the presence of SOD, the activity can be tested indirectly spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity was reported as SOD units/mg protein.

2.3.3 Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel (1981)[20], using *tert*-butylhydroperoxide as substrate. The decomposition of NADPH was controlled in a spectrophotometer (UV-vis Shimadzu) at 340 nm for 3 minutes and 30 seconds. 90 µL of each sample were added to the medium containing 800 µL of buffer, 20 µL of 2.0 mM GSH, 30 µL of 0.15 U/mL GSH reductase, 10 µL of 0.4 mM azide, and 10 µL of 0.1 mM NADPH. The absorbance was counted every 10 seconds for 1 minute and 30 seconds. Subsequently, 50 µL of 0.5 mM *tert*-butylhydroperoxide were added and the absorbance was read for a further 2 minutes. One GSH-Px unit is characterized as 1 µmol of NADPH consumed per minute and the specific activity was defined as GSH-Px units/mg of protein.

2.3.4 Total Sulfhydryl Content

The total sulfhydryl content was measured following the method of Aksenov & Markersbery (2001)[21], based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, yielding a yellow derivative (TNB), which is evaluated spectrophotometrically at 412 nm. For the assay, 50 µL of homogenate was added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction started with the addition of 30 µL of 10 mM DTNB and incubated for 30 minutes at room temperature in the dark. Analyses of a blank (DTNB absorbance) were also performed. The results are presented as nmol TNB/mg protein.

2.3.5 Thiobarbituric acid reactive substances (TBA-RS)

TBA-RS were defined according to the method of Ohkawa *et al*. (1979)[22]. The methodology for the study of TBA-RS measures malondialdehyde (MDA), resulting from lipoperoxidation, provided predominantly by hydroxyl free radicals. At first, the cerebral cortex, cerebellum and hippocampus, in 1.15% KCl, were mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBA-RS were determined by the absorbance at 535 nm. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was exposed to the same treatment as that of the supernatants. TBA-RS content was presented in nanomoles of MDA formed per milligram of protein.

2.3.6 Pyruvate kinase activity (PK)

Pyruvate kinase activity was assayed essentially as described by Leong *et al*. (1981)[23]. The incubation medium consisted of 0.1 M Tris–HCl buffer, pH 7.5, 10.0 mM $MgCl_2$, 0.16 mM NADH, 75.0 mM KCl, 5.0 mM ADP, 7.0 units of L-lactate dehydrogenase, 0.1% (v/v) Triton X-100, and 10.0 µL of the mitochondria-free supernatant in a final volume of 0.5 mL. Unless otherwise stated, the reaction was started after 30 min of pre-incubation by the addition of 1.0 mM phosphoenolpyruvate (PEP). All assays were performed in duplicate at 25ºC. Results were expressed as µmol of pyruvate formed per min per mg of protein.

2.3.7 Citrate synthase activity (CS)

The activity of citrate synthase was assessed using spectrophotometry, as described by Alp *et al*. (1976)[24]. Homogenized cerebral cortex and cerebellum samples (10µL) were added to cuvettes with 800 µL buffer (1M Tris-HCL, pH 8), 100 µL 1mM DTNB, 40 µL 2.5 mM acetyl-CoA and 10 µL 10% Triton. The reaction was started by the addition of 50 µL of oxaloacetate (4 mM) and changes in absorbance were observed during 3 minutes at 412 nm.

2.3.8 SDH and complex II activities (CII)

The activities of succinate:phenazine oxyreductase (soluble SDH) and complex II (succinate: DCIP oxyredutase) were measured in the cerebral cortex and cerebellum homogenates by following the decrease in absorbance due to the reduction of 2,6-dichloroindophenol (DCIP) at 600 nm with 700 nm as the reference wavelength (ε=19.1 mM^{-1} cm^{-1}) in the presence of phenazine methasulphate (PMS), according to Fischer *et al*. (1985)[25]. The reaction mixture, consisting of 40.0 mM potassium phosphate, pH 7.4, 16.0 mM succinate and 8 μM DCIP, was preincubated with 40-80 μg homogenate protein at 30°C for 20 min. Subsequently, for complex II activity, 4.0 mM sodium azide and 7 μM rotenone were added and the reaction was initiated by the addition of 40 μM DCIP and monitored for 5 min. The activity of SDH was accessed in the same incubation medium by the addition of 1.0 mM PMS and monitored for 5 min.

2.3.9 Cytochrome *C* Oxidase (COX) activity

The activity of cytochrome c oxidase was measured according to Rustin *et al*. (1994)[26]. Enzymatic activity was measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm with 580 nm as the reference wavelength (ε = 19.1 mM^{-1}x cm^{-1}). The reaction buffer contained 10.0 mM potassium phosphate, pH 7.0, 0.6 mM *n*-dodecyl-β-D-maltoside, 2-4 μg homogenate protein and the reaction was initiated by the addition of 0.7 μg reduced cytochrome *c*. The activity of cytochrome *c* oxidase was measured at 25°C for 10 min.

2.3.10 Protein determination

Protein was measured by the Lowry *et al*. (1951)[27] method, using serum bovine albumin as standard.

2.4 Statistical Analysis

Statistical treatment was performed using the R software version 4.0.3 with the aid of the Car and Desctools packages[28–30]. A multiple linear regression model was applied, followed by Analysis of Variance (ANOVA) for unbalanced data, in order to verify whether there were differences in the outcome variables. The normality assumption of the model residuals was verified using the Shapiro-Wilk normality test.

Homoscedasticity was assessed using graphs and Levene's test. To identify differences between groups, the Duncan's test was used for multiple comparisons.

For the statistical analysis of body weight and adipose tissue of rats, ANOVA with repeated measures was applied to analyze the relationship between the independent variables and the outcome variable over the period of analysis. To verify the sphericity, the Mauchly test was applied and the Greenhouse-Gueisser correction was applied, if necessary. For the multiple comparison tests, the t test with Bonferroni adjustment was applied. To analyze the association between the independent variables and the weight of adipose tissue in the final week, a single-way ANOVA was applied. In the multiple comparison, the Duncan´s test was used.

Values of $p<0.05$ were considered significant. Results are expressed as means ± SD for eight independent experiments (animals) performed in duplicate.

3 RESULTS

3.1 Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats

We initially verified the effects of high fat-diet (HFD), high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats, with the aim of validating our obesity model. Fig.2A shows that there were no significant differences on rats´ starting weight between the experimental groups, and shows that in the 14th week, the HFD increased the body weight in HFD-UNT, HFD-UNT-C and HFD-HIIT-C groups, when compared to ND-UNT group. Fig.2A also shows that HIIT protocol, isolated or in association with L-Carnitine supplementation, in the 24th week, were able to revert the increase in this parameter.

With regard to adipose tissue, Fig.2B shows that the HFD increased this parameter in the HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C groups, when compared to the ND-UNT groups, and that the HIIT protocol, when used alone or in association with L-Carnitine supplementation, decreased this parameter, when compared to the HFD-UNT-C group.

3.2 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on parameters of oxidative stress in the brain of obese rats

We initially verified the effects of the HIIT protocol and L-Carnitine supplementation on TBA-RS, total sulfhydryl content and on the activities of antioxidant enzymes in the cerebral cortex, cerebellum and hippocampus of obese rats.

Fig.3A shows that neither HFD, HIIT protocol or L-Carnitine supplementation, isolated or associated, altered TBA-RS levels in the cerebral cortex and hippocampus of obese rats, while in the cerebellum, HFD enhanced this parameter, when compared to ND-group, and L-Carnitine supplementation alone, HIIT protocol, isolated or associated with L-Carnitine supplementation, were able to decrease and prevent the increase in this parameter, respectively.

With regard to total sulfhydryl content, Fig.3B shows that the HFD did not alter this parameter in the cerebral cortex, cerebellum and hippocampus of obese rats, when compared to ND-group. Otherwise, in the cerebral cortex and hippocampus, the association between L-Carnitine supplementation and the HIIT protocol significantly increased total sulfhydryl content, when compared to the HFD-UNT and HFD-UNT-C groups, and to HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT groups, respectively.

With regard to antioxidant enzyme activities, Fig.4A shows that the HFD increased CAT activity in the cerebral cortex and hippocampus, while in the cerebellum, it decreased this enzyme´s activity, when compared to the ND-UNT group. Fig.4A also shows that HIIT protocol, isolated or in association with L-Carnitine supplementation, reduced this increase, when compared to HFD-UNT and HFD-UNT-C groups, in the cerebral cortex and hippocampus of obese rats, while in the cerebellum, HIIT protocol alone reversed this decrease, and HIIT protocol associated with L-Carnitine supplementation was able to enhance CAT activity.

As regards SOD activity, Fig.4B shows that HFD did not alter this parameter, while HIIT protocol associated to L-Carnitine supplementation was able to increase this enzyme´s activity in the cerebral cortex, when compared to all other experimental groups. Additionally, in the cerebellum, Fig.4B shows that HFD enhanced SOD activity, and that HIIT protocol and L-Carnitine supplementation, isolated or in association, were not able to reverse this increase. About hippocampus, Fig.4B shows that L-

Carnitine and HIIT protocol, alone or associated, increased this enzyme´s activity, while HFD did not alter this parameter.

Fig.4C demonstrates that HFD decreased GSH-Px activity in the cerebral cortex, cerebellum and hippocampus, when compared to the ND-UNT group. In the cerebral cortex, HIIT protocol associated with L-Carnitine supplementation partially reversed this alteration and, in the hippocampus, HIIT protocol isolated or associated with L-Carnitine reversed this alteration, when compared to the ND-UNT group, HFD-UNT, and ND-UNT, HFD-UNT-C, respectively. Otherwise, in the cerebellum, HIIT protocol isolated or associated with L-Carnitine was able to reverse the alteration in this parameter, when compared to HFD-UNT and HFD-UNT-C.

3.3 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on parameters of energy metabolism in the brain of obese rats

Subsequently, the effects of HIIT and L-Carnitine supplementation on parameters of energy metabolism were also analyzed in the cerebral cortex and cerebellum of obese rats. These analyzes were not performed in the hippocampus due to lack of sample.

As can be seen in Fig.5A, neither HFD, HIIT protocol or L-Carnitine supplementation altered pyruvate kinase (PK) activity in the cerebral cortex, while in the cerebellum, the association between HIIT protocol and L-Carnitine supplementation enhanced this enzyme´s activity, when compared to the other experimental groups. With regard to citrate synthase (CS) activity, Fig.5B shows that there were no significantly differences between the experimental groups in both structures.

Fig.5C shows that, in the cerebral cortex, the HIIT protocol, when used together with L-Carnitine supplementation, promoted an increase in the activity of complex II, when compared to the other experimental groups. Conversely, in the cerebellum, Fig.5C shows that the HFD reduced this parameter and shows that the HIIT protocol, when used alone or in association with L-Carnitine supplementation, was able to reverse the activity of complex II, when compared to the HFD-UNT and HFD-UNT-C groups.

With regard to SDH activity, Fig.5D shows that, in the cerebral cortex, the L-Carnitine supplementation alone, the HIIT protocol alone and the association between

the HIIT protocol and L-Carnitine supplementation, increased SDH activity, when compared to the HFD-UNT and ND-UNT groups, while there were no significant differences in this enzyme´s activity in the cerebellum.

Finally, Fig.5E shows that there were no significant differences between the experimental groups in the cerebral cortex, and also shows that HFD by itself reduced cytochrome *c* oxidase (COX) activity in the cerebellum, when compared to the ND-UNT group, and that the HIIT protocol alone and in association with L-Carnitine supplementation, reversed the alteration in this parameter, when compared to the HFD-UNT and HFD-UNT-C groups.

4 DISCUSSION

In the present study we investigated whether HIIT protocol and L-Carnitine supplementation could prevent or protect against oxidative stress and energy metabolism dysfunction in the brain of obese rats. First of all, our results showed that HFD increased body weight and promoted adipose tissue gain in the experimental groups, validating our methodology. Our results also showed that HFD promoted alterations on the activities of antioxidant enzymes in the cerebral cortex, cerebellum and hippocampus and alterations on energy metabolism parameters and lipoperoxidation in the cerebellum. Furthermore, we showed that the HIIT protocol, when used alone and, sometimes, when associated with L-Carnitine supplementation, was able to reverse and even improve these alterations.

Initially, we evaluated TBA-RS levels and total sulfhydryl content in the brain of obese rats. ROS-induced lipid peroxidation is associated with multiple acute and chronic brain disorders, and the TBA-RS assay, which measures MDA reactivity, is a useful indicator for evaluating it[31]. The measurement of TBA-RS levels serves as a lipoperoxidation parameter and indicates damage to cell membranes, which may cause changes in its structures resulting in cell death, while the determination of the total sulfhydryl content is a parameter that indicates protein damage, which can increase protein fragmentation, making them more likely to be degradated[32–34].

According to our results, HFD alone did not alter TBA-RS levels in the cerebral cortex and hippocampus of obese rats, possibly linked to the exposure time to HFD, that may not have been enough to promote oxidative stress that would significantly

affect this parameter. However, in the cerebellum, HFD enhanced TBA-RS levels in the HFD-UNT and HFD-UNT-C groups, and L-Carnitine supplementation alone, HIIT protocol, isolated or associated with L-Carnitine supplementation, reversed the alteration in this parameter.

The alteration only in cerebellum´s TBA-RS levels can be explained by its increased vulnerability to oxidative stress, due to its high oxygen consumption, the presence of polyunsaturated fatty acids in its molecular structure, and a restricted antioxidant capacity[35]. In a study carried out by Hazzaa *et al*. (2021)[36], it was observed that rats fed with HFD presented an increased brain tissue MDA concentration, when compared with the control group, as an indicative of lipoperoxidation, corroborating our findings.

Chadorneshin *et al*. (2021)[37] conducted a study to compare the impact of HIIT and Continuous Training (CT) on antioxidant enzyme activity and MDA levels in the rat brain. Unlike our data, the study did not find significantly changes on brain MDA levels in the HIIT and CT groups, however their HIIT protocol was performed only for 6 weeks, while in our study, it was performed for 10 weeks, which may explain the divergence of results[37].

Our study suggests that the protective effect of L-Carnitine against lipid peroxidation can be attributed to its promotion of fatty acid oxidation, which may have reduced the availability of fatty acids for peroxidation[38]. Furthermore, our findings indicate that the efficiency of L-Carnitine in the transport of fatty acids and subsequent oxidation is enhanced when it is combined with HIIT, likely due to the higher energy demand. Therefore, L-Carnitine supplementation appears to be more effective when used in conjunction with HIIT.

With regard to total sulfhydryl content, our data show that the HFD did not alter this parameter in the brain of obese rats, but that HIIT protocol and L-Carnitine supplementation significantly increased this parameter in the cerebral cortex and cerebellum. These results suggest that the HIIT protocol and L-Carnitine exert protective effects against protein damage by increasing the total sulfhydryl content in the cerebral cortex and cerebellum of obese rats. Unfortunately, we did not find any studies relating the protective role of HIIT against protein damage in the rat brain.

With regard to antioxidant enzyme activities, our results show that the HFD increased CAT activity in the cerebral cortex and hippocampus and reduced its activity in the cerebellum, did not alter SOD activity in cerebral cortex and hippocampus and increased its activity in the cerebellum, and decreased GSH-Px activity in all brain structures.

The enzymatic antioxidant system is composed of three principal enzymes: CAT, SOD and GSH-Px. CAT is responsible for neutralizing H_2O_2 into water and molecular oxygen, SOD for the reduction of superoxide anion and GSH-Px for the elimination of hydroperoxides[39–41]. Any alteration is these enzymes activities may suggest an increase in the production of ROS. According to Travacio and Llesuy (1996), in face of oxidative stress, an antioxidant enzyme can increase its activity, with the aim of reducing or preventing any damage caused by free radicals[42].

The increase in CAT activity in the cerebral cortex and hippocampus and in SOD activity in the cerebellum may suggest that the HFD induced an increase in the production of H_2O_2 and superoxide radical, respectively, and as a consequence, an increase in the antioxidant enzyme´s activities related to the attempt of minimizing the damages caused by these radicals, which attack lipids, DNA and protein[40]. While in the cerebellum, the decrease may suggest that the increase in the production of ROS induced by HFD caused a depletion in the CAT activity. In addition, higher levels of superoxide radical may enhance the production of H_2O_2, which could explain the decrease in GSH-Px activity in the brain of obese rats. Besides that, the increase in CAT activity in the cerebral cortex and hippocampus may have occurred to compensate the enzymatic reduction of GSH-Px.

When comparing brain tissue GSH concentrations, Hazzaa *et al*. (2021)[36] observed a significantly decrease in this parameter in feeding rats HFD, when compared with the control group. The reduction of GSH concentration may compromise the enzymatic activity of GSH-Px, since it is a cofactor of the enzyme.

The HIIT protocol, isolated and associated with L-Carnitine supplementation, partially reversed the alteration in CAT activity in the cerebral cortex and hippocampus, while in the cerebellum, HIIT protocol, when used alone, reversed the alteration, and HIIT protocol associated with L-Carnitine supplementation increased CAT activity. About SOD activity, in the cerebral cortex HIIT protocol isolated, and in the

hippocampus, HIIT isolated and in association with L-Carnitine supplementation, were able to enhance and potentiate this enzyme´s activity. Regarding GSH-Px, HIIT protocol alone and associated with L-Carnitine reversed the alteration in the cerebellum and hippocampus; and HIIT protocol associated with L-Carnitine partially reversed the alteration in the cerebral cortex.

In 2019, Freitas *et al*.[31] studied the effects of HIIT in the improvement of cerebellar antioxidant capacity in rats and found a significant increase of cerebellar SOD activity after 6 weeks of HIIT, but no effect on cerebral cortex. Chadorneshin *et al*. (2021)[37] compared the effects of HIIT and continuous training (CT) on memory and its correlation with antioxidant enzyme activity in the rat brain. The results showed that HIIT significantly increased SOD activity, but did not alter GSH-Px and CAT activities in the rat brain, when compared with the untrained group, while in our study, HIIT protocol, isolated and in association with L-Carnitine supplementation, was able to reserve the increase in CAT activity in all brain structures, and about GSH-Px activity, HIIT protocol *per se* and associated with L-Carnitine reversed the decrease in this parameter in the cerebellum and hippocampus, and associated with L-Carnitine supplementation, reversed the decrease in GSH-Px activity in the cerebral cortex of rats[37]. The differences in the results of both studies may be related to the differences between HIIT protocols. In the study conducted by Chadorneshin (2021)[37], HIIT protocol was performed in even day with 30 seconds of high-intensity training, followed by 60 seconds of active rest, with 3 repetitions and 4.5 minutes each day, and in odd day with 3 minutes of high-intensity training, followed by 60 seconds of active rest, with 2 repetitions and 8 minutes each day, while in our study, HIIT protocol was performed 5 days a week with a cycle of 3 minutes at 60% intensity, followed by 4 minutes at 85% of the maximum test speed, repeating this cycle seven times, totaling 49 minutes of training.

Subsequently, we evaluated energy metabolism parameters in the cerebral cortex and cerebellum. Although some studies have investigated changes in brain mitochondria following exposure to a high-fat diet, these investigations are limited[43].

Our results show that HFD *per se* did not alter PK activity in both structures, while HIIT protocol in association with L-Carnitine supplementation was able to enhance this parameter in the cerebellum. The PK enzyme plays a crucial role in glycolysis by

catalyzing the conversion of phosphoenolpyruvate to ATP and pyruvate[44,45]. An increase in the activity of this enzyme can positively impact both aerobic and anaerobic pathways of energy metabolism.

With regard to citrate synthase (CS) activity, there were no significantly differences between the experimental groups in both brain structures. With the aim of investigating whether long-term HF feeding impacts on mitochondrial respiratory function in the brain, Jørgensen *et al*. (2015)[43] examined mitochondrial respiratory function in skeletal muscle and brain tissue from the same rats following a 1-year HFD. Their results showed no significantly differences between HFD and control animals in citrate synthase activity in brain tissue, corroborating our data.

Complex II was not alter by HFD in the cerebral cortex, but was decreased by the HFD in the cerebellum. In the cerebral cortex, HIIT protocol, when used together with L-Carnitine supplementation, enhanced the activity of complex II, while in the cerebellum, HIIT protocol, alone and associated with L-Carnitine supplementation, reversed the alteration.

With regard to SDH activity, HFD *per se* did not alter this parameter in both brain structures, but in the cerebral cortex, L-Carnitine supplementation alone, HIIT protocol alone, as well their association, increased the activity of this enzyme.

Regarding COX activity, the HFD did not alter this parameter in the cerebral cortex; in contrast, HFD decreased the activity of this enzyme in the cerebellum, and HIIT protocol, isolated and in association with L-Carnitine supplementation, reversed and increased COX activity, suggesting that physical exercises can enhance mitochondrial energy efficiency, and that the association with L-Carnitine contributes by favoring the transport and oxidation of long-chain fatty acids, increasing the efficiency of energy production in mitochondria[9].

The reduction in oxidative stress caused by the HIIT protocol and its association with L-Carnitine possibly contributed to the reversal of the reduction caused in the protein complexes of the mitochondrial respiratory chain in the cerebellum.

Although our studies did not show significantly differences in mitochondrial respiratory chain in the cerebral cortex and cerebellum of obese rats, the study carried out by Raza *et al.* (2015)[46] found negatively changes in Zucker diabetic fatty rats, when

compared with the lean model, showing complex I, II/III, and IV activities significantly reduced in the brain of these rats.

In summary, our findings shed light on the role of the HFD in causing oxidative stress and impairing energy metabolism in the brain. Moreover, our study suggests that the combination of HIIT protocol and L-Carnitine supplementation could be effective in preventing and even reversing this damage.

Conflict of interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

Acknowledgements

This work was supported by grants from Fundo de Apoio à Pesquisa da Universidade da Região de Joinville, Fundo de Apoio à Pesquisa de Santa Catarina (FAPESC), Fundação Universidade Regional de Blumenau (FURB) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

We thank the statistics teacher, Dra. Elisa Henning (Udesc/Joinville), for statistical analysis.

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Fig.1. HIIT Protocol

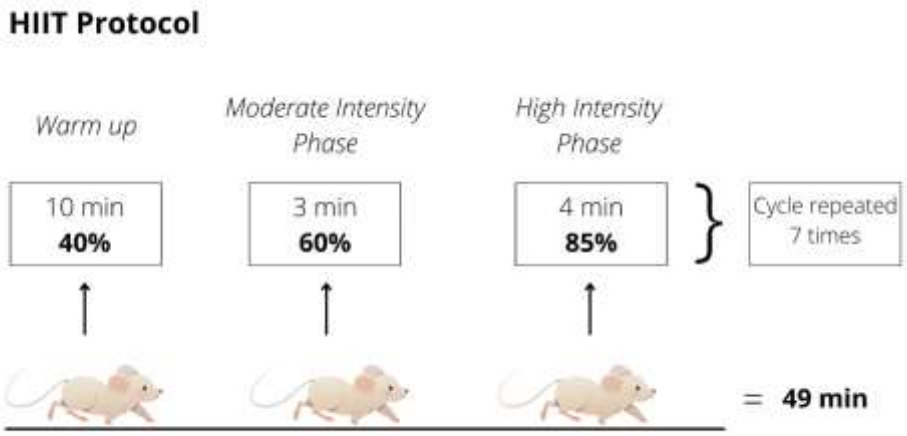
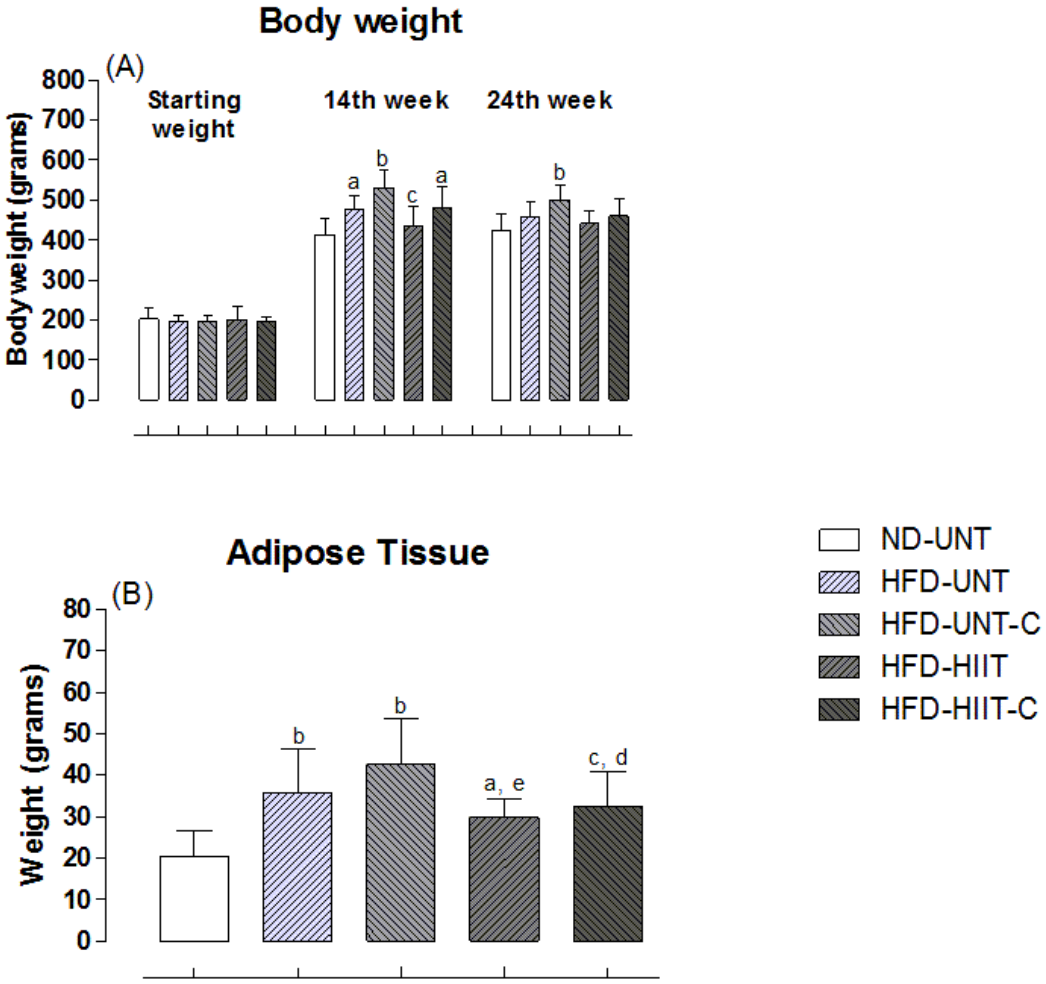
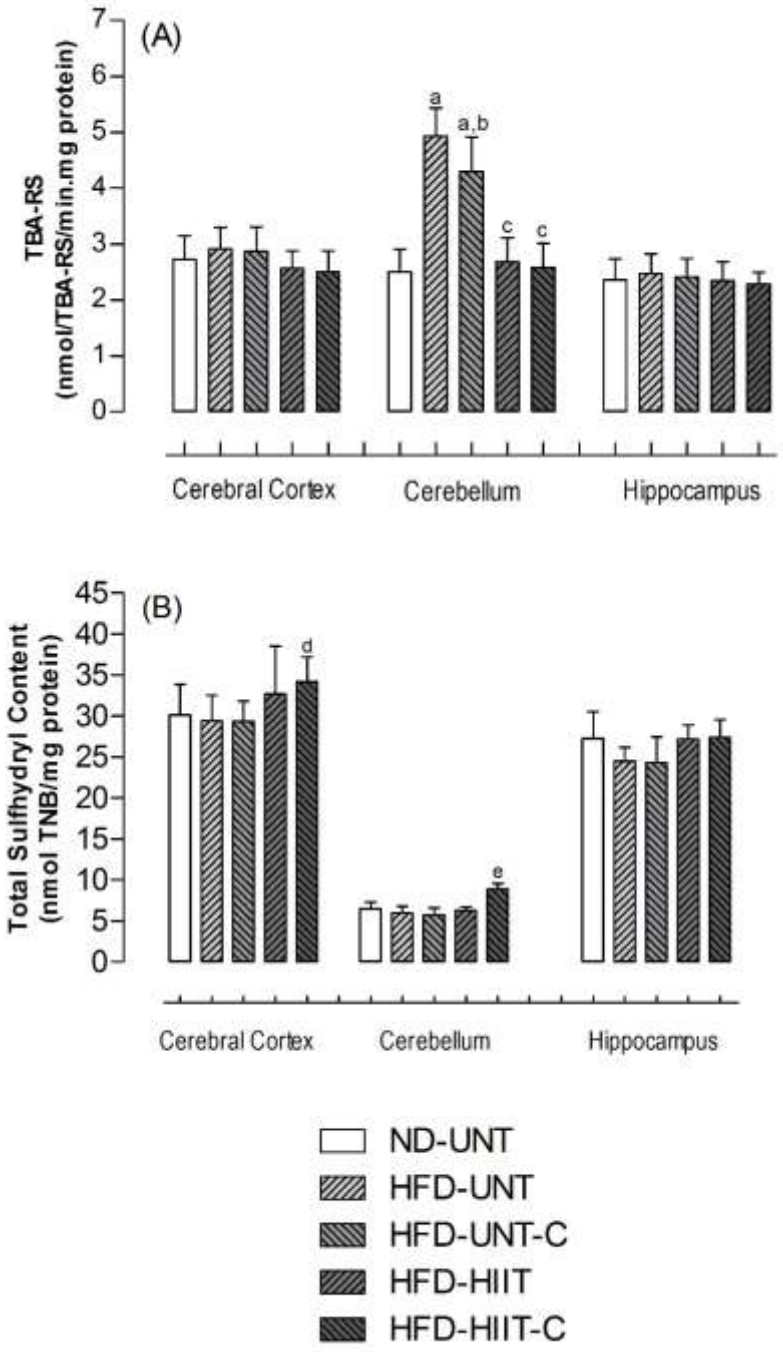


Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats submitted to different experimental protocols.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-Intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-Intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* ND-UNT; [b], $p<0.001$ *vs* ND-UNT; [c], $p<0.05$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-UNT; [e], $p<0.01$ *vs* HFD-UNT-C.

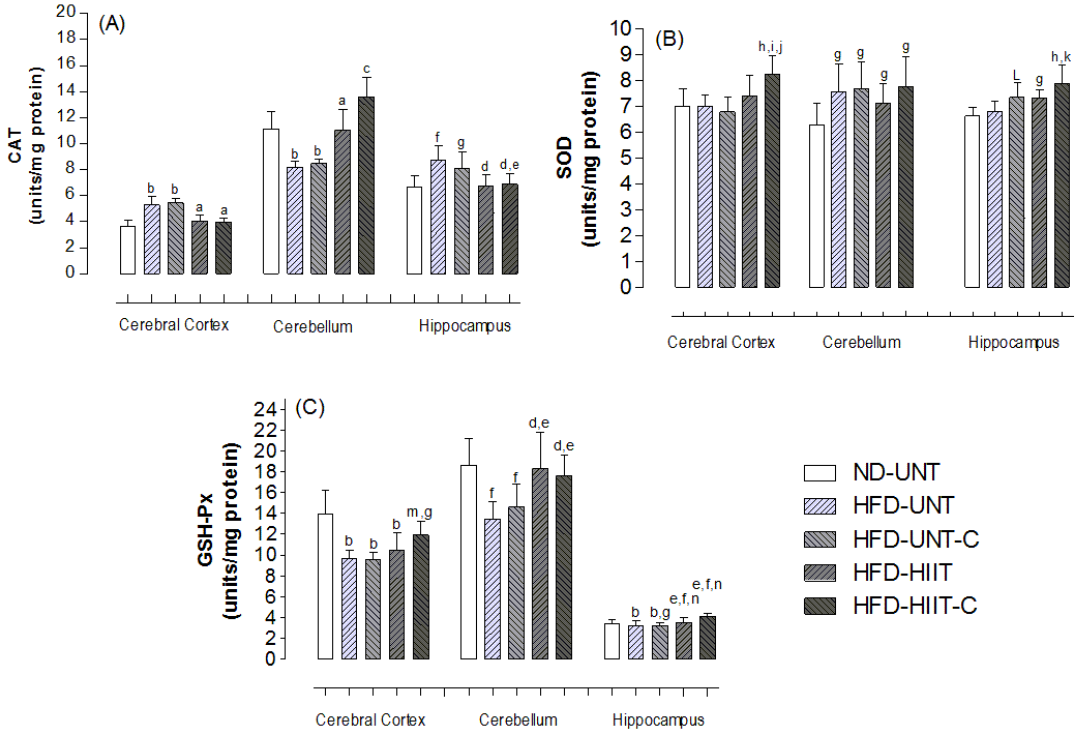
Fig.3. Effects of high-intensity interval training protocol and L-Carnitine supplementation on oxidative stress parameters in the cerebral cortex, cerebellum and hippocampus of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.001$ *vs* ND-UNT; [b], $p<0.05$ *vs* HFD-UNT; [c],

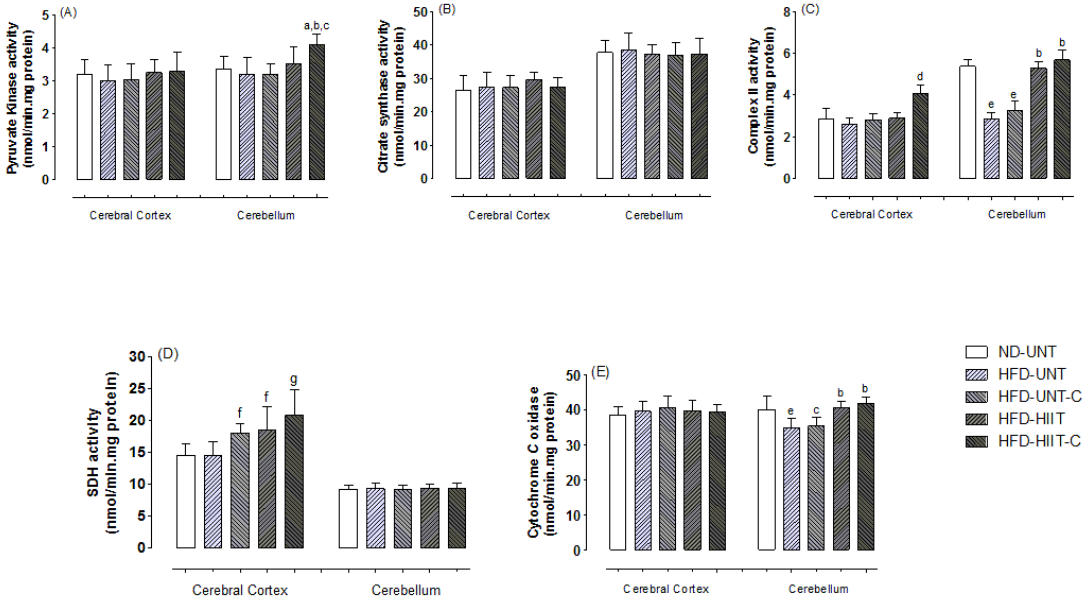
p<0.001 *vs* HFD-UNT and HFD-UNT-C; [d], p<0.05 *vs* HFD-UNT and HFD-C-UNT; [e], p<0.001 *vs* HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT.

Fig.4. Effects of high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the cerebral cortex, cerebellum and hippocampus of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + Carnitine. [a], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.001 *vs* HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT; [d], p<0.01 *vs* HFD-UNT; [e], p<0.05 *vs* HFD-UNT-C; [f], p<0.01 *vs* ND-UNT; [g], p<0.05 *vs* ND-UNT; [h], p<0.05 *vs* HFD-HIIT; [i], p<0.01 *vs* HFD-UNT and ND-UNT; [j], p<0.001 *vs* HFD-UNT-C; [k], p<0.001 *vs* HFD-UNT and ND-UNT; [L], p<0.05 *vs* HFD-UNT and ND-UNT; [m], p<0.01 *vs* HFD-UNT and HFD-UNT-C; [n], p<0.001 *vs* HFD-UNT.

Fig.5. Differences between experimental groups on the activities of pyruvate kinase, citrate synthase, complex II, succinate dehydrogenase (SDH) and cytochrome *c* oxidase in the cerebral cortex and cerebellum of rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* HFD-HIIT; [b], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [c], p<0.01 *vs* ND-UNT; [d], p<0.001 *vs* HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT; [e], p<0.001 *vs* ND-UNT; [f], p<0.05 *vs* HFD-UNT and ND-UNT; [g], p<0.001 *vs* HFD-UNT and ND-UNT.

Legends to figures

Fig.1. HIIT Protocol.

Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* ND-UNT; [b], p<0.001 *vs* ND-UNT; [c], p<0.05 *vs* HFD-UNT-C; [d], p<0.01 *vs* ND-UNT; [e], p<0.01 *vs* HFD-UNT-C.

Fig.3. Effects of high-intensity interval training protocol and L-Carnitine supplementation on TBA-RS (A) and Total Sulfhydryl Content (B) in the cerebral cortex, cerebellum and hippocampus of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* HFD-UNT; [b], p<0.001 *vs* HFD-UNT; [c], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [d], p<0.001 *vs* ND-UNT; [e], p<0.05 *vs* HFD-UNT and HFD-C-UNT; [f], p<0.001 *vs* HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT.

Fig.4. Effects of high-intensity interval training protocol and L-Carnitine supplementation on the activities of catalase (A), superoxide dismutase (B) and glutathione peroxidase (C) in the cerebral cortex, cerebellum and hippocampus of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [b], p<0.001 *vs* ND-UNT; [c], p<0.001 *vs* HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT; [d], p<0.01 *vs* HFD-UNT; [e], p<0.05 *vs* HFD-UNT-C; [f], p<0.01 *vs* ND-UNT; [g], p<0.05 *vs* ND-UNT; [h], p<0.05 *vs* HFD-HIIT; [i], p<0.01 *vs* HFD-UNT and ND-UNT; [j], p<0.001 *vs* HFD-UNT-C; [k], p<0.001 *vs* HFD-UNT and ND-UNT; [L], p<0.05 *vs* HFD-UNT and ND-UNT; [m], p<0.01 *vs* HFD-UNT and HFD-UNT-C; [n], p<0.001 *vs* HFD-UNT.

Fig.5. Differences between experimental groups on the activities of pyruvate kinase (A), citrate synthase (B), complex II (C), succinate dehydrogenase (SDH) (D) and cytochrome *c* oxidase (E) in the cerebral cortex and cerebellum of rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training

+ L-Carnitine. [a], p<0.05 *vs* HFD-HIIT; [b], p<0.001 *vs* HFD-UNT and HFD-UNT-C; [c], p<0.01 *vs* ND-UNT; [d], p<0.001 *vs* HFD-HIIT, HFD-UNT-C, HFD-UNT and ND-UNT; [e], p<0.001 *vs* ND-UNT; [f], p<0.05 *vs* HFD-UNT and ND-UNT; [g], p<0.001 *vs* HFD-UNT and ND-UNT.

6.5 Artigo:

HIGH-INTENSITY INTERVAL TRAINING AND L-CARNITINE SUPPLEMENTATION REVERT OXIDATIVE STRESS AND ENERGY METABOLISM ALTERATIONS IN THE GASTROCNEMIUS MUSCLE AND ADIPOSE TISSUE OF OBESE RATS

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ABSTRACT

The purpose of this research was to assess the protective effects of L-Carnitine supplementation and high-intensity interval training (HIIT) on energy metabolism and oxidative stress parameters in the gastrocnemius muscle and adipose tissue of obese rats. The animals were divided into five groups: Normal Diet + Untrained (ND-UNT), High-Fat Diet + Untrained (HFD-UNT), High-Fat Diet + Untrained + L-Carnitine (HFD-UNT-C), High-Fat Diet + High-intensity Interval Training (HFD-HIIT), and High-Fat Diet + High-intensity Interval Training + L-Carnitine (HFD-HIIT-C). The HFD groups were fed with a high-fat diet for 14 weeks to induce obesity, while the ND groups were provided with a standard diet. The HFD-UNT-C and HFD-HIIT-C groups were administered L-Carnitine (300 mg/kg) via gavage at the start of the HIIT protocol, which was carried out five days per week. The UNT group engaged in 40% intensity walking twice a week. After 10 weeks of training, the animals were sacrificed by decapitation, and the adipose tissue and gastrocnemius muscle were extracted and homogenized in a suitable buffer. The study evaluated catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) antioxidant activities, thiobarbituric acid reactive substances (TBA-RS) levels, total sulfhydryl and protein carbonyl contents in both structures, as well as pyruvate kinase, citrate synthase, succinate dehydrogenase (SDH), complex II and cytochrome *c* oxidase activities in the gastrocnemius muscle. Results showed that the HFD promoted oxidative stress and alterations in mitochondrial function. The HIIT protocol, when used alone and, sometimes, when associated with L-Carnitine, reverted these alterations in the gastrocnemius muscle and adipose tissue of obese rats.

Keywords: Obesity; Oxidative stress; Energy Metabolism; L-Carnitine; High-intensity Interval Training, adipose tissue, gastrocnemius muscle.

1 INTRODUCTION

Obesity is a multifactorial disease characterized by the excessive accumulation of body fat due to an imbalance between energy intake and expenditure[1]. It is a global pandemic and has been linked to the development of serious complications, such as cardiovascular issues (e.g. arterial hypertension, coronary insufficiency, atherosclerosis), respiratory problems, skeletal muscle disorders, endocrine-metabolic system disorders (e.g. diabetes mellitus and dyslipidemia), and even certain types of neoplasms[1,2].

Some studies have suggested that obesity and high intake of saturated fatty acids can enhance the development of skeletal muscle atrophy in various muscles, such as the gastrocnemius, soleus and tibialis anterior muscles, and can worsen sarcopenia, which is the combination of reduced muscle mass and increased body fat commonly observed in older adults[3–6]. Sarcopenic obesity is a growing concern worldwide and poses a serious public health threat, and its pathophysiology is associated with many factors such as oxidative stress, inflammatory cytokines, mitochondrial dysfunction, insulin resistance, and physical inactivity[5,7].

The development of these disorders in obese individuals is largely due to oxidative stress, which results in an excessive production of free radicals, such as reactive oxygen species (ROS), and in a greater depletion of the antioxidant defense system[8,9]. This group of people typically experiences an imbalance between the levels of body fat, body weight, lipoproteins, and lipids, leading to an increase in metabolic needs and oxygen consumption[10]. As a result, there is a rise in the production of ROS, such as superoxide and hydrogen peroxide[10].

Furthermore, studies have suggested that, aside from the increased production of free radicals, obesity is linked to a decline in the activity of antioxidant enzymes[11]. Olusi and collaborators conducted a study in 2002 on 250 obese individuals, measuring the activities of superoxide dismutase and glutathione peroxidase enzymes and the findings revealed reduced erythrocyte antioxidant enzyme activities in obese patients, which could lead to progressive cellular damage and pose a significant risk factor for the development of various pathologies associated with obesity[11]. In terms of changes in energy metabolism, research has linked obesity with alterations in

mitochondrial respiratory chain function and adverse effects on carbohydrate metabolism[11].

To minimize harmful effects on both cellular and systemic levels, complementary substances, such as L-Carnitine, have been proposed as adjuvant treatments for obesity due to their potential to reduce the oxidative stress caused by this condition[12]. Several studies indicate that L-Carnitine, an ammonium compound, can aid in *post*-workout muscle recovery and protect cell membranes and DNA from oxidative stress, reason why it is considered an essential nutrient[13]. Additionally, L-Carnitine has been described to have antioxidant properties, protecting against ROS[13].

Along with oral supplementation, physical exercise appears to be a helpful strategy for modulating metabolism[14]. High intensity interval training (HIIT) is a highly popular form of exercise due to its ability to promote high energy expenditure in a shorter amount of time when compared to moderate intensity continuous training (MICT), making it an easy-to-include alternative in everyday activities[15]. Thus, many studies have found significant benefits of HIIT in comparison to sedentary groups, highlighting its protective effects in obese patients against functional impairment of various organs and systems caused by obesity and oxidative stress[16].

Given these findings, this study aimed to evaluate the protective effects of HIIT protocol and L-Carnitine supplementation on oxidative stress and energy metabolism parameters in the adipose tissue and gastrocnemius muscle of obese rats.

2 MATERIALS AND METHODS

2.1 Animals and Reagents

The experiments utilized 60-day-old male Wistar rats from the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil. Prior to the study, the animals were accommodated and acclimatized for seven days to adapt to their new environment. The rats were kept in rooms with a 12-hour light/dark cycle, with the temperature maintained between 20-22°C, and were given free access to food and water. The light/dark cycle was reversed to optimize the animals' active period for training. The rats were housed in cages, with a maximum of four animals per cage, and the bedding was replaced every two days. The animal care was conducted in

compliance with Law No. 11794 (October 8, 2008) and other applicable regulations regarding the use of animals in research and teaching, specifically the Normative Resolutions of the National Council for the Control of Animal Experimentation - CONCEA[17,18].

The room lighting, accommodation, and nutrition provided to the animals followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals[19].

For the *in vivo* experiments, the animals were divided into groups of equal numbers (n=8), as follows: Normal Diet + Untrained (ND-UNT); High-Fat Diet + Untrained (HFD-UNT); High-Fat Diet + Untrained + L-Carnitine (HFD-UNT-C); High-Fat Diet + High-intensity Interval Training (HFD-HIIT); High-Fat Diet + High-intensity Interval Training + L-Carnitine (HFD-HIIT-C).

The experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 012/2017. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2 Experimental Protocols

2.2.1 Dietary induction of obesity

The experimental HFD groups were given a high-fat diet consisting of 20% calories from carbohydrates, 20% from proteins, and 60% from lipids (Prag Soluções Biosciences, Jaú, São Paulo-SP) for fourteen weeks to induce obesity. The control group (ND) received a standard diet (70% carbohydrates, 20% proteins, and 10% lipids) (Quimtia, Curitiba, Paraná, Brazil). All animals had access to water *ad libitum*.

2.2.2 L-Carnitine supplementation

The animals from the experimental groups HFD-UNT-C and HFD-HIIT-C received L-Carnitine supplementation by gavage, in a concentration of 300 mg/ kg of body mass per day, while animals from the experimental groups ND-UNT, HFD-UNT and HFD-HIIT, received saline by gavage once a day.

2.2.3 High-Intensity Interval Training Protocol

A maximum effort tolerance test was applied in order to define the animals' maximum speed and then prescribe the training intensity. This data served as a parameter for the prescription of the training speeds of the HIIT protocol.

The maximum effort test was performed at three times: at the beginning, after four weeks and during the eighth week of the training protocol. Following the protocol of Ferreira *et al*., the test consisted of a running on a treadmill (model KT-4000, IMBRAMED), with an inclination of 20 degrees, with an initial speed of 6 m/min and an increase of 3 m/min every three minutes, until exhaustion of the animal (visible fatigue)[20]. Once the maximum speed was found, distance and speed were computed to calculate training intensity.

The HIIT training protocol was carried out on five days a week, using a 20° inclination of the treadmill, with intensities defined using the exercise tolerance test. HIIT was performed for three minutes at 60% intensity, followed by four minutes at 85% of the maximum test speed[20]. This cycle was repeated seven times, totaling 49 minutes of training (Fig.1).

The animals of the untrained group (UNT) performed a 40% intensity walk, twice a week, in order to maintain the animals' ability to walk for subsequent physical tests.

2.2.4 Tissue preparation

The animals were sacrificed at the end of the 10th week of training (24 weeks into the experiment) by decapitation without anesthesia, since the use of anesthetics can interfere with the determination of oxidative parameters, 48 hours after their last training session[21,22]. The gastrocnemius muscle and adipose tissue were then dissected and homogenized in a suitable buffer. After centrifugation at x 3,000 g and 4°C for 15 min to remove cellular debris, the supernatant was stored in aliquots at -80°C for the analysis of oxidative stress and energy metabolism parameters.

2.3 Biochemical studies

2.3.1 Catalase Assay (CAT)

CAT activity was determined by the method of Aebi (1984)[23]. This method is based on the disappearance of hydrogen peroxide (H_2O_2) in a reaction medium

composed of 25µL of sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. The absorbance was counted every 10 seconds for 1 minute and 40 seconds at 240 nm using a UV-vis Shimadzu spectrophotometer. One CAT corresponds to 1 µmol of H_2O_2 consumed per minute and the specific activity was calculated as CAT units/mg protein.

2.3.2 Superoxide Dismutase Assay (SOD)

The activity of SOD was assayed by the method described by Marklund (1985)[24], using a process highly dependent on superoxide ($O2^{\bullet-}$), which is a substrate for SOD. Sample (15 µL) was added to 215 µL of a mixture containing 50 µM Tris buffer, 1 µM EDTA, pH 8.2, and 30 µM CAT. Subsequently, 20 µL of pyrogallol were added and the absorbance was measured every 30 seconds for 3 minutes at 420 nm using a UV-vis Shimadzu spectrophotometer. Inhibition of the auto-oxidation of pyrogallol occurs in the presence of SOD, the activity can be tested indirectly spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity was reported as SOD units/mg protein.

2.3.3 Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel (1981)[25], using *tert*-butylhydroperoxide as substrate. The decomposition of NADPH was controlled in a UV-vis Shimadzu spectrophotometer at 340 nm for 3 minutes and 30 seconds. 90 µL of each sample were added to the medium containing 800 µL of buffer, 20 µL of 2.0 mM GSH, 30 µL of 0.15 U/mL GSH reductase, 10 µL of 0.4 mM azide, and 10 µL of 0.1 mM NADPH. The absorbance was counted every 10 seconds for 1 minute and 30 seconds. Subsequently, 50 µL of 0.5 mM *tert*-butylhydroperoxide were added and the absorbance was read for a further 2 minutes. One GSH-Px unit is characterized as 1 µmol of NADPH consumed per minute and the specific activity was defined as GSH-Px units/mg of protein.

2.3.4 Total Sulfhydryl Content

The total sulfhydryl content was measured following the method of Aksenov & Markersbery (2001)[26], based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, yielding a yellow derivative (TNB), which is evaluated spectrophotometrically at 412 nm. For the assay, 50 μL of homogenate were added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction started with the addition of 30 μL of 10 mM DTNB and incubated for 30 minutes at room temperature in the dark. Analyses of a blank (DTNB absorbance) were also performed. The results were presented as nmol TNB/mg protein.

2.3.5 Thiobarbituric acid reactive substances (TBA-RS)

TBA-RS were defined according to the method of Ohkawa *et al.* (1979)[27]. The methodology for the study of TBA-RS measures malondialdehyde (MDA), resulting from lipoperoxidation, provided predominantly by hydroxyl free radicals. At first, samples, in 1.15% KCl, were mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBA-RS were determined by the absorbance at 535 nm. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was exposed to the same treatment as that of the supernatants. TBA-RS content was presented in nanomoles of MDA formed per milligram of protein.

2.3.6 Protein carbonyl content

Protein Carbonyl content was tested using the methodology detailed by Reznick & Packer (1994)[28], based on the reaction of protein carbonyls with dinitrophenylhydrazine, in order to form dinitrophenylhydrazone, a yellow compound that is measured spectrophotometrically at 370 nm. Briefly, 200 μL of the gastrocnemius muscle and adipose tissue homogenate were added to plastic tubes containing 400 μL of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). Samples were kept in the dark for 1 h and vortexed every 15 min. Afterwards, 500 μL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 14 000 rpm for 3 min and the resulting supernatant was excluded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed and centrifuged

at 14 000 rpm for 3 min. The supernatant was discarded and the pellet re-suspended in 600 µL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), pre-vortexed and incubated at 60ºC for 15 min. Thus, samples were centrifuged at 14 000 rpm for 3 min and the supernatant was used to measure absorbance at 370 nm (UV) in a quartz cuvette. Results were described as nmol of carbonyl content /mg protein.

2.3.7 Pyruvate kinase activity (PK)

Pyruvate kinase activity was assayed essentially as described by Leong *et al.* (1981)[29]. The incubation medium consisted of 0.1 M Tris–HCl buffer, pH 7.5, 10 mM $MgCl_2$, 0.16 mM NADH, 75 mM KCl, 5 mM ADP, 7.0 units of L-lactate dehydrogenase, 0.1% (v/v) Triton X-100, and 10 µL of the mitochondria-free supernatant in a final volume of 0.5 mL. Unless otherwise stated, the reaction was started after 30 min of pre-incubation by the addition of 1 mM phosphoenolpyruvate (PEP). All assays were performed in duplicate at 25ºC. Results were expressed as µmol of pyruvate formed per min per mg of protein.

2.3.8 Citrate synthase activity (CS)

The activity of citrate synthase was assessed using spectrophotometry, as described by Alp *et al.* (1976)[30]. Homogenized gastrocnemius muscle samples (10µL) were added to cuvettes with 800 µL buffer (1M Tris-HCL, pH 8), 100 µL of 1mM DTNB, 40 µL of 2.5 mM acetyl-CoA and 10 µL of 10% Triton. The reaction was started by the addition of 50 µL of oxaloacetate (4 mM) and changes in absorbance were observed during 3 minutes at 412 nm. Results were expressed as nmol of substrate consumed per minute per mg of protein.

2.3.9 SDH and complex II activities (CII)

The activities of succinate:phenazine oxyreductase (soluble SDH) and complex II (succinate: DCIP oxyredutase) were measured in gastrocnemius muscle homogenates by following the decrease in absorbance due to the reduction of 2,6-dichloroindophenol (DCIP) at 600 nm with 700 nm as the reference wavelength (ε=19.1 mM^{-1} cm^{-1}) in the presence of phenazine methasulphate (PMS), according to Fischer

et al. (1985)[31]. The reaction mixture, consisting of 40 mM potassium phosphate, pH 7.4, 16 mM succinate and 8 μM DCIP, was preincubated with 40-80 μg homogenate protein at 30°C for 20 min. Results were expressed as nmol of substrate consumed per minute per mg of protein. Subsequently, for complex II activity, 4 mM sodium azide and 7 μM rotenone were added and the reaction was initiated by the addition of 40 μM DCIP and monitored for 5 min. The activity of SDH was accessed in the same incubation medium by the addition of 1 mM PMS and monitored for 5 min. Results were expressed as nmol of substrate consumed per minute per mg of protein.

2.3.10 Cytochrome *C* Oxidase (COX) activity

The activity of cytochrome *c* oxidase was measured according to Rustin *et al*. (1994)[32]. Enzymatic activity was measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm with 580 nm as the reference wavelength (ε = 19.1 $mM^{-1}x\ cm^{-1}$). The reaction buffer contained 10 mM potassium phosphate, pH 7.0, 0.6 mM *n*-dodecyl-β-D-maltoside, 2-4 μg homogenate protein and the reaction was initiated by the addition of 0.7 μg reduced cytochrome *c*. The activity of cytochrome *c* oxidase was measured at 25°C for 10 min. Results were expressed as nmol of substrate consumed per minute per mg of protein.

2.3.11 Protein determination

Protein was measured by the Lowry *et al.* (1951)[33] method, using serum bovine albumin as standard.

5.4 Statistical Analysis

Statistical treatment was performed using the R software version 4.0.3 with the aid of the Car and Desctools packages[34–36]. A multiple linear regression model was applied, followed by Analysis of Variance (ANOVA) for unbalanced data, in order to verify whether there were differences in the outcome variables. The normality assumption of the model residuals was verified using the Shapiro-Wilk normality test. Homoscedasticity was assessed using graphs and Levene's test. To identify differences between groups, the Duncan's test was used for multiple comparisons.

For the statistical analysis of body weight and adipose tissue of rats, ANOVA with repeated measures was applied to analyze the relationship between the independent variables and the outcome variable over the period of analysis. To verify the sphericity, the Mauchly test was applied and the Greenhouse-Gueisser correction was applied, if necessary. For the multiple comparison tests, the *t* test with Bonferroni adjustment was applied. To analyze the association between the independent variables and the weight of adipose tissue in the final week, a single-way ANOVA was applied. In the multiple comparison, the Duncan´s test was used.

Values of $p<0.05$ were considered significant. Results were expressed as means ± SD for eight independent experiments (animals) performed in duplicate.

6 RESULTS

3.1 Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats

We initially verified the effects of high fat-diet, high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats, with the aim of validating our obesity model. Fig.2A shows that there were no significant differences on rats´ starting weight between the experimental groups, and shows that in the 14th week, HFD increased the body weight in HFD-UNT, HFD-UNT-C and HFD-HIIT-C groups, when compared to ND-UNT group. Fig.2A also shows that HIIT protocol, isolated or in association with L-Carnitine supplementation, in the 24th week, was able to reverse the increase in this parameter.

With regard to adipose tissue, Fig.2B shows that HFD increased this parameter in HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C, when compared to ND-UNT groups, and that HIIT protocol, isolated or associated with L-Carnitine supplementation, decreased this parameter, when compared to HFD-UNT-C group.

3.2 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on oxidative stress parameters in the adipose tissue and gastrocnemius muscle of obese rats

We initially verified the effects of the HIIT protocol and L-Carnitine supplementation on TBA-RS, total sulfhydryl content, protein carbonyl content and on the activities of antioxidant enzymes in the adipose tissue and gastrocnemius muscle of obese rats.

Fig.3A shows that the HFD significantly increased TBA-RS levels in the gastrocnemius muscle, but alone did not altered this parameter in the adipose tissue of obese rats, when compared to ND-UNT group. With regard to HIIT protocol and L-Carnitine supplementation, Fig.3A shows that isolated L-Carnitine supplementation, HIIT protocol alone or in association with L-Carnitine supplementation, significantly reversed the increase in TBA-RS levels in the gastrocnemius muscle, when compared to HFD-UNT, while the HIIT protocol associated with L-Carnitine supplementation also was able to reduce this parameter, when compared to ND-group. With regard to the adipose tissue, HIIT protocol, alone or associated with L-Carnitine supplementation, reduced TBA-RS levels, when compared to ND-UNT, HFD-UNT and HFD-UNT-C groups.

Regarding the total sulfhydryl content, Fig.3B shows that the HFD did not alter this parameter in the gastrocnemius muscle, but decreased this parameter in the adipose tissue of obese rats, when compared to ND-group. Fig.3B also shows that in the gastrocnemius muscle, HIIT protocol associated with L-Carnitine supplementation increased the total sulfhydryl content, while in the adipose tissue, HIIT protocol, isolated and associated with L-Carnitine supplementation, reversed the decrease in this parameter, when compared to HFD-UNT and HFD-UNT-C groups, and HIIT protocol associated with L-Carnitine supplementation increased the total sulfhydryl content in the adipose tissue when compared to ND-group. Fig.3C also shows that there were no significant differences between experimental groups in carbonyl protein content in both structures.

With regard to antioxidant enzyme´s activities, Fig.4A shows that the HFD, isolated L-Carnitine supplementation, HIIT protocol, isolated and associated with L-Carnitine supplementation, significantly increased CAT activity in the gastrocnemius muscle, when compared to ND-group. In the adipose tissue, HFD *per se* did not alter this enzyme´s activity, while HIIT protocol, alone and associated with L-Carnitine supplementation, significantly increased this parameter.

As can be seen in Fig.4B, HFD, L-Carnitine supplementation, HIIT protocol, alone or associated with L-Carnitine supplementation, increased SOD activity in the gastrocnemius muscle and adipose tissue of obese rats, when compared to ND-group.

About GSH-Px activity, Fig.4C shows that HFD reduced this enzyme´s activity in the gastrocnemius muscle and adipose tissue of obese rats, when compared to ND-group. Fig.4C also shows that HIIT protocol associated with L-Carnitine supplementation partially reversed this decrease in the gastrocnemius muscle when compared to ND-UNT and HFD-UNT group, and HIIT protocol alone and associated with L-Carnitine supplementation partially reversed the decrease when compared to ND-UNT, HFD-UNT and HFD-UNT-C groups in the adipose tissue of obese rats.

3.2 Effects of the high-intensity interval training protocol and L-Carnitine supplementation on energy metabolism parameters in the gastrocnemius muscle of obese rats

Subsequently, the effects of HIIT and L-Carnitine supplementation on energy metabolism parameters were also analyzed in the gastrocnemius muscle of obese rats. As can be seen in Fig.5A, the HIIT protocol, when used alone or together with L-Carnitine supplementation, promoted an increase in pyruvate kinase (PK) activity in the gastrocnemius muscle of obese rats, when compared to HFD-UNT and HFD-UNT-C groups.

Fig.5B shows that HFD isolated did not alter citrate synthase (CS) activity, while L-Carnitine supplementation alone and HIIT protocol, isolated and associated with L-Carnitine supplementation, increased this parameter, when compared to ND-UNT, HFD-UNT and HFD-UNT-C group. With regard to complex II enzyme (CII) and SDH activities, Fig.5C e 5D show that there were no significantly differences between experimental groups.

Finally, Fig.5E shows that HFD by itself reduced cytochrome *c* oxidase (COX) activity in the gastrocnemius muscle, when compared to the ND-UNT group, and that the HIIT protocol when used alone, and in association with L-Carnitine supplementation, reversed this alteration when compared to the HFD-UNT and HFD-UNT-C groups.

7 DISCUSSION

In the present study, we investigated whether a HIIT protocol and L-Carnitine supplementation could prevent or protect against oxidative stress and energy metabolism dysfunction in the gastrocnemius muscle and adipose tissue of obese rats. First of all, our results showed that HFD increased body weight and promoted adipose tissue gain in the experimental groups, validating our methodology. Our findings indicated that HFD led to disruptions in antioxidant enzymes´ activities and in the mitochondrial respiratory chain, protein damage and lipoperoxidation in both structures' groups. Furthermore, we observed that the HIIT protocol, either on its own or in conjunction with L-Carnitine supplementation, was capable of reversing or ameliorating these disruptions.

Initially, we assessed levels of TBA-RS, total sulfhydryl and carbonyl protein content in the gastrocnemius muscle and adipose tissue of obese rats. TBA-RS levels are a measure of lipid peroxidation and can indicate damage to cell membranes, which can lead to cell death due to changes in lipid structure, permeability and membrane transport[37–39]. Alterations on the total sulfhydryl and carbonyl protein contents can serve as an indicator of protein damage. Alterations in protein structures can increase their fragmentation and aggregation, as well as make them more vulnerable to degradation by proteasomes[38,39].

Our results showed that HFD increased TBA-RS levels in the gastrocnemius muscle, promoting lipoperoxidation, while L-Carnitine supplementation, HIIT protocol, isolated and associated with L-Carnitine supplementation, reversed this alteration, and HIIT associated with L-Carnitine supplementation decreased TBA-RS levels in the gastrocnemius muscle. In a study carried out in 2014, Xie and collaborators found significantly higher levels of MDA in the gastrocnemius muscle of mice fed high-fat diet when compared to the control group, which corroborates our data[40].

In the adipose tissue, HIIT protocol, alone and associated with L-Carnitine supplementation, also reduced TBA-RS levels. These data suggest that HIIT protocol and L-Carnitine have a protective role against lipoperoxidation in both structures, probably by increasing enzymatic antioxidant activity, reducing free radicals, such as hydroxyl radical, and for stabilization cell membranes. Although our results showed that HFD *per se* did not increase TBA-RS levels in this structure, Charradi *et al*. (2013) found that the HFD promoted a 74% increase in MDA levels while investigating the

effects of a 6-week high fat diet on plasma and white adipose tissue lipid deposition in rats[41]. Regarding to L-Carnitine supplementation, several studies have reported that it can improve the stabilization of cell membranes due to its ability to improve the acetylation of membrane phospholipids, protecting them from lipoperoxidation[42].

The reversal of lipoperoxidation caused by HFD in the gastrocnemius muscle and the reduction in TBA-RS levels in the adipose tissue promoted by the HIIT protocol can be related to a lower production of ROS, to an increase in the expression or in the activity of antioxidant enzymes or even to the fact that physical training can lead to more resistance to oxidative stress, since the tissues or organs became more exposed to ROS, creating mechanisms to adapt to oxidative stress[43].

Regarding the total sulfhydryl content, our data showed that the HFD did not alter this parameter in the gastrocnemius muscle; in contrast, it decreased this parameter in the adipose tissue of obese rats, suggesting protein damage to this structure. In addition, our data also showed that in the gastrocnemius muscle, HIIT protocol associated with L-Carnitine supplementation enhanced this parameter, while in the adipose tissue, HIIT protocol, isolated and associated with L-Carnitine supplementation, reverted the decrease, and HIIT protocol associated with L-Carnitine supplementation increased the total sulfhydryl content in the adipose tissue. These findings indicate that both HIIT protocol and L-Carnitine supplementation have a protective impact on protein damage, as they reverse the decrease and elevate the total sulfhydryl content in the both structures.

Results also shows that there were no significant differences between experimental groups in carbonyl protein content in both structures. Corroborating our data, in 2013, Charradi *et al*. investigated the effects of a 6-week high fat diet on white adipose tissue in rats and also did not find any significant carbonylation[41]. Conversely, Xie and collaborators (2014) reported an increase in carbonyl protein content in the gastrocnemius muscle of mice fed high-fat diet, showing that HFD promoted protein damage in this case[40].

Furthermore, we assessed the antioxidant activities of CAT, SOD and GSH-Px in the gastrocnemius muscle and adipose tissue of obese rats. Our data showed that HFD, L-Carnitine supplementation, HIIT protocol, isolated and in association with L-Carnitine supplementation, increased CAT and SOD activities and decreased GSH-Px

activity in the gastrocnemius muscle, while HIIT protocol associated with L-Carnitine supplementation partially reversed this decrease.

In contrast with our data, Xie and collaborators (2014) showed that mice fed high-fat diet presented decreased SOD, CAT and GSH-Px activities in the gastrocnemius muscle[40]. The differences in SOD and CAT activities between our and their results may be due to differences in the total experiment time, with our animals being fed high-fat diet for 24 weeks, while theirs were fed for 20 weeks.

In the adipose tissue, HFD *per se* did not alter CAT activity, while HIIT protocol, alone and associated with L-Carnitine supplementation, significantly increased CAT activity. About SOD activity, HFD, isolated L-Carnitine supplementation, HIIT protocol, alone or associated with L-Carnitine supplementation, increased SOD activity in obese rats, while HFD, isolated L-Carnitine supplementation, HIIT protocol, alone or associated with L-Carnitine supplementation, reduced GSH-Px activity, alteration that was partially reversed by HIIT protocol alone and associated with L-Carnitine supplementation. Conversely, Charradi *et al.* (2013) reported a decrease by 31% and 67% in SOD and GSH-Px activity, respectively, and no alteration on CAT activity in white adipose tissue of rats fed with HFD[41]. Similar to the differences between our and Xie and collaborators results in the gastrocnemius muscle, these differences in antioxidant enzymes´ activities in the adipose tissue may be due to differences in the total experimental time, with our animals being fed high-fat diet for 24 weeks, while Charradi *et al*. (2013) animals were fed only for 6 weeks[41].

The enhanced SOD activity in our study may suggest that HFD promoted an increase in ROS production, such as the superoxide radical, in the gastrocnemius muscle and adipose tissue, and as higher levels of superoxide radical lead to higher levels of hydrogen peroxide, this could explain the increase in CAT activity in the gastrocnemius muscle. Additionally, the reduction in GSH-Px activity may have contributed to the increase in CAT activity in this structure and an enzymatic saturation due to the need to neutralize hydrogen peroxide in the adipose tissue. Corroborating our data, Travacio and Llesuy (1996) describes that in order to minimize or prevent oxidative damage, the antioxidant activity of an enzyme can be increased[44].

Regarding to the alterations in antioxidant enzymes´ activities promoted by the HIIT protocol in both structures, Steinbacher and Eckl (2015), carried out a review

evaluating the available evidences on the effects of ROS in exercising muscle and found out that, in general, several studies pointed to a link between increased antioxidant protein levels and antioxidant activity induced by exercise[45]. They also found out that endurance training promotes an enhance in SOD, GSH-Px and CAT activities in skeletal muscle, probably to a greater need of oxygen consumption, corroborating our data[46–48].

With regard to the energy metabolism of the gastrocnemius muscle, HFD *per se* did not alter pyruvate kinase (PK) activity. The PK enzyme plays a crucial role in glycolysis by oxidizing phosphoenolpyruvate to ATP and pyruvate[49,50]. A decline in its activity can hamper both aerobic and anaerobic pathways of energy metabolism, thereby affecting overall energy production[49,50]. However, the HIIT protocol, either alone or in conjunction with L-Carnitine, was found to augment PK activity in the gastrocnemius muscle, which suggests that mitochondrial energy efficiency can be improved by exercise.

About citrate synthase (CS) activity, citric acid cycle enzyme, our data showed that HFD did not alter this enzyme´s activity, while isolated L-Carnitine supplementation and HIIT protocol, alone and associated with L-Carnitine supplementation, increased this parameter, suggesting greater efficiency in aerobic metabolism. In contrast, there were no significantly differences between the experimental groups with regard to complex II enzyme (CII) and SDH activities. Corroborating our data, Yokota *et al*. (2009) also did not find significant differences in the activities of complex II and IV, while investigating the effects of the HFD in the skeletal muscles of diabetic and obese mice[50].

Regarding cytochrome *c* oxidase (COX) activity, HFD by itself decreased this parameter and HIIT protocol, alone or in association with L-Carnitine supplementation, reversed this decrease, improving energy metabolism. With regard to changes caused by the HIIT protocol in CS and COX activities, several studies also reported an increase in these enzymes' activities in mice and rats, corroborating our data, and also demonstrating that HIIT improves mitochondrial content in skeletal muscles[52–56]. We suggest that the HIIT protocol and L-Carnitine supplementation increases the expression and/or activity of antioxidant enzymes, reducing ROS and, consequently, lipoperoxidation and mitochondrial dysfunction in gastrocnemius muscle and adipose

tissue of rats. These alterations contribute to lower oxidative stress and better efficiency of the electron transport chain in obese animals, probably reducing the inflammatory process and insulin resistance associated with obesity.

Collectively, our findings shed light on the role of the HFD in inducing oxidative stress and disrupting energy metabolism in the gastrocnemius muscle and adipose tissue of obese rats. Moreover, the implementation of the HIIT protocol and L-Carnitine supplementation may offer promising strategies for mitigating and potentially even reversing such damage.

Conflict of interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

Acknowledgements

This work was supported by grants from Fundo de Apoio à Pesquisa da Universidade da Região de Joinville, Fundo de Apoio à Pesquisa de Santa Catarina (FAPESC), Fundação Universidade Regional de Blumenau (FURB), Uniedu/Pipe/Article 170 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

We thank the statistics teacher, Dra. Elisa Henning (Udesc/Joinville), for statistical analysis.

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training increases intrinsic rates of mitochondrial fatty acid oxidation in rat red and white skeletal muscle. *Appl Physiol Nutr Metab*. 2013;38:326-333.

Fig.1. HIIT Protocol

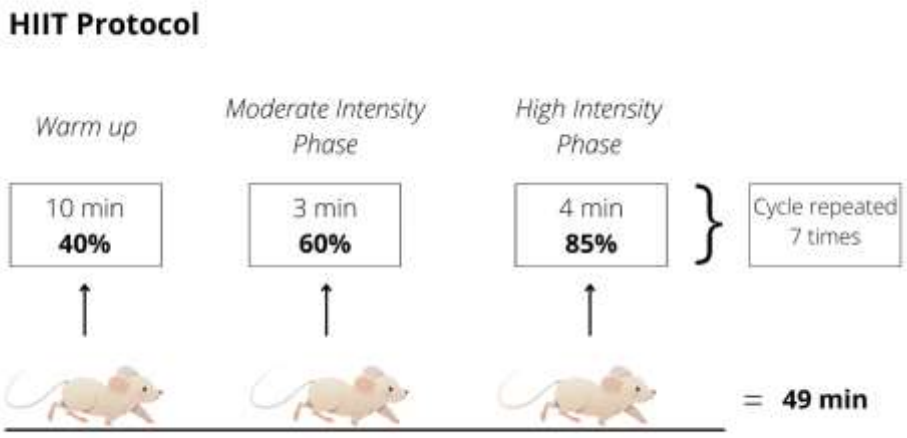
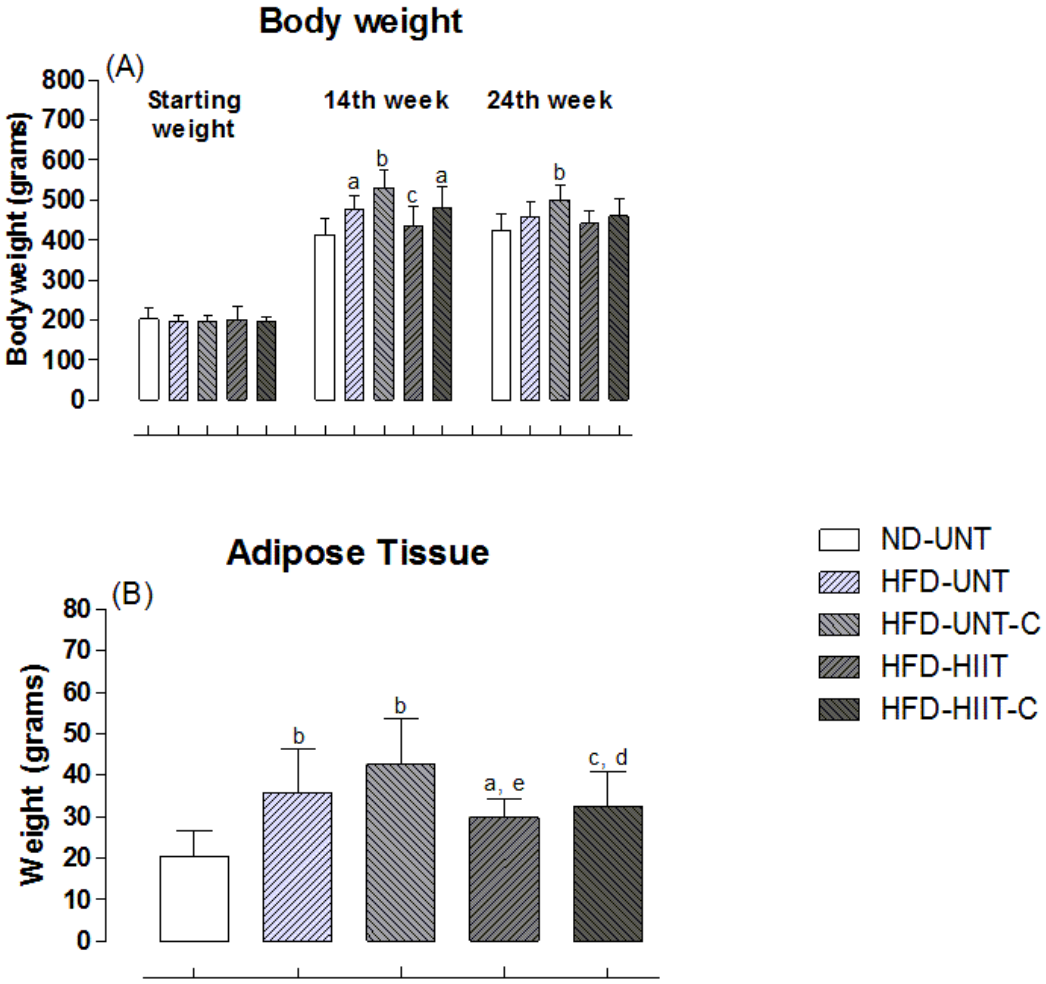
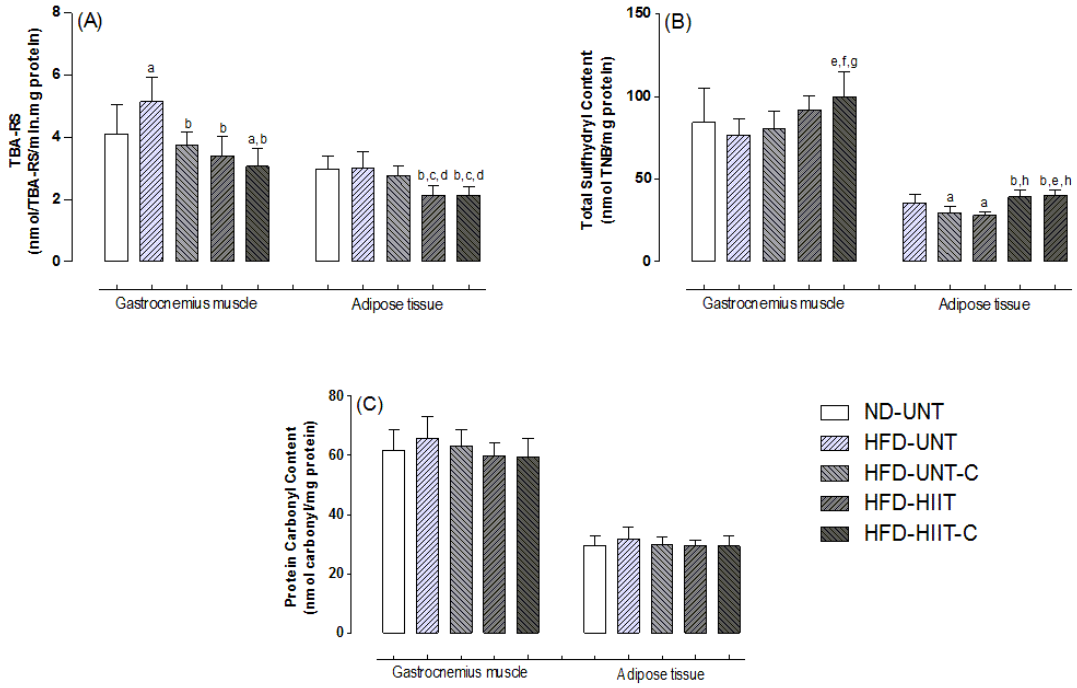


Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats submitted to different experimental protocols.



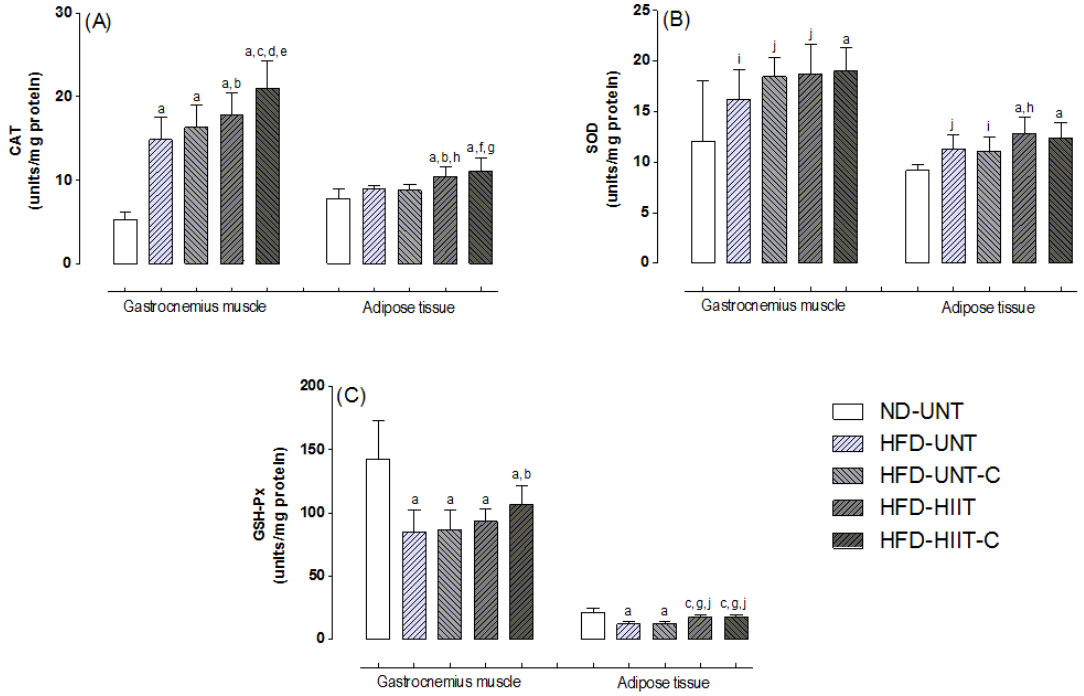
Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* ND-UNT; [b], $p<0.001$ *vs* ND-UNT; [c], $p<0.05$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-UNT; [e], $p<0.01$ *vs* HFD-UNT-C.

Fig.3. Effects of high-intensity interval training protocol and L-Carnitine supplementation on TBA-RS (A), Total Sulfhydryl Content (B) and Protein Carbonyl Content in the gastrocnemius muscle and adipose tissue of obese rats.



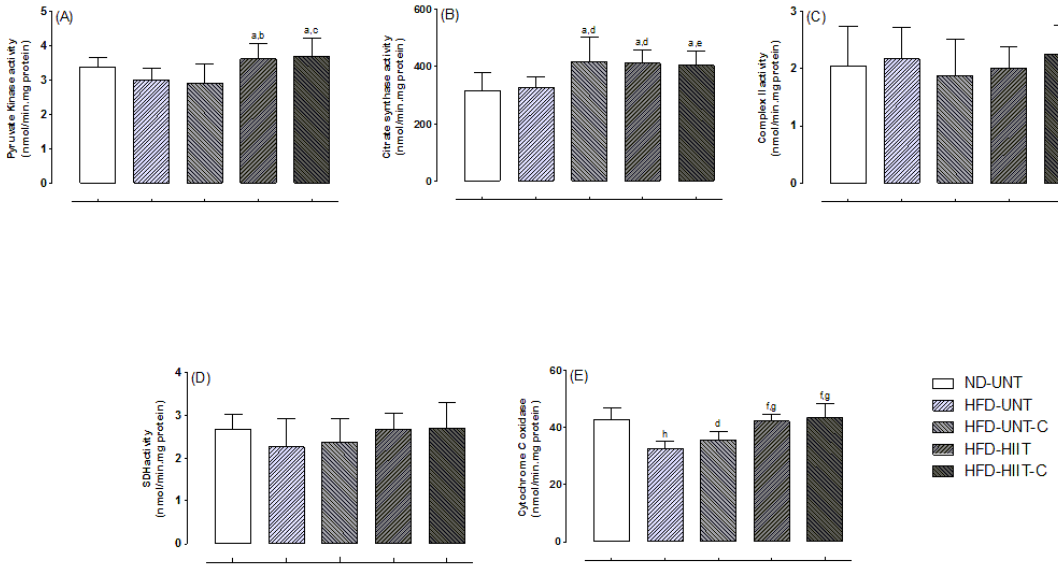
Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.01$ *vs* ND-UNT; [b], $p<0.001$ *vs* HFD-UNT; [c], $p<0.001$ *vs* ND-UNT; [d], $p<0.01$ *vs* HFD-UNT-C; [e], $p<0.05$ *vs* ND-UNT; [f], $p<0.01$ *vs* HFD-UNT; [g], $p<0.05$ *vs* HFD-UNT-C; [h], $p<0.001$ *vs* HFD-UNT-C.

Fig.4. Effects of high-intensity interval training protocol and L-Carnitine supplementation on the activities of catalase (A), superoxide dismutase (B) and glutathione peroxidase (C) in the gastrocnemius muscle and adipose tissue of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.001 *vs* ND-UNT; [b], p<0.05 *vs* HFD-UNT; [c], p<0.001 *vs* HFD-UNT; [d], p<0.01 *vs* HFD-UNT-C; [e], p<0.05 *vs* HFD-HIIT; [f], p<0.01 *vs* HFD-UNT; [g], p<0.001 *vs* HFD-UNT-C; [h], p<0.05 *vs* HFD-UNT-C; [i], p<0.05 *vs* ND-UNT; [j], p<0.01 *vs* ND-UNT.

Fig.5. Differences between experimental groups on the activities of pyruvate kinase (A), citrate synthase (B), complex II (C), succinate dehydrogenase (SDH) (D) and cytochrome c oxidase (E) in the gastrocnemius muscle of obese rats.



Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* HFD-UNT; [b], p<0.05 *vs* HFD-UNT-C; [c], p<0.001 *vs* HFD-UNT-C; [d], p<0.01 *vs* ND-UNT; [e], p<0.05 *vs* ND-UNT; [f], p<0.001 *vs* HFD-UNT; [g], p<0.01 *vs* HFD-UNT-C; [h], p<0.001 *vs* ND-UNT.

Legends to figures

Fig.1. HIIT Protocol.

Fig.2. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], p<0.05 *vs* ND-UNT; [b], p<0.001 *vs* ND-UNT; [c], p<0.05 *vs* HFD-UNT-C; [d], p<0.01 *vs* ND-UNT; [e], p<0.01 *vs* HFD-UNT-C.

Fig.3. Effects of high-intensity interval training protocol and L-Carnitine supplementation on TBA-RS (A) and Total Sulfhydryl Content (B) in the gastrocnemius muscle and adipose tissue of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.01$ *vs* ND-UNT; [b], $p<0.001$ *vs* HFD-UNT; [c], $p<0.001$ *vs* ND-UNT; [d], $p<0.01$ *vs* HFD-UNT-C; [e], $p<0.05$ *vs* ND-UNT; [f], $p<0.01$ *vs* HFD-UNT; [g], $p<0.05$ *vs* HFD-UNT-C; [h], $p<0.001$ *vs* HFD-UNT-C.

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Fig. 5. Effects of high-intensity interval training protocol and L-Carnitine supplementation on the activities of pyruvate kinase (A), citrate synthase (B), complex II (C), succinate dehydrogenase (SDH) (D) and cytochrome *c* oxidase (E) in the gastrocnemius muscle of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + L-Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + L-Carnitine. [a], $p<0.05$ *vs* HFD-UNT; [b], $p<0.05$ *vs* HFD-UNT-C; [c], $p<0.001$ *vs* HFD-UNT-C; [d], $p<0.01$ *vs* ND-

UNT; [e], p<0.05 *vs* ND-UNT; [f], p<0.001 *vs* HFD-UNT; [g], p<0.01 *vs* HFD-UNT-C; [h], p<0.001 *vs* ND-UNT.

7 CONSIDERAÇÕES FINAIS

O presente estudo mostrou que a ingestão de uma dieta hiperlipídica promoveu um quadro de estresse oxidativo, uma vez que alterou a atividade enzimática dos sistemas antioxidantes endógenos e alterou parâmetros oxidativos, como TBA-RS, conteúdo total de sulfidrilas e proteínas carboniladas, em várias das estruturas analisadas. Além disso, nossos dados demonstraram alterações em parâmetros bioquímicos, como dosagem de insulina e colesterol total no soro, e de metabolismo energético nos músculos esqueléticos e estruturas cerebrais de ratos obesos.

Com isso foi possível evidenciar que a ingestão de uma dieta hiperlipídica promoveu lipoperoxidação, danos às proteínas, alterações nas atividades das enzimas antioxidantes e da cadeia respiratória mitocondrial e alterações bioquímicas, contribuindo assim para elucidar o papel do estresse oxidativo e dos distúrbios no metabolismo energético mitocondrial e metabolismo de lipídeos e carboidratos na fisiopatogênese da obesidade.

Ao estudar os efeitos do treinamento intervalado de alta intensidade (HIIT) e da suplementação com L-Carnitina sobre o estresse oxidativo, metabolismo energético, parâmetros bioquímicos e inflamação causados pela obesidade, evidenciou-se a capacidade antioxidante e o efeito protetor do protocolo HIIT, isolado ou em associação com a L-Carnitina, sobre as alterações no estado redox, no metabolismo energético e parâmetros bioquímicos, uma vez que o mesmo se mostrou capaz de reverter total ou parcialmente a maioria dos efeitos deletérios causados pela ingestão da dieta hiperlipídica.

Em conclusão, nossos dados indicam que a patogênese da obesidade está associada à um quadro de estresse oxidativo e disfunção energética mitocondrial, relacionado ao aumento de radicais livres, e que o protocolo HIIT, isolado e/ou associado a suplementação com L-Carnitina apresentou papel protetor e em algumas situações, preventivo, dos danos oxidativos causados pela obesidade.

Esses dados podem servir para uma maior compreensão sobre a fisiopatologia da obesidade e adoção de novas estratégias para o combate às síndromes metabólicas.

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APÊNDICE A – Artigo publicado como capítulo no livro: “Medicina do Exercício e do Esporte: evidências científicas para uma abordagem multiprofissional” - Protective effects of HIIT and L-Carnitine supplementation against obesity-induced oxidative stress and biochemical alterations in the blood of rats

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PROTECTIVE EFFECTS OF HIIT AND L-CARNITINE SUPPLEMENTATION AGAINST OBESITY-INDUCED OXIDATIVE STRESS AND BIOCHEMICAL ALTERATIONS IN THE BLOOD OF RATS

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doi 10.37885/230312516

ABSTRACT

Objective: This study evaluated the effects of High-intensity Interval Training (HIIT) and L-Carnitine supplementation on oxidative stress and biochemical parameters in the blood of obese rats. **Methods:** Animals were divided into five groups: Normal Diet-Untrained (ND-UNT), High-Fat Diet-Untrained (HFD-UNT), High-Fat Diet-Untrained + Carnitine (HFD-UNT-C), High-Fat Diet + HIIT (HFD-HIIT) and High-Fat Diet + HIIT + Carnitine (HFD-HIIT-C). To induce obesity, animals in the HFD groups were fed on a high-fat diet for 14 weeks, while animals in the ND groups were fed on a standard diet. Animals in the HFD-UNT-C and HFD-HIIT-C groups received L-Carnitine by gavage as soon as the HIIT protocol was started (5 days a week) and animals in the UNT group walked at 40% intensity (twice a week). After the end of the 10th week of training, animals were sacrificed by decapitation and their blood was collected and prepared according to the technique. The antioxidant activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), levels of thiobarbituric acid reactive substances (TBARS), total sulfhydryl and protein carbonyl content, glucose, insulin, triglycerides, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c) and total cholesterol were determined. **Results:** HFD promoted lipoperoxidation, protein damage, and alterations in the activities of antioxidant enzymes, increased LDL-c and insulin levels; the HIIT protocol, isolated and sometimes associated with L-Carnitine, prevented these alterations. **Conclusion:** These results indicate that the HFD promoted oxidative stress and alterations in biochemical parameters, and that the HIIT protocol, isolated and associated with L-Carnitine, has antioxidant potential.

Keywords: Obesity, Oxidative Stress, Blood, L-Carnitine, High-Intensity Aerobic Training.

INTRODUCTION

Obesity, considered a global public health problem, is a polygenic and multifactorial disease, characterized by an excess of body fat and is identified as a risk factor for the development of cardiometabolic disorders, diabetes, dyslipidemia, atherosclerosis, cancer, respiratory disease and other inflammatory diaseases[1,2]. Several studies have shown that obese patients present higher levels of circulating cytokines, promoting an inflammatory state, that may also be related to insulin resistance, hyperlipidemia and metabolic syndrome[3].

Furthermore, obesity has been associated with oxidative stress by several researchers. According to França *et al.* (2013), obese patients present some biological alterations that make them more susceptible to oxidative damage, probably due to an imbalance between the amounts of fat, body weight, lipoproteins and lipids, an imbalance that promotes an increase in metabolic need and oxygen consumption and, consequently, an increased production of reactive oxygen species (ROS), such as superoxide and peroxides of hydrogen[4].

The association between obesity, inflammation and oxidative stress is mediated by different physicochemical pathways, such as an increase in blood glucose levels, an increase in the generation and storage of lipids, stimulation in fatty acid oxidation and also an increase in proinflammatory cytokines, promoting oxidative stress and metabolic disorders[2].

Among the various forms of obesity prevention and treatment, great importance has been given to the regular practice of physical activity, due to its beneficial results and low cost[1,5,6]. According to Aparicio *et al.* (2016), high-intensity interval training (HIIT) may be more beneficial than moderate intensity exercise to improve body composition and metabolic syndrome alterations[5]. In addition, L-Carnitine has been studied as a supplement option in these cases and can be used as a supporting treatment for dyslipidemia, due to its potential antioxidant effects, and its ability to participate in the transfer reactions of free fatty acids, improving their oxidation[7].

Considering that obesity is related to oxidative stress, inflammatory and biochemical alterations, and that studies indicate that the HIIT protocol and L-Carnitine supplementation can promote benefits in obese patients, this study evaluated the protective effects of HIIT and L-Carnitine supplementation on oxidative stress and biochemical parameters in the blood of obese rats.

METHODS

Animals and Reagents

Sixty-day-old male Wistar rats from the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil, were used in the experiments. Before the experiments, animals were accommodated and acclimatized for 7 days to adapt to their new environment. Animals were kept in rooms with a 12h light/dark cycle with the temperature maintained between 20-22°C and free access to food and water. The 12h light/dark cycle was inverted for better use of the animals' active period for training. The animals were kept in cages with a maximum number of four per cage; box exchange was performed every 2 days. Animal care was carried out in accordance with Law No. 11794 (October 8, 2008), and other regulations applicable to the use of animals in teaching and/or research, especially the Normative Resolutions of the National Council for the Control of Animal Experimentation – CONCEA[8,9]. Room lighting, accommodation and nutrition used followed the recommendations of the Guide for the Care and Use of Laboratory Animals[10].

For the *in vivo* experiments, animals were divided, in equal numbers, into the following groups:

1) Normal Diet-Untrained (ND-UNT, n = 8);
2) High-Fat Diet-Untrained (HFD-UNT, n = 8);
3) High-Fat Diet-Untrained + L-Carnitine (HFD-UNT-C, n = 8);
4) High-Fat Diet + High-intensity Interval Training (HFD-HIIT, n = 8);
5) High-Fat Diet + High-intensity Interval Training + L-Carnitine (HFD-HIIT-C, n = 8).

The experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 012/2017. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

Experimental Protocols

Dietary induction of obesity

The animals in the experimental groups (HFD) were fed with a high-fat diet, composed of 20% of calories from carbohydrates, 20% of proteins and 60% of lipids (Prag Soluções Biosciences, Jaú, São Paulo-SP), for fourteen weeks, in order to induce the condition of obesity. The animals in the control group (ND) were treated with a standard diet (70%

carbohydrates, 20% proteins and 10% lipids) (Quimtia, Curitiba, Paraná, Brazil). All animals received water ad libitum.

L-Carnitine supplementation

The animals from the experimental groups HFD-UNT-C and HFD-HIIT-C received L-Carnitine supplementation by gavage, in a concentration of 300 mg/kg of body mass per day, while animals from the experimental groups ND-UNT, HFD-UNT and HFD-HIIT, received saline by gavage once a day.

High Intensity Interval Training Protocol

The maximum effort tolerance test was performed to find the maximum speed that each rat could run. This data served as a parameter for the prescription of the training speeds of the HIIT protocol. The maximum effort test was performed in three moments: at the beginning, after four weeks and in the eighth week of the training protocols. Following the protocol of Ferreira *et al*., the test consisted of running on a treadmill (model KT-4000, IMBRAMED), with an inclination of 20 degrees, with an initial speed of 6 m/min that increased by 3 m/min every three minutes, until exhaustion of the animal (visible fatigue)[11]. Once the maximum speed was found, distance and speed were computed to calculate training intensity.

The HIIT protocol was administered with a frequency of five days a week, 20° inclination of the treadmill and intensities defined from the exercise tolerance test. HIIT was applied in three minutes at 60% intensity followed by four minutes at 85% of the maximum test speed[11]. This cycle was repeated seven times, totaling 49 minutes of training.

The animals of the untrained group (UNT) performed a 40% intensity walk for 10 minutes, twice a week, in order to maintain the animals' ability to walk for subsequent physical tests.

Preparation of samples

After the end of the 10th week of training (24 weeks of experiment), the animals were fasted for 12 hours, before being sacrificed (48 hours after the last training session) by decapitation, without anesthesia, since the use of anesthetics can interfere with the determination of oxidative parameters[12,13], and peripheral whole blood was collected and processed, according to the technique to be used.

For erythrocyte separation, peripheral blood was collected and transferred to heparinized tubes, which were centrifuged at 1,000 rpm; the plasma was then removed by aspiration and maintained frozen at –80 °C until assay. Erythrocytes were washed three times with cold saline solution (0.153 mol/L sodium chloride) and lysates were prepared by the addition of

1 mL of distilled water to 100 µL washed erythrocytes and maintained frozen at –80 °C until determination of the activities of antioxidant Enzyme[14]. For the determination of antioxidant enzyme activity, erythrocyte lysates were frozen and thawed three times, and centrifuged at 13,500 rpm for 10 min. The supernatant was diluted in order to contain approximately 0.5 mg/mL of protein[14].

Serum was prepared from blood samples obtained from rats. The peripheral blood was rapidly collected and transferred to tubes without anticoagulant and centrifuged at 1,000 rpm for 10 min; the serum was then separated and used for the measurement of glucose, insulin, triglycerides, total cholesterol, HDL-cholesterol (HDL-c), LDL-c, TNF-α, IL-6 and IL-1β[14].

Biochemical studies

Analysis of oxidative stress parameters

Catalase assay (CAT)

CAT activity was determined by the method of Aebi[15], using a UV-vis Shimadzu spectrophotometer. The method used is based on the disappearance of hydrogen peroxide (H_2O_2) at 240 nm in a reaction medium containing 25µL of sample and 600µL of 10 mM potassium phosphate buffer, pH 7.0, 20mM H_2O_2. The absorbance was measured every 10 seconds for 1 minute and 40 seconds at 240 nm using a spectrophotometer. One CAT unit is defined as 1µmol of H_2O_2 consumed per minute and the specific activity is calculated as CAT units/mg protein.

Superoxide dismutase assay (SOD)

The activity of SOD was assayed by the method described by Marklund[16], using a process highly dependent on superoxide ($O_2^{\bullet-}$), which is a substrate for SOD. Sample (15µL) was added to 215µL of a mixture containing 50µM Tris buffer, 1µM EDTA, pH 8.2, and 30µM CAT. Subsequently, 20µL of pyrogallol were added and the absorbance was measured every 30 seconds for 3 minutes at 420 nm using a UV-vis Shimadzu spectrophotometer. Inhibition of the auto-oxidation of pyrogallol occurs in the presence of SOD, the activity of which can be tested indirectly spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity is reported as SOD units/mg protein.

Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel[17] using *tert*-butyl hydroperoxide as substrate. The decomposition of NADPH was monitored in a spectrophotometer at 340 nm for 3 minutes and 30 seconds using a UV-vis Shimadzu spectrophotometer. The medium contained 90µL of each sample, 800µL of buffer, 20µL of 2.0 mM GSH, 30µL of 0.15 U/mL GSH reductase, 10µL of 0.4 mM azide and 10µL of 0.1 mM NADPH. The absorbance was determined every 10 seconds for 1 minute and 30 seconds. Afterwards, 50µL of 0.5 mM *ter-t*-butylhydroperoxide were added and the absorbance was read for another 2 minutes. One GSH-Px unit is defined as 1µmol of NADPH consumed per minute and the specific activity is reported as GSH-Px units/mg of protein.

Total sulfhydryl content

The total sulfhydryl content was determined, according to the method described by Aksenov and Markersbery[18], which is based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, generating a yellow derivative (TNB) that is measured spectrophotometrically at 412 nm. For the assay, 50µL of plasma were added to 1mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction was started with the addition of 30µL of 10 mM DTNB and incubated for 30 minutes at room temperature in the dark. Analyses of a blank (DTNB absorbance) were also performed. The results were expressed as nmol TNB/mg protein.

Thiobarbituric acid reactive substances (TBA-RS)

TBA-RS were determined according to the method described by Ohkawa *et al.*[19]. The TBA-RS methodology measures malondialdehyde (MDA), a product of lipoperoxidation, generated mainly by OH· radicals. TBA-RS were determined by the absorbance at 535 nm. At first, plasma in 1.15% KCl was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid, and heated in a boiling water bath for 60 min. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was exposed to the same treatment as that of the supernatants. The results were expressed in nmol of MDA/mg protein.

Protein carbonyl content

Protein carbonyl content was assayed by a method described by Reznick & Packer (1994)[20], based on the reaction of protein carbonyls with dinitrophenylhydrazine to form

dinitrophenylhydrazone, a yellow compound that is measured spectrophotometrically at 370 nm. Initially, 200μL of plasma were added to plastic tubes containing 400μL of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). Samples were kept in the dark for 1h and vortexed every 15 min. Subsequently, 500μL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 14,000 rpm for 3 min and the resulting supernatant was discarded. The pellet was washed with 1mL ethanol/ethyl acetate (1:1 v/v), vortexed and centrifuged at 14,000 rpm for 3 min. The supernatant was discarded and the pellet re-suspended in 600μL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), pre-vortexed and incubated at 60°C for 15 min. Afterwards, samples were centrifuged at 14,000 rpm for 3 min and the supernatant was used to measure absorbance in a quartz cuvette. Results were reported as carbonyl content (nmol/mg protein).

Protein determination

Protein was measured by the Lowry *et al.* (1951)[21] method, using serum bovine albumin as standard.

Analysis of biochemical parameters

Determination of glucose, triglycerides, total cholesterol, HDL-c, LDL-c and insulin

The measurements of glucose, triglycerides, total cholesterol and HDL-c were performed in serum samples collected at the time of animal sacrifice, using specific kits from the Labtest brand. Absorbance was determined using a Shimadzu UV-visible spectrophotometer, according to the manufacturer's guidelines. LDL-c levels were determined using Friedewald´s formula.

The measurement of insulin was performed in serum samples collected at the time of animal sacrifice, using the Advia Centaur CP Immunoassay System (Siemens Healthineers).

Statistical analysis

Statistical analysis was performed using the R software version 4.0.3 with the aid of the Car and Desctools packages[22–24]. A multiple linear regression model was applied, followed by Analysis of Variance (ANOVA) for unbalanced data, in order to verify whether there were differences in the outcome variables. The normality assumption of the model residuals was verified using the Shapiro-Wilk normality test. Homoscedasticity was assessed using graphs and Levene's test. To identify differences between groups, Duncan's test was used for multiple comparisons.

For the statistical analysis of body weight and adipose tissue of rats, ANOVA with repeated measures was applied to analyze the relationship between the independent variables and the outcome variable over the period of analysis. To verify the sphericity, the Mauchly test was applied and the Greenhouse-Gueisser correction was applied, if necessary. For the multiple comparison tests, the *t* test with Bonferroni adjustment was applied. To analyze the association between the independent variables and the weight of adipose tissue in the final week, a single-way ANOVA was applied. In the multiple comparison, the Duncan´s test was used.

Values of $p<0.05$ were considered significant. Results are expressed as means ± SD for eight independent experiments (animals) performed in duplicate.

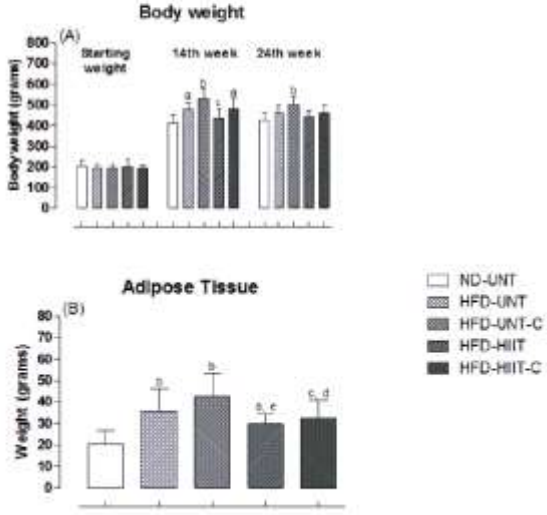
■ RESULTS

Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats

We initially verified the effects of high fat-diet, high-intensity interval training (HIIT) protocol and L-Carnitine supplementation on the body weight and adipose tissue of rats, with the aim of validating our obesity model. Figure 1A shows that there were no significant differences on rats´ starting weight between the experimental groups, and shows that in the 14th week, HFD increased the body weight in HFD-UNT, HFD-UNT-C and HFD-HIIT-C groups, when compared to ND-UNT group. Figure 1A also shows that HIIT protocol, isolated or in association with L-Carnitine supplementation, in the 24th week, were able to revert the increase in this parameter.

With regard to adipose tissue, Figure 1B shows that HFD increased this parameter in HFD-UNT, HFD-UNT-C, HFD-HIIT and HFD-HIIT-C, when compared to ND-UNT groups, and that HIIT protocol, isolated or associated with L-Carnitine supplementation, decreased this parameter, when compared to HFD-UNT-C.

Figure 1. Effects of high fat-diet, high-intensity interval training protocol and L-Carnitine supplementation on the body weight (A) and adipose tissue (B) of rats submitted to different experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + Carnitine. a, p<0.05 *vs* ND-UNT; b, p<0.001 *vs* ND-UNT; c, p<0.05 *vs* HFD-UNT-C; d, p<0.01 *vs* ND-UNT; e, p<0.01 *vs* HFD-UNT-C.



Effects of the high-intensity interval training protocol and L-Carnitine supplementation on parameters of oxidative stress in the blood of obese rats

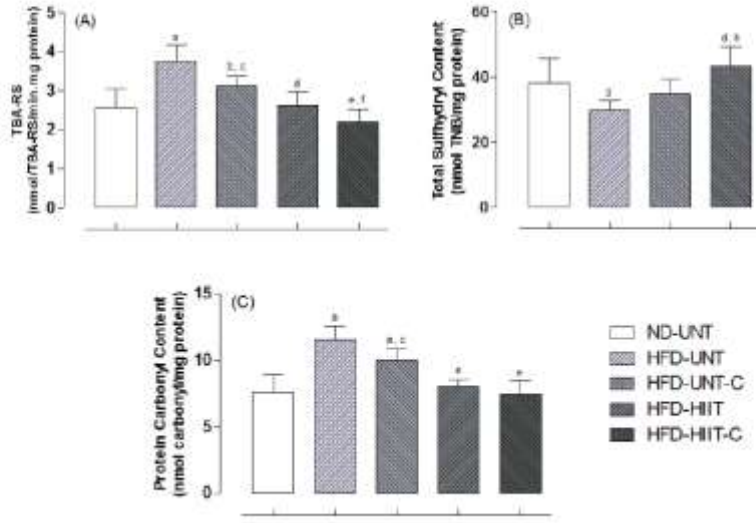
Subsequently, we verified the effects of the HIIT protocol and L-Carnitine supplementation on TBA-RS, total sulfhydryl content, protein carbonyl content and on the activities of antioxidant enzymes in the plasma and erythrocytes of obese rats.

Figure 2A shows that the HFD significantly increased TBA-RS levels in the plasma ($p<0.001$), when compared with ND-UNT group, while L-Carnitine supplementation, on its own, partially reversed this increase. The HIIT protocol, alone and when associated with L-Carnitine supplementation, reversed this alteration.

With regard to the total sulfhydryl content, Figure 2B shows that the HFD significantly decreased this parameter levels in the plasma ($p<0.01$), when compared with the ND-UNT group; furthermore, the HIIT protocol, when used alone or in association with L-Carnitine, was able to reverse this alteration.

In addition, Figure 2C shows that, HFD increased the protein carbonyl content ($p<0.001$) in the plasma, when compared with the ND-UNT group. L-Carnitine supplementation, when used alone, partially reduced this increase, and the HIIT protocol, in isolation or in association with L-Carnitine, reversed this alteration.

Figure 2. Oxidative stress biomarkers in the plasma of obese rats submitted to experimental protocols. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + Carnitine. a, $p<0.001$ *vs* ND-UNT; b, $p<0.05$ *vs* ND-UNT; c, $p<0.01$ *vs* HFD-UNT; d, $p<0.001$ *vs* HFD-UNT; e, $p<0.001$ *vs* HFD-UNT and HFD-UNT-C; f, $p<0.01$ *vs* HFD-HIIT; g, $p<0.01$ *vs* ND-UNT; h, $p<0.01$ *vs* HFD-UNT-C.

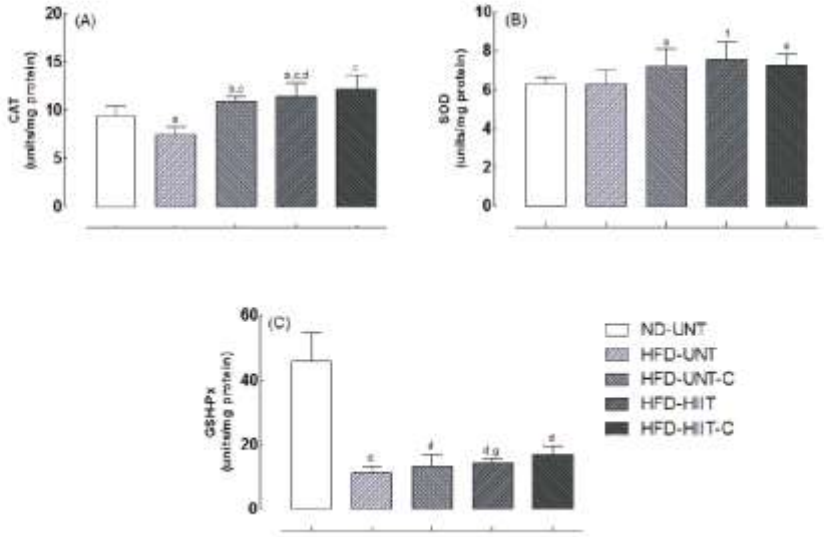


With regard to antioxidant enzymes activities, Figure 3A shows that the HFD reduced CAT activity in the erythrocytes ($p<0.001$), when compared with the ND-UNT group. L-Carnitine supplementation and the HIIT protocol (in isolation and in association with L-Carnitine) were able to reverse and increase this parameter.

Figure 3B shows that L-Carnitine on its own and the HIIT protocol, alone or in association with L-Carnitine, enhanced SOD activity in the erythrocytes ($p<0.05$), when compared with ND-UNT group.

As can be seen in Figure 3C, HFD decreased GSH-Px activity in the erythrocytes of obese rats ($p<0.001$), while neither L-Carnitine supplementation nor the HIIT protocol, alone or when associated with L-Carnitine, were able to reverse this alteration. The HIIT protocol isolated partially reversed this alteration, when compared with ND-UNT.

Figure 3. Effects of the high-intensity interval training protocol and L-Carnitine supplementation on the activities of antioxidant enzymes in the blood of obese rats. Data are presented as means ± SD for 8 independent experiments (animals), performed in duplicate. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + Carnitine. a, p<0.001 *vs* ND-UNT; b, p<0.05 *vs* ND-UNT; c, p<0.001 *vs* HFD-UNT; d, p<0.001 *vs* ND-UNT; e, p<0.05 *vs* ND-UNT and HFD-UNT; f, p<0.01 *vs* ND-UNT and HFD-UNT; g, p<0.05 *vs* HFD-UNT.



Effects of the high-intensity interval training protocol and L-Carnitine supplementation on biochemical parameters in the blood of obese rats

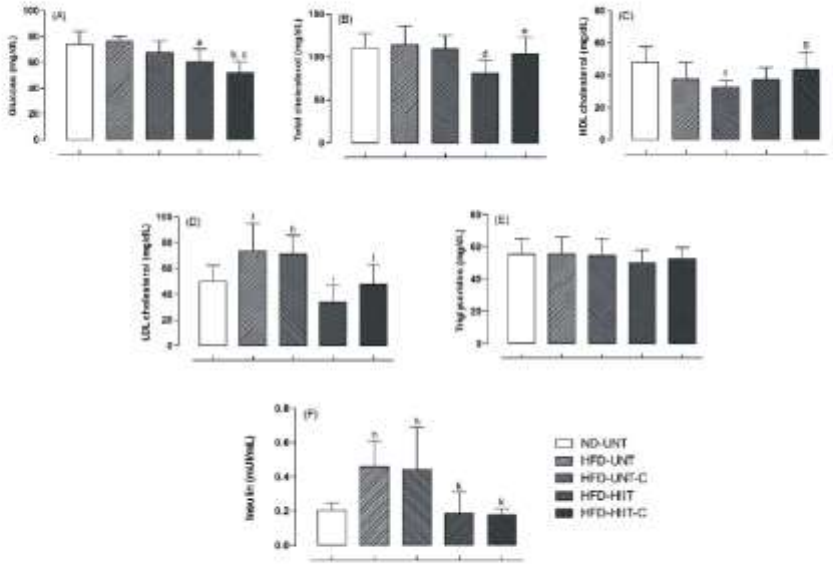
Finally, this study evaluated the effects of the HIIT protocol and L-Carnitine supplementation on biochemical parameters, such as glucose, insulin, triglycerides, HDL-c, LDL-c and total cholesterol levels, in the serum of obese rats. Figure 4A shows that HFD did not alter glucose; however, when we analyzed the other experimental groups, the HIIT protocol, when used alone and in association with L-Carnitine supplementation, reduced blood glucose, when compared with the ND-UNT, HFD-UNT and HFD-UNT-C groups.

The HIIT protocol decreased total cholesterol levels (Figure 4B; p<0.01), when compared with the ND-UNT, HFD-UNT and HFD-UNT-C groups. With regard to the HDL-c levels, Figure 4C also shows that HFD, associated with L-Carnitine supplementation, reduced this parameter (p<0.01), when compared to the ND-UNT group, and that the HIIT protocol in association with L-Carnitine was able to partially reverse this alteration in the serum of obese rats. Figure 4D shows that the HFD, on its own and when associated with L-Carnitine, significantly enhanced LDL-c levels in the serum of obese rats (p<0.01 and p<0.05, respectively), when compared to the ND-UNT group. In contrast, the HIIT protocol, when used alone and in association with L-Carnitine supplementation, reversed this alteration. Figure 4E also shows that neither

HFD, the HIIT protocol nor L-Carnitine supplementation, in isolation or in association, were able to alter triglyceride levels in the serum of obese rats.

Regarding insulin levels, Figure 4F shows that the administration of HFD alone and in association with L-Carnitine supplementation increased this parameter ($p<0.05$), when compared with the ND-UNT; the HIIT protocol was able to reverse the alteration in this parameter.

Figure 4. Effects of high-intensity interval training protocol and L-Carnitine supplementation on glucose, lipid profile and insulin parameters in the serum of obese rats. ND-UNT, Normal Diet-Untrained; HFD-UNT, High-Fat Diet-Untrained; HFD-UNT-C, High-Fat Diet – Untrained + Carnitine; HFD-HIIT, High-Fat Diet + High-intensity Interval Training; HFD-HIIT-C, High-Fat Diet + High-intensity Interval Training + Carnitine. a, $p<0.01$ *vs* ND-UNT and HFD-UNT; b, $p<0.01$ *vs* ND-UNT and HFD-UNT-C; c, $p<0.001$ *vs* HFD-UNT; d, $p<0.01$ *vs* ND-UNT, HFD-UNT and HFD-UNT-C; e, $p<0.05$ *vs* HFD-HIIT; f, $p<0.01$ *vs* ND-UNT; g, $p<0.01$ *vs* HFD-UNT-C; h, $p<0.05$ *vs* ND-UNT; i, $p<0.001$ *vs* HFD-UNT and HFD-UNT-C; j, $p<0.01$ *vs* HFD-UNT and HFD-UNT-C; k, $p<0.05$ *vs* HFD-UNT.



DISCUSSION

The present study contributes valuable information regarding the effects of the HIIT protocol, when used alone or in combination with L-Carnitine supplementation, on oxidative stress and biochemical parameters in the blood of obese rats. We found that these blood parameters are sensitive to the positive effects of exercise training and L-Carnitine supplementation, which protected animals against obesity-induced oxidative stress and biochemical disorders. First of all, our results showed that HFD increased body weight and promoted adipose tissue gain in the experimental groups, validating our methodology. Furthermore, we showed that the HIIT protocol, when used alone or in association with L-Carnitine, improved the function of the antioxidant enzyme system, thereby decreasing lipoperoxidation and protein

damage. In addition, this treatment (exercise and/or L-Carnitine supplementation) decreased HFD-induced alterations in glucose, total cholesterol, LDL-cholesterol and insulin, therefore protecting against metabolic syndrome.

Since oxidative stress can cause damage to lipids, protein and DNA, TBA-RS is an important parameter that is indicative of lipid peroxidation and plasma membrane damage[25–27]. Furthermore, the determination of total sulfhydryl and carbonyl protein contents serves as an indication of increased protein fragmentation and aggregation, and thereby protein damage, as a result of changes in protein structures, which could make proteins more susceptible to degradation by proteasomes[25–27].

Our results show that HFD increased TBA-RS and protein carbonyl content levels, and decreased the total sulfhydryl content; the HIIT protocol, when used alone or in association with L-Carnitine supplementation, was able to reverse these alterations in the plasma of obese rats. These data suggest that the HFD promoted lipoperoxidation and protein damage, while the HIIT protocol and L-Carnitine supplementation provide protective effects against lipids and protein damage by reversing the alterations in these parameters in the blood of obese rats.

In 2018, Lima *et al.* evaluated the effects of moderate-intensity continuous training (MICT) and HIIT protocols on the alterations in oxidative stress parameters caused by HFD. Authors reported that a HFD increased TBA-RS levels and protein carbonyl content and decreased total sulfhydryl content in the plasma of obese rats[2]. They also identified that the HIIT protocol prevented the increase in TBA-RS levels and totally reversed the increase in protein carbonyl content, but did not correct the alteration in total sulfhydryl content[2]. Also corroborating the findings of our investigation, a study carried out in 2015 identified an increase in TBA-RS levels in the blood of male rats fed on a high-fat diet, which were partially reversed in animals submitted to physical exercise (treadmill running protocol for 60 min, 5 times a week, for 8 weeks)[28].

With regard to antioxidant enzyme activities, our results show that the HFD reduced CAT and GSH-Px activities, but did not alter SOD activity. The HIIT protocol, in isolation or associated with L-Carnitine supplementation, was able to prevent the alteration in CAT activity, but not in GSH-Px activity. We suggest that a longer exercise time, associated with L-Carnitine supplementation, could increase GSH-Px activity, thereby reversing the decrease caused by HFD. With regard to SOD activity, our results show that both L-Carnitine and the HIIT protocol, alone or in association with L-Carnitine, enhanced this enzyme's activity in the erythrocytes of obese rats.

The decreases in CAT and GSH-Px activities suggest that the HFD promoted an increase in the production of ROS, such as hydrogen peroxide, and a consequent depletion in the activity of these antioxidant enzymes. In contrast, the significant increase in SOD activity, in

response to HIIT, may suggest that this protocol enhanced the production of the superoxide radical, since several studies show that high or exhausting intensities of exercise may lead to an increase in the production of ROS, due to the greater need for oxygen consumption and supply, which may corroborate our findings[25,29].

However, several studies demonstrate that chronic and moderate intensity physical exercises promote a reduction in ROS production, a decrease in oxidative stress, or an increase in antioxidant enzymes and repair processes, a fact that may justify the increase in CAT and GSH-Px activities found in the blood of HIIT protocol experimental groups[30].

A study carried out by Lima *et al.* (2018) also identified HFD-induced decreases in CAT and GSH-Px activities that were prevented by a HIIT protocol[2]. In addition, they also reported that the HIIT protocol increased the activity of SOD, probably due to a greater removal of superoxide radical and the formation of hydrogen peroxide, in agreement with our data[2].

Finally, this study evaluated the effects of the HIIT protocol and L-Carnitine supplementation on biochemical parameters, such as glucose, insulin, triglycerides, HDL-c, LDL-c and total cholesterol levels, in the serum of obese rats. Initially, we evaluated blood glucose levels in the serum of obese rats. Our data show that HFD alone was not able to alter this parameter. However, when we analyzed the other experimental groups, we found that the HIIT protocol, alone and in association with L-Carnitine supplementation, was able to reduce blood glucose. With regard to the insulin levels in the serum of rats, HFD increased this parameter and HIIT protocol reversed the alteration in this parameter. Our results show that chronic ingestion of a HFD causes insulin resistance and suggest that prolonged periods of ingestion would probably cause hyperglycemia.

With regard to the lipid profile, our study shows that the HIIT protocol decreased total cholesterol levels, while HFD decreased the HDL-c level, which was reversed by the HIIT protocol, in association with L-Carnitine, in the serum of obese rats. With regard to the LDL-c level, our data show that the HFD alone and when associated with L-Carnitine significantly enhanced this parameter and that the HIIT protocol, in isolation and in association with L-Carnitine supplementation, was able to reverse this alteration. Our data show no significant differences in triglyceride levels between groups.

In a study carried out by Yokota *et al.* (2009), mice were fed on normal diet or high fat diet (HFD) for 8 weeks[31]. Animals from the HFD group presented significantly increased fasting blood glucose, plasma insulin and triglyceride levels, but no difference was found in total cholesterol[31]. Zambon *et al.* (2009) investigated the effects of two different types of swimming exercise on adiposity and lipid profile in rats with exogenous obesity[32]. Their results show that the high fat diet increased serum concentrations of triglycerides, total cholesterol and high--density lipoprotein (HDL). In addition, Wang *et al.* (2017) demonstrated that rats submitted

to the HIIT protocol 5 days/week for 8 weeks presented significant decreases in triglycerides, and total and LDL-c levels, when compared to sedentary groups, but no differences in HDL-c between groups[1]. Aparicio *et al.* (2015) investigated whether interval aerobic training, combined with strength-endurance exercise, improves metabolic markers, when used together with caloric restriction in Zucker rats. Authors found a decrease in fasting glucose, serum insulin, total cholesterol, LDL and HDL-c in the exercise groups, compared to the sedentary groups, corroborating our data showing that the HIIT protocol is an important alternative for preventing changes in the glycemic and lipid profile caused by HFD[5].

CONCLUSION

In summary, our findings show that the HFD alters antioxidant defenses and biochemical parameters in the blood of rats, inducing lipoperoxidation, oxidative damage to proteins, alterations in antioxidant enzyme activities and modulating glucose, insulin, HDL-c, LDL-c and total cholesterol. In turn, we demonstrated that the HIIT protocol, when used alone and in association with L-Carnitine, is an important tool for combating the damage caused by obesity, since this training protocol was able to reverse most of the deleterious effects of HFD ingestion, in the blood of obese rats, thereby, protecting against metabolic syndrome.

Acknowledgements

This work was supported by grants from Fundo de Apoio à Pesquisa da Universidade da Região de Joinville, Fundo de Apoio à Pesquisa de Santa Catarina (FAPESC) Public call notice No. 06/2017, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank statistics teacher, Dra. Elisa Henning (Udesc/Joinville), for statistical analysis.

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ANEXO A – Parecer Consubstanciado de Comitê de Ética em Pesquisa no Uso de Animais

FUNDAÇÃO EDUCACIONAL DA REGIÃO DE JOINVILLE - FURJ
UNIVERSIDADE DA REGIÃO DE JOINVILLE

UNIVILLE
UNIVERSIDADE

UNIVERSIDADE DA REGIÃO DE JOINVILLE – UNIVILLE
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA EM PESQUISA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO

1) Dados Gerais

Protocolo nº. 012/1117

Data de entrada no CEUA: 14/11/2017

Comitê: ( ) Humanas (X) Animais

Área Temática Especial: (X) Não

( ) Sim. Qual? Não se aplica.

Projeto de pesquisa vinculado ao Programa de Pós-Graduação em Saúde e Meio Ambiente.

Fonte Financiadora: Carta-convite

2) Título

EFEITO DO TREINAMENTO AERÓBICO DE ALTA INTENSIDADE E DA SUPLEMENTAÇÃO COM L-CARNITINA SOBRE O ESTRESSE OXIDATIVO, METABOLISMO ENERGÉTICO E INFLAMAÇÃO EM RATOS OBESOS.

3) Responsável

Ariene Sampaio Souza Farias Ulbricht

4) Participantes

Daniela Delwing de Lima

Carla Werlang Coelho

Débora Delwing Dal Magro

Alunos de Iniciação Científica (em seleção)

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Unidade Centro - Joinville
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Unidade São Francisco do Sul
Campus São Bento do Sul

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UNIVERSIDADE DA REGIÃO DE JOINVILLE

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UNIVERSIDADE

5) Sumário e Análise Crítica

A pesquisadora responsável pelo projeto respondeu todas as pendências em carta-resposta com data de 20 de janeiro de 2018. Foi incluído o Profº. Eduardo Manoel Pereira na equipe executora do projeto. Assim sendo, o referido projeto foi aprovado para execução conforme os documentos apresentados no momento do protocolo e que serviram de base para aprovação. A pesquisadora informou que utilizará **50 ratos Wistar** neste projeto de pesquisa, informação disponibilizada no Formulário Unificado para solicitação de autorização para uso de animais em ensino e/ou pesquisa, página 09, impresso e arquivado na sala B 117 da Univille Universidade.

6) PARECER (SINTÉTICO)

( ) Não aprovado;
( ) Com Pendências;
(X) Aprovado

Joinville, 16 de fevereiro de 2018

Profª. Drª. Márcia Luciane Lange Silveira
Presidente do Comitê de Ética em Pesquisa no Uso de Animais

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Orientador: Daniela Delwing de Lima Coorientador: Débora Delwing Dal-Magro
Data de Defesa: 26/05/2023
Título: Efeito do treinamento aeróbico de alta intensidade e da suplementação com L-Carnitina sobre o estresse oxidativo, metabolismo energético e inflamação em ratos obesos
Instituição de Defesa: Universidade da Região de Joinville - UNIVILLE

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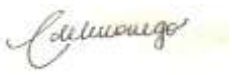

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