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TESE DE DOUTORADO

**EFEITOS DE UMA SESSÃO DE EXERCÍCIO AERÓBIO, EM ESTADO
ALIMENTADO OU EM JEJUM, SOBRE MARCADORES METABÓLICOS E
MOLECULARES EM RATOS MACHOS MAGROS E OBESOS**

Éverton Lopes Vogt

Porto Alegre, 07 de novembro de 2022.

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ALIMENTADO OU EM JEJUM, SOBRE MARCADORES METABÓLICOS E
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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, do Departamento de Fisiologia do Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), como requisito parcial para obtenção do grau de Doutor em Fisiologia.

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RESUMO

A obesidade está implicada em uma variedade de comorbidades, como doenças cardiovasculares, hipertensão, dislipidemia, resistência insulínica e inflamação crônica. Como resultado do tecido adiposo em expansão, a deposição ectópica de lipídeos e infiltração de células imunes no tecido adiposo estão envolvidas na produção de citocinas pró-inflamatórias e desenvolvimento de inflamação/resistência insulínica. Uma das principais medidas não farmacológicas de combate à obesidade é a realização de exercícios físicos. A oxidação de lipídeos imposta pelo exercício, em jejum ou não, em indivíduos obesos, é capaz de melhorar a relação entre mobilização e oxidação lipídica através da ativação da via AMPK-SIRT1-PGC1 α . Apesar disso, é de fundamental importância o papel da HSP70 no combate à inflamação, onde seus efeitos sobre a inflamação/resistência insulínica se dão, em parte, por sua ação inibitória sobre citocinas pró-inflamatórias. Dessa maneira, o objetivo do presente trabalho foi comparar os efeitos agudos e subagudos de uma sessão de exercício aeróbio de intensidade moderada, realizado em estado alimentado ou em jejum em animais magros e obesos sobre marcadores metabólicos, oxidação de diferentes substratos, expressão gênica e imunoconteúdo das vias AMPK-SIRT1-PGC1 e HSF1-HSP70. Dois artigos resultaram do presente trabalho. No artigo "*Metabolic and Molecular Subacute Effects of a Single Moderate-Intensity Exercise Bout, Performed in the Fasted State, in Obese Male Rats*" foi avaliado o efeito de uma sessão de exercício aeróbio moderado em jejum em ratos obesos 12h após a intervenção. Os achados mostraram que não houve interação entre exercício e jejum, embora o exercício tenha provocado respostas positivas no imunoconteúdo das proteínas avaliadas, bem como promissores resultados de redução da lipídemia dos animais mesmo depois de 12h da realização do exercício. Já no artigo "*Acute Effects of a Single Moderate-Intensity Exercise Bout Performed in Fast or Fed States on Cell Metabolism and Signaling: Comparison between Lean and Obese Rats*", a associação de jejum e exercício apresentou diferentes efeitos no gastrocnêmio em relação ao sóleo em animais obesos imediatamente após o exercício. No sóleo, a combinação de jejum e exercício teve um efeito poupador de glicogênio associado ao aumento da oxidação de palmitato, enquanto no gastrocnêmio, aumentou a oxidação de glicose. Além disso, a expressão de AMPK foi mais sensível às intervenções em animais magros do que em obesos e sua resposta foi diferente entre os músculos oxidativos e glicolíticos. Apesar da dificuldade da extrapolação destes achados em ratos para a população humana obesa, eles contribuem para o conhecimento sobre os efeitos do exercício em jejum. No entanto, as alterações metabólicas induzidas na lipídemia e o fato de perdurarem por até 12 horas, são efeitos que precisam ser investigados em humanos. Diante do exposto, o atual trabalho apresenta como perspectiva a elucidação do mecanismo de mobilização de substratos e ativação de vias controladoras do metabolismo glicídico e lipídico.

ABSTRACT

Obesity is implicated in a variety of comorbidities, such as cardiovascular disease, hypertension, dyslipidemia, insulin resistance, and chronic inflammation. In consequence of the adipose tissue expansion, ectopic lipid deposition and infiltration of immune cells into adipose tissue are involved in the production of pro-inflammatory cytokines and development of inflammation and insulin resistance. Physical exercise is one of the main non-pharmacological measures to combat obesity. The lipid oxidation imposed by exercise, fasting or not, in obese individuals, is able to improve the relationship between mobilization and lipid oxidation through the activation of the AMPK-SIRT1-PGC1 α pathway. The heat shock response, another molecular pathway triggered by exercise, has a fundamental role against inflammation because it inhibits pro-inflammatory cytokines and improve insulin resistance. Thus, the objective of the present study was to compare the acute and sub-acute effects of a moderate-intensity aerobic exercise session, performed in a fed or fasted state, in lean and obese animals on metabolic markers, oxidation of different substrates, gene and protein expression of the AMPK-SIRT1-PGC1 and HSF1-HSP70 pathways. Two papers resulted from the present work. In the paper "Metabolic and Molecular Subacute Effects of a Single Moderate-Intensity Exercise Bout, Performed in the Fasted State, in Obese Male Rats", the effect of a fasted moderate aerobic exercise session in obese rats 12 h after the intervention was evaluated. The findings showed that there was no interaction between exercise and fasting, although exercise caused positive responses in the immunocontent of the proteins evaluated, as well as promising lipidemia results in the animals even after 12 hours of exercise. In the article "Acute Effects of a Single Moderate-Intensity Exercise Bout Performed in Fast or Fed States on Cell Metabolism and Signaling: Comparison between Lean and Obese Rats", the association of fasting and exercise had different effects on the gastrocnemius in relation to the soleus in obese animals immediately after exercise. In the soleus, combination of fasting and exercise had a glycogen-sparing effect associated with increased palmitate oxidation, whereas in the gastrocnemius, it increased glucose oxidation. Furthermore, AMPK expression was more sensitive to interventions in lean animals than in obese animals and its response was different between oxidative and glycolytic muscles. Despite the difficulty of extrapolating these findings in rats to the obese human population, they contribute to the knowledge about the effects of exercise in the fasted state. The metabolic changes induced in lipidemia by exercise while fasting and the fact that they last for up to 12 hours are effects that need to be further investigated in humans. Furthermore, the endocrine and molecular pathways that control these metabolic switches should also be investigated.

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LISTA DE ABREVIATURAS E SIGLAS

ACC2: *Acetyl-CoA Carboxylase 2*

AKT: *Protein Kinase B*

AMP: *Adenosina Monofosfato*

AMPK: *AMP-activated Protein Kinase*

ATGL: *Adipose Triglyceride Lipase*

ATP: *Adenosina Trifosfato*

BABA: *β -aminoisobutyric Acid*

CaMKIV: *Ca²⁺/Calmodulin-Dependent Protein Kinase IV*

CD36: *Cluster of Differentiation 36*

CPT1: *Carnitine Palmitoyltransferase 1*

CREB: *cAMP Response Element-Binding Protein*

CRTC2: *CREB Regulated Transcription Coactivator 2*

DAG: *Diacylglycerol*

DM2: *Diabetes melito tipo 2*

FABP3: *Fatty Acid-Binding Protein 3*

FABPpm: *Plasma Membrane Associated Fatty Acid Binding Protein*

FBP: *Fructose 1,6-Bisphosphate*

FGF21: *Fibroblast Growth Factor 21*

FOXO1A: *Forkhead Box O1A*

GLUT1: *Glucose Transporter 1*

GLUT4: *Glucose Transporter 4*

GPAT: *Glycerol-3-Phosphate Acyltransferase*

HCAR-1: *Hydroxycarboxylic Acid Receptor 1*

HKII: *Hexokinase II*

HSF1: *Heat Shock Factor 1*

HSL: *Hormone-Sensitive Lipase*

HSP70: *70 kilodalton Heat Shock Protein*

IGF-1: *Insulin-like Growth Factor 1*

IkB α : *Nuclear Factor of Kappa-Light-Polypeptide Gene Enhancer in B-Cells Inhibitor α*

IKK- β : *IkB kinase- β*

IL-1ra: *Interleukin-1 Receptor Antagonist*

IL-6: *Interleucina 6*

IMC: *Índice de Massa Corporal*

IRS1: *Insulin Receptor Substrate 1*

JNK: *c-Jun N-terminal Kinase*

LPL: *Lipoprotein Lipase*

MAPK: *Mitogen-Activated Protein Kinase*

NADH: *Nicotinamide Adenine Dinucleotide em sua forma reduzida*

NAD⁺: *Nicotinamide Adenine Dinucleotide em sua forma oxidada*

NEFA: *Non-Esterified Fatty Acids*

NF-kB: *Nuclear Factor of Kappa-Light-Polypeptide Gene Enhancer in B-Cells*

NRF1: *Nuclear Respiratory Factor 1*

NRF2: *Nuclear Factor Erythroid 2-Related Factor 2*

TAG: *Triacilglicerol*

TGIH: *Triacilglicerol Intra-Hepático*

TGIM: *Triacilglicerol Intramuscular*

PDK4: *Pyruvate Dehydrogenase Kinase 4*

PGC1 α : *Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha*

PI3K: *Phosphoinositide 3-Kinase*

PKC: *Protein Kinase C*

PKC ϵ : *Protein Kinase C Epsilon*

PKC ζ : *Protein Kinase C Zeta*

PLIN2: *Perilipina 2*

PLIN3: *Perilipina 3*

PLIN5: *Perilipina 5*

PPAR α : *Peroxisome Proliferator-Activated Receptor α*

PPAR δ : *Peroxisome Proliferator-Activated Receptor δ*

PP2A: *Protein Phosphatase 2A*

SIRT1: *Sirtuína 1*

SREBP1: *Sterol Regulatory Element Binding Protein 1*

TBC1D: *TBC Domain Family Member 1*

TNF α : *Tumor Necrosis Factor α*

TXNIP: *Thiredoxin-Interacting Protein*

UCP3: *Uncoupling Protein 3*

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1. INTRODUÇÃO

1.1. Obesidade

Obesidade é o resultado direto de um desequilíbrio entre ingesta e gasto energético, e é definida por um Índice de Massa Corporal (IMC) $\geq 30\text{kg/m}^2$ ou um percentual de gordura corporal $> 25\%$ para homens e $> 32\%$ para mulheres. Entretanto, apesar de medição mais fácil e prática quando comparada a avaliações de composição corporal, o IMC não consegue determinar diretamente o risco cardiometabólico de medidas de adiposidade central, como a circunferência da cintura, por exemplo (HESHKA; ALLISON, 2001; STIENSTRA *et al.*, 2007; YUSUF *et al.*, 2005).

É bem sabido que a obesidade está implicada em uma variedade de problemas de saúde, como desenvolvimento de doenças cardiovasculares e musculoesqueléticas, hipertensão, dislipidemia, prejuízo na qualidade de vida, resistência insulínica e que apresenta uma íntima relação com diabetes melito tipo 2 (DM2) e inflamação (CARBONE *et al.*, 2019; CHOBOT *et al.*, 2018; HERZOG, 2020; NIMPTSCH; KONIGORSKI; PISCHON, 2019; STIENSTRA *et al.*, 2007; WIRTH; WABITSCH; HAUNER, 2014). O tecido adiposo não é mais considerado apenas um local de estoque energético, mas sim um órgão endócrino ativo intrinsecamente ligado ao metabolismo intermediário (WIRTH; WABITSCH; HAUNER, 2014).

Embora indivíduos obesos apresentem uma diminuída capacidade muscular oxidativa, uma das principais medidas não farmacológicas de combate à obesidade é a realização regular de exercícios físicos devido à sua capacidade de melhorar os perfis insulinêmico e trigliceridêmico e capacidade oxidativa de

seus praticantes (Figura 1) (CARBONE *et al.*, 2019; FUJITA *et al.*, 2018; HOUMARD, 2008; KIM *et al.*, 2000; WIRTH; WABITSCH; HAUNER, 2014).


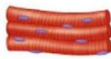

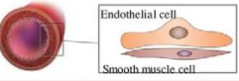


	Mitochondrial 	Skeletal Muscle 	Cardiac Muscle 	Conduit Arteries 
Sedentary behaviors and Physical Inactivity 	<ul style="list-style-type: none"> • ↑ mitochondrial DNA deletions and mutations • Electron transport chain abnormalities • ↑ mitochondrial fission • ↓ mitochondrial content • ↓ respiration 	<ul style="list-style-type: none"> • ↑ IL-6 and CRP • Activation of proteolytic systems • Inactivation of the PI3K/Akt/mTOR pathway • ↓ lean muscle mass • Greater proportion of hybrid fibers possibly due to dysregulation in MHC isoform expression 	<ul style="list-style-type: none"> • ↑ AGE accumulation indicative of collagen cross-linking • ↑ Left ventricular stiffness • β-adrenergic receptor desensitization resulting in impaired inotropic and chronotropic responses to adrenergic stimulation • ↓ SERCA2a contributes to prolonged calcium transients 	<ul style="list-style-type: none"> • ↓ sympathetic baroreflex sensitivity and ↓ sympathetic activation • ↑ NOS uncoupling, ↓ NO bioavailability, thereby ↑ oxidative stress • Extracellular matrix remodeling through elastin degradation by MMPs and formation of AGEs • Endothelial dysfunction
Physical Activity and Exercise Training 	<ul style="list-style-type: none"> • ↑ mitochondrial protein turnover through degradation of damaged proteins and de novo synthesis of new functional proteins • ↑ expression of PGC-1α • ↑ SIRT3 content • ↑ mitochondrial volume 	<ul style="list-style-type: none"> • ↑ metabolic enzymes profile: citrate synthase, β-HAD, glycogen phosphorylase • ↓ catabolic mRNA expression (FOXO3a, MuRF-1, Atrogin-1, myostatin) • ↑ capillary-to-fiber ratio • ↑ insulin sensitivity 	<ul style="list-style-type: none"> • ↑ SERCA2a mRNA & protein expression • ↑ phosphorylation of threonine-17 residue of phospholamban allowing for faster reuptake of cytoplasmic calcium • ↑ contractility and relaxation due to faster systolic rise and diastolic decay time of calcium • ↓ Left ventricular stiffness 	<ul style="list-style-type: none"> • Lower expression of the transcription factor p53 which is associated with senescence compared to sedentary counterparts • Lower markers of senescence (p21 and p16) • ↓ expression of nitrotyrosine and NADPH oxidase (prooxidant) • ↑ expression of manganese SOD (antioxidant)

Figura 1. Alterações metabólicas promovidas pelo comportamento sedentário e inatividade física e os efeitos benéficos da atividade física e do treinamento físico nas mitocôndrias, músculo esquelético, miocárdio e circulação. AGE, *advanced glycation end products*; AKT, *protein kinase B*; β-HAD, *beta-hydroxyacyl-CoA dehydrogenase*; CRP, *C-reactive protein*; FOXO3a, *forkhead box O3a*; DNA, *deoxyribonucleic acid*; IL-6, *interleukin-6*; MHC, *myosin heavy chain*; mRNA, *messenger ribonucleic acid*; MuRF-1, *muscle RING-finger protein-1*; MMP, *matrix metalloproteinase*; mTOR, *mammalian target of rapamycin*; NADPH, *nicotinamide adenine dinucleotide phosphate*; NO, *nitric oxide*; NOS, *nitric oxide synthase*; PGC-1α, *peroxisome proliferator-activated receptor gamma coactivator 1-alpha*; PI3K, *phosphoinositide 3-kinase*; SERCA2a, *sarcoplasmic reticulum calcium adenosine triphosphatase*; SIRT3; *nicotinamide adenine dinucleotide dependente deacetylase sirtuin-3* SOD (CARBONE *et al.*, 2019).

1.1.1. Obesidade, inflamação e resistência insulínica

Como resultado do tecido adiposo em expansão, a deposição de lipídeos fora deste tecido é caracterizada como um acúmulo ectópico e se dá pela formação de gotículas lipídicas citoplasmáticas, podendo resultar em disfunção celular pelo seu excesso e culminar com morte celular, o que é descrito na literatura como lipotoxicidade (BANDET *et al.*, 2019; BOSMA *et al.*, 2012; MENGESTE; RUSTAN; LUND, 2021; MONTGOMERY; DE NARDO; WATT, 2019; SCHAFFER, 2003; SZTALRYD; KIMMEL, 2014). Todavia, é importante

que ácidos graxos livres liberados de forma excessiva pela lipólise do tecido adiposo possam ser armazenados, a curto prazo, em órgãos como músculo esquelético, fígado, coração, rins e pâncreas, atuando assim de forma tamponante (ZACHAREWICZ; HESSELINK; SCHRAUWEN, 2018). Este tamponamento de ácidos graxos livres, de maneira geral, é realizado por macrófagos infiltrados nesse tecido adiposo em expansão (VERBOVEN *et al.*, 2018). Os macrófagos infiltrados no tecido adiposo estão envolvidos no desenvolvimento da inflamação crônica, incluindo obesidade induzida por inflamação, visto que são o principal local de produção de citocinas pró-inflamatórias (CHEN *et al.*, 2015; NIMPTSCH; KONIGORSKI; PISCHON, 2019). Um aumento na captação de ácidos graxos por esses macrófagos, decorrente de uma dieta rica em lipídeos, pode alterar seu fenótipo, promovendo assim uma polarização inflamatória (M2 para M1) e consequente inflamação do tecido adiposo (KLEIN-WIERINGA *et al.*, 2013; LUMENG *et al.*, 2007; MORIGNY *et al.*, 2016; PRIEUR *et al.*, 2011). Embora a maior fonte de citocinas pró-inflamatórias na obesidade seja o próprio tecido adiposo, os adipócitos, *per se*, apresentam uma discreta participação na síntese destas substâncias comparados à grande produção realizada pelos macrófagos infiltrados neste tecido (RODRÍGUEZ-HERNÁNDEZ *et al.*, 2013).

Em indivíduos obesos, a hidrólise de triacilgliceróis (TAG) se apresenta normal pela *adipose triglyceride lipase* (ATGL), resultando na formação de diacilgliceróis (DAG). Entretanto, a hidrólise de DAG pela *hormone-sensitive lipase* (HSL) encontra-se reduzida nesta população devido a uma redução no conteúdo da enzima, culminando com acúmulo do substrato (Figura 2) (JOCKEN *et al.*, 2010; MENGESTE; RUSTAN; LUND, 2021; ZACHAREWICZ;

HESSELINK; SCHRAUWEN, 2018). Todavia, a literatura nos mostra que, em indivíduos obesos, não há modificação na maquinaria que controla o equilíbrio entre síntese e degradação lipídica, mas sim uma aumentada captação de ácidos graxos, capaz de ser observada por um maior conteúdo de transportadores de ácidos graxos em suas membranas celulares, resultando assim em um maior acúmulo de lipídeos intracelulares (BERGMAN *et al.*, 2018; KELLEY *et al.*, 1999).

Mais relevantes ainda do que o conteúdo de triacilgliceróis intramusculares (TGIM), são os conteúdos de espécies específicas de DAG e ceramidas intracelulares, componentes que efetivamente são capazes de alterar a sinalização insulínica (JOCKEN *et al.*, 2010). Embora alguns estudos tenham mostrado uma piora na sinalização insulínica correlacionada ao conteúdo de DAG, outros mostraram uma correlação positiva entre acúmulo de DAG intramuscular e sensibilidade insulínica (BERGMAN *et al.*, 2018; BONI; RANDO, 1985; HANNUN *et al.*, 1986). Dessa maneira, o conteúdo de DAG total não está necessariamente envolvido em um prejuízo na sinalização hormonal (MORALES; BUCAREY; ESPINOSA, 2017). Essas diferenças de resultados podem ser atribuídas ao tipo de DAG formado e o tipo de célula onde se encontram. Embora o subtipo de DAG que apresenta marcadas propriedades dessensibilizadoras de insulina seja o 1,2DAG formado pela hidrólise do fosfatidilinositol da membrana celular, tanto a hidrólise de fosfatidilcolina como a hidrólise incompleta de TAG resulta em elevadas concentrações de 2,3DAG e 1,3DAG (ASAOKA *et al.*, 1992; BONI; RANDO, 1985). Em indivíduos acometidos por resistência insulínica hepática, há um acúmulo de triacilgliceróis intra-hepáticos (TGIH) que, por sua vez, está correlacionado positivamente com um

acúmulo de DAG (PETERSEN; SHULMAN, 2017). Esse DAG acumulado nos hepatócitos ativa a *protein kinase C ε* (PKCε) ancorada na membrana celular. A PKCε, por sua vez, fosforila o receptor de insulina em um resíduo de treonina, resultando em uma inibição de sua capacidade de tirosina quinase (PETERSEN; SHULMAN, 2017, 2018). Dessa maneira então, toda cascata de sinalização adiante fica prejudicada.

Sendo assim, o DAG é capaz de alterar a sinalização insulínica por ativação de diferentes isoformas de PKC, dependendo do tecido em que se encontra. Porém, deve ser levado em consideração que as concentrações intracelulares aumentadas de DAG podem ser resultado tanto da hidrólise de fosfatidilinositol como da reesterificação de ácidos graxos livres captados pela célula, como o que acontece na obesidade (PETERSEN; SHULMAN, 2018).

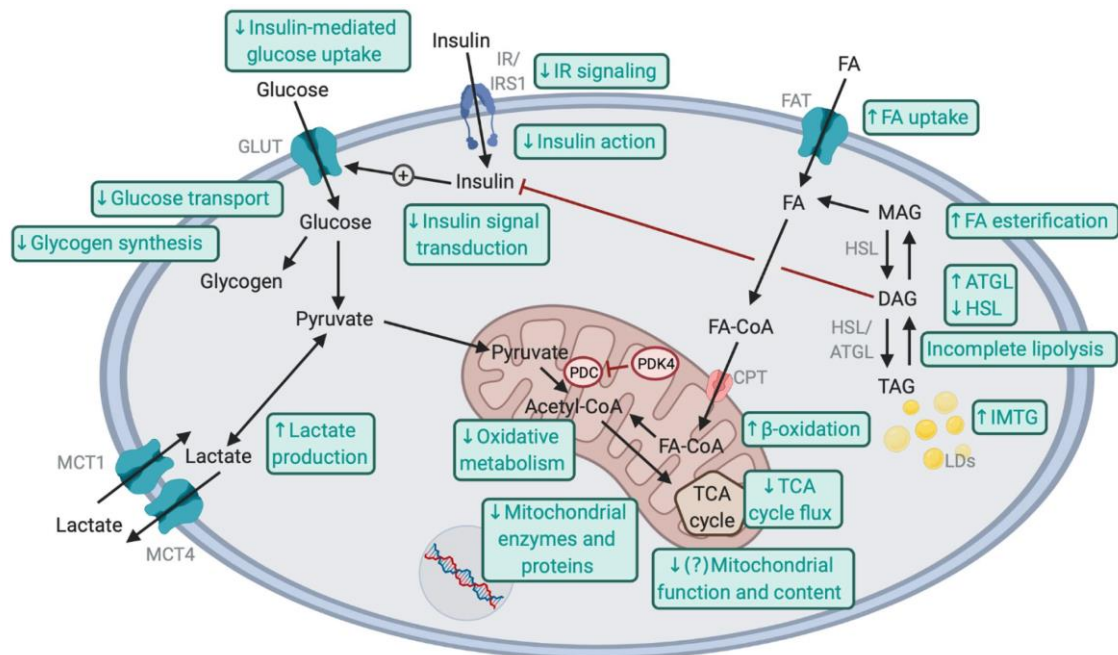


Figura 2. Alterações metabólicas causadas pela obesidade no músculo esquelético. Aumento na captação de ácidos graxos (FA) circulantes, prejuízo na sinalização insulínica, diminuição do metabolismo oxidativo, aumento do fluxo glicolítico e concomitante aumento na formação de lactato são alguns dos efeitos causados pela obesidade no músculo esquelético. ATGL, *adipose triglyceride lipase*; CPT, *carnitine palmitoyltransferase*; DAG, *diacylglycerol*; FA, *fatty acid*; GLUT, *glucose transporter*; HSL, *hormone-sensitive lipase*; IMTG, *intramuscular/intramyoelétrico triacylglycerol*; IR, *insulin receptor*; IRS1, *insulin receptor substrate 1*; LD, *lipid droplet*; MAG, *monoacylglycerol*; MCT, *monocarboxylate transporter*; PDC, *pyruvate dehydrogenase complex*; PDK4, *pyruvate dehydrogenase lipoamide kinase 4*; TAG, *triacylglycerol*; TCA, *tricarboxylic acid* (MENGESTE; RUSTAN; LUND, 2021).

Além do DAG formado, ceramidas são moléculas lipídicas bioativas que também estão relacionadas ao desenvolvimento de resistência insulínica sendo originadas da hidrólise de lipídeos de membrana, como esfingomielina, pela ação de uma esfingomielinase ativada por citocinas pró-inflamatórias ou sintetizadas através da condensação entre serina e palmitoil-CoA (GOMEZ-MUÑOZ *et al.*, 2016; LARSEN; TENNAGELS, 2014; PERALDI *et al.*, 1996; PETERSEN; SHULMAN, 2018; SAMUEL; SHULMAN, 2016; SAWAI; HANNUN, 1999).

Concentrações plasmáticas de ceramidas encontram-se elevadas em indivíduos obesos e diabéticos tipo 2 (DM2) relacionando-se intimamente com a capacidade de captação de glicose mediada por insulina em tecidos periféricos. Dessa maneira, parece óbvio que as ceramidas sejam os metabólitos que interconectam obesidade, diabetes e inflamação, relacionando-se ainda a mecanismos como apoptose, formação de espécies reativas de oxigênio, estresse de retículo endoplasmático e autofagia (Figura 3) (FUCHO *et al.*, 2017; GOMEZ-MUÑOZ *et al.*, 2016).

Além destas moléculas contribuírem para a estabilidade estrutural da célula, agem como lipídeos bioativos em diferentes rotas de sinalização celular e são capazes de inibir a captação de glicose, síntese de glicogênio e ativação da *protein kinase B* (AKT) através da ativação de enzimas como a *protein phosphatase 2A* (PP2A) (ITANI *et al.*, 2000; KURZ *et al.*, 2019; LARSEN; TENNAGELS, 2014; MAHFOUZ *et al.*, 2014). Esta enzima, cuja atividade está estimulada no músculo esquelético de humanos obesos e hepatócitos de ratos resistentes à insulina, é capaz de inibir a sinalização insulínica. (DOBROWSKY *et al.*, 1993; GALBO *et al.*, 2011).

Parte do efeito produzido pela PP2A se deve à defosforilação e inativação da AKT, proteína envolvida na sinalização da *fosfatidilinositol-3-kinase* (PI3K), rota esta que é crucial para a correta sinalização insulínica (GALBO *et al.*, 2011; GOMEZ-MUÑOZ *et al.*, 2016). Ceramidas também são capazes de ativar a PKC- ζ , proteína responsável pela inibição da translocação da AKT até a membrana dos hepatócitos. A ausência da AKT na membrana celular impede a proteína de participar corretamente na sinalização insulínica, resultando em prejuízo na resposta hormonal (PETERSEN; SHULMAN, 2017). Uma diminuição na ativação

da AKT (Figura 3) resulta em uma atividade prejudicada da insulina em suprimir a liberação de glicose hepática e diminuição na captação de glicose nos tecidos muscular e adiposo (GALBO *et al.*, 2011). Dessa maneira, essa hiperglicemia estimula ainda mais secreção de insulina, resultando em um quadro de hiperinsulinemia. Apesar de uma prejudicada capacidade de captação de glicose, a insulina mantém inalterada sua capacidade lipogênica através da via intacta PKC-SREBP1C. Fatores como *sterol regulatory element binding protein 1C* (SREBP1C) aumentam a expressão da enzima glicerol-3-fosfato aciltransferase (GPAT), responsável pela iniciação da cascata de síntese de TAG (WENDEL *et al.*, 2013). Por fim, hiperglicemia seguida de hiperinsulinemia resulta em maior atividade lipogênica, criando assim um ciclo “obesogênico” vicioso.

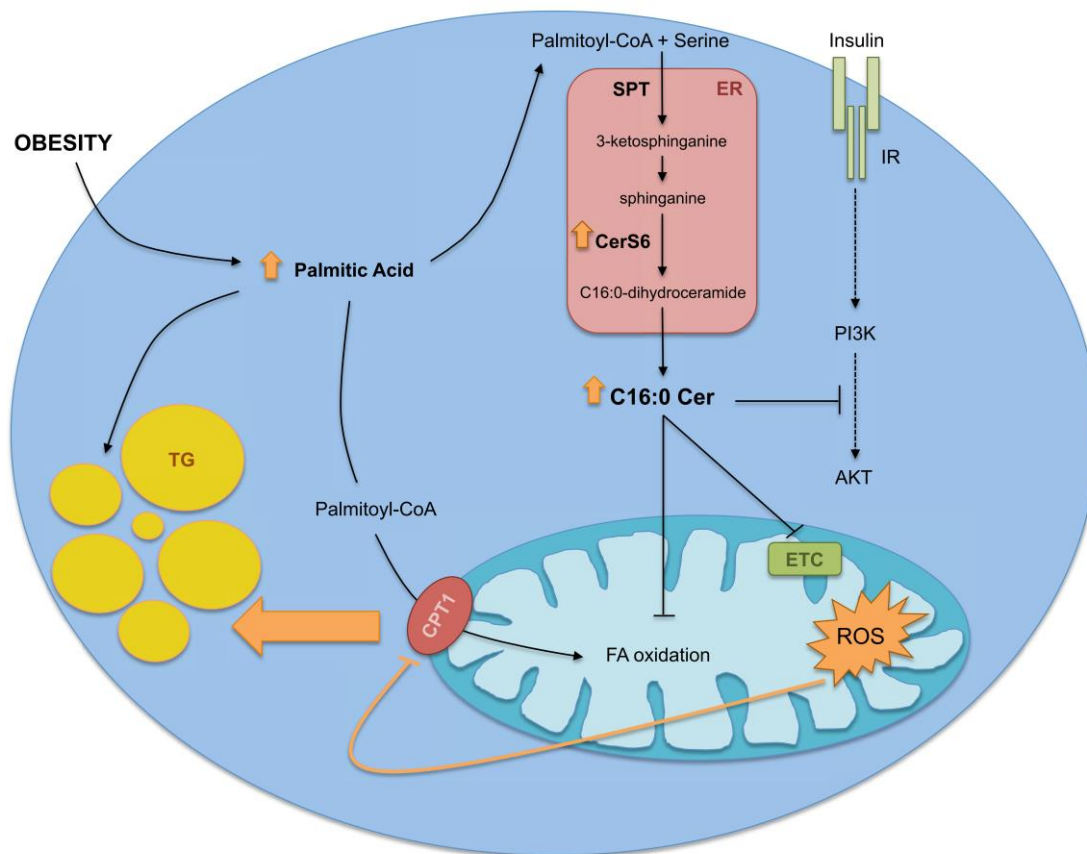


Figura 3. Regulação da oxidação de ácidos graxos pelas ceramidas na obesidade. A formação de ceramidas (C16:0) inibe diretamente a cadeia transportadora de elétrons (ETC), estimula a formação de espécies reativas de oxigênio (ROS) e inibe enzimas relacionadas à oxidação de ácidos graxos, como a CPT1. SPT, *serine palmitoyltransferase*; ER, *endoplasmic reticulum*; IR, *insulin receptor*; CerS6, *ceramide synthase 6*; PI3K, *phosphoinositide 3-kinase*; AKT, *protein kinase B*; ETC, *electron transport chain*; ROS, *reactive oxygen species*; CPT1, *carnitine palmitoyltransferase 1*; TG, *triglyceride* (FUCHO *et al.*, 2017).

1.1.2. Capacidade oxidativa de indivíduos obesos

Indivíduos obesos apresentam uma lipólise basal diminuída tanto no tecido adiposo subcutâneo como no visceral, bem como uma diminuída sensibilidade a catecolaminas e um menor fluxo sanguíneo no tecido adiposo (GOODPASTER; SPARKS, 2017; HOROWITZ, 2001; VERBOVEN *et al.*, 2018). Embora pareça contraditório, essa menor capacidade lipolítica em indivíduos obesos apresenta benefícios metabólicos. Um menor fluxo de ácidos graxos livres advindos da lipólise do tecido adiposo conserva um equilíbrio entre o

aporte destes ácidos e a capacidade oxidativa dos tecidos (HOROWITZ, 2001). Uma grande concentração de ácidos graxos livres na circulação, somada à sua menor capacidade oxidativa, é capaz de induzir a um quadro de resistência insulínica através da inibição da captação de glicose, reduzindo assim tanto sua oxidação como a síntese de glicogênio muscular (HOUMARD, 2008; KIM *et al.*, 2000; SHULMAN, 2000). Além de reduzida atividade de enzimas relacionadas à oxidação de ácidos graxos, alterações morfológicas mitocondriais também podem acontecer na obesidade (HOUMARD, 2008).

Além da polarização no perfil de seus macrófagos, como citado anteriormente, indivíduos obesos também apresentam atividade diminuída de enzimas relacionadas ao metabolismo oxidativo, como a *carnitine palmitoyltransferase 1* (CPT1), enzima que regula a entrada dos ácidos graxos na mitocôndria e que representa um passo limitante no processo de oxidação destes (GALIC *et al.*, 2018; HOROWITZ, 2001; KELLEY *et al.*, 1999; KIM *et al.*, 2000, 2002; ZACHAREWICZ; HESSELINK; SCHRAUWEN, 2018). Este desequilíbrio entre mobilização e oxidação de ácidos graxos pode explicar o acúmulo de TGIM, podendo contribuir para o surgimento ou manutenção de resistência insulínica (PERSEGHIN *et al.*, 1999). A captação diminuída de glicose tem uma importante função de proteção contra lipotoxicidade, já que uma concentração reduzida de glicose intracelular disponibiliza menos substrato para liponeogênese, gerando menos efeitos deletérios de seus metabólitos (UNGER, 2003).

Embora estes TGIM apresentem efeitos deletérios em algumas populações, está bem estabelecido que atletas apresentam conteúdos de TGIM maiores do que seus controles sedentários, obesos ou diabéticos, não

apresentando quaisquer prejuízos na sinalização insulínica (GEMMINK *et al.*, 2018; GOODPASTER *et al.*, 2001). De fato, atletas apresentam uma sensibilidade insulínica melhorada quando comparada a seus controles, sugerindo que os TGIM não são lipotóxicos, embora sejam importantes marcadores de acúmulo de intermediários do metabolismo de ácidos graxos (BOSMA *et al.*, 2012; ZACHAREWICZ; HESSELINK; SCHRAUWEN, 2018). Esta situação onde há um aumento no conteúdo de TGIM e uma melhora, ao invés de um esperado prejuízo, na sinalização insulínica é bem descrita na literatura como o 'paradoxo do atleta' (GOODPASTER *et al.*, 2001; WOLINS; MITTENDORFER, 2018; ZACHAREWICZ; HESSELINK; SCHRAUWEN, 2018). Esta sensibilidade insulínica não alterada deve-se, em grande parte, a um maior conteúdo de proteínas de revestimento lipídico, conhecidas como perilipinas. Indivíduos treinados apresentam maior conteúdo de perilipina 5 (PLIN5) no músculo esquelético quando comparados a indivíduos sedentários, obesos ou diabéticos, por exemplo. Este maior conteúdo está positivamente correlacionado à sensibilidade insulínica e sua capacidade oxidativa. Além disso, as gotículas lipídicas intramusculares apresentam marcada função protetora contra resistência insulínica induzida por lipídeos, já que são capazes de sequestrar os intermediários lipídicos capazes de prejudicar a sinalização hormonal (DAEMEN; VAN POLANEN; HESSELINK, 2018; MORALES; BUCAREY; ESPINOSA, 2017).

1.2. Jejum

O estado de jejum é caracterizado por um *switch* metabólico coordenado na utilização de substratos energéticos com o objetivo de poupar glicose às custas de um aumento na mobilização de lipídeos para garantir a demanda energética de células dependentes de glicose (BERTHOLDT *et al.*, 2018; STEINHAUSER *et al.*, 2018). O jejum é capaz de ativar uma série de fatores transcricionais que estimulam a transcrição de genes responsáveis pela codificação de enzimas com papel crucial no metabolismo hepático, como gliconeogênese, oxidação de ácidos graxos e cetogênese, por exemplo (HILDEBRANDT; NEUFER, 2000). Durante o jejum, quando o organismo prioriza a oxidação de ácidos graxos, fatores como o *peroxisome proliferator-activated receptor α* (PPAR α) promovem uma maior captação e oxidação destes pelo tecido hepático produzindo substratos para cetogênese com o intuito de prover energia para os tecidos periféricos (PAWLAK; LEFEBVRE; STAELS, 2015). O PPAR α , um receptor nuclear ativado por ácidos graxos não esterificados (NEFAs), é um fator crucial para as respostas adaptativas normais ao jejum capaz de regular negativamente vias de sinalização pró-inflamatórias (HOTTA *et al.*, 2009; PAWLAK; LEFEBVRE; STAELS, 2015; STIENSTRA *et al.*, 2007). O PPAR α ainda é capaz de aumentar a expressão hepática de hormônios como o *fibroblast growth factor 21* (FGF21), um importante regulador metabólico envolvido no controle da homeostase glicídica e do metabolismo energético (HOTTA *et al.*, 2009; VILLARROYA *et al.*, 2018). Tendo o fígado como seu principal local de síntese e secreção, o FGF21 é capaz de regular a lipólise no tecido adiposo branco, embora exerça um efeito estimulatório sobre o escurecimento desse tecido (FISHER *et al.*, 2012; HOTTA *et al.*, 2009; KEIPERT

et al., 2014; LEE *et al.*, 2014; VILLARROYA *et al.*, 2018). Esse escurecimento do tecido adiposo branco, conhecido por *browning*, se dá pelo aumento do número de suas mitocôndrias, fenômeno que pode ser estimulado por irisina, β -*aminoisobutyric acid* (BABA), interleucina 6 (IL-6), além do próprio FGF21 (SEVERINSEN; PEDERSEN, 2020).

O PPAR α estimula a expressão de genes relacionados à oxidação de ácidos graxos no tecido muscular e ainda regula a biogênese mitocondrial tendo como alvos que medeiam esse *shift* da utilização de glicose para ácidos graxos os genes responsáveis pela codificação das proteínas *cluster of differentiation 36* (CD36), *fatty acid-binding protein 3* (FABP3), *uncoupling protein 3* (UCP3), *peroxisome proliferator-activated receptor gamma coactivator 1-alpha* (PGC1 α), *pyruvate dehydrogenase kinase 4* (PDK4) e *forkhead box O1A* (FOXO1A) (ANTON *et al.*, 2018). É principalmente expresso em tecidos metabolicamente ativos, como fígado, coração, músculo esquelético e tecido adiposo marrom (GRYGIEL-GÓRNIAK, 2014; PAWLAK; LEFEBVRE; STAELS, 2015; STIENSTRA *et al.*, 2007). Além destes efeitos lipolíticos, estes autores afirmam que o PPAR α apresenta um importante efeito anti-inflamatório através da sua capacidade de estimular a expressão de genes como *interleukin-1 receptor antagonist* (IL-1ra) e *NF-kB inhibitor α* (IkB α), um inibidor citoplasmático do NFkB.

O aumento da oferta de ácidos graxos livres a tecidos periféricos também é resultado de outros processos como autofagia mediada por chaperonas. Este fenômeno de autofagia é descrito como o reconhecimento das proteínas de revestimento da gota lipídica perilipina 2 (PLIN2) e perilipina 3 (PLIN3) pela *Heat Shock Protein 70* (HSP70). Após o reconhecimento das proteínas, a HSP70 controla o tráfego das perilipinas até os lisossomos para sua posterior

degradação (MORIGNY *et al.*, 2016). Essa diminuição da presença de perilipinas sobre a gotícula lipídica permite o recrutamento da ATGL e posterior ativação de seus intermediários específicos, culminando com uma maior atividade lipolítica (MORIGNY *et al.*, 2016). O *switch* metabólico ocorre geralmente entre 12 e 36 horas após a última refeição, dependendo da concentração de glicogênio hepático e do gasto energético individual durante o jejum (exercício ou não). Após isso, os lipídeos são então metabolizados até ácidos graxos livres e liberados na corrente sanguínea (ANTON *et al.*, 2018; THIERION; ODOM, 2018). Parte destes ácidos graxos formam cetonas no fígado e estas, por sua vez, são transportadas aos tecidos ativos como músculos e neurônios onde serão reconvertidas a acetil-CoA, que participa do ciclo de Krebs para a formação de energia. Dessa forma, parece que quando o *switch* é acionado, a primeira fonte de energia corporal é trocada de glicose para ácidos graxos livres advindos do tecido adiposo e cetonas do fígado, preservando assim o tecido muscular (ANTON *et al.*, 2018).

Ainda durante o jejum, proteínas como a sirtuína 1 (SIRT1) são ativadas neste manejo metabólico de glicogenólise para cetogênese, o que leva à desacetilação e degradação do *CREB Regulated Transcription Coactivator 2* (CRTC2), principal mediador de gliconeogênese hepática (WALKER *et al.*, 2010; WANG *et al.*, 2009). A SIRT1 ainda é capaz de aumentar a oxidação de ácidos graxos através da desacetilação de fatores como PGC1 α e ativação do PPAR α (CANTÓ *et al.*, 2010; PURUSHOTHAM *et al.*, 2009; RODGERS, Joseph T *et al.*, 2005).

1.3. Exercício e jejum

Muitos ajustes metabólicos são similares entre o jejum e o exercício físico prolongado, como depleção dos estoques de glicogênio, ativação de vias metabólicas como lipólise, cetogênese e gliconeogênese, bem como mobilização de proteínas estruturais para sua utilização como substratos. O exercício físico tem a capacidade de alterar as maquinarias bioquímica e molecular para modificar a mobilização de substratos energéticos e essa flexibilidade metabólica entre a mobilização de glicose e ácidos graxos durante o exercício em indivíduos saudáveis se dá principalmente pela intensidade e volume do exercício (GOODPASTER; SPARKS, 2017). Em situações de déficit energético, uma disponibilidade limitada de glicose para oxidação tem de ser compensada pelo aumento da oxidação de ácidos graxos com o intuito de preservar concentrações adequadas de glicose sanguínea para o correto funcionamento de tecidos dependentes deste substrato, como o cérebro (ZACHAREWICZ; HESSELINK; SCHRAUWEN, 2018). Durante o exercício, a manutenção da glicemia é garantida pela mobilização dos estoques de glicogênio hepático, enquanto a ingestão de glicose durante ou previamente ao exercício pode criar uma situação atípica onde genes envolvidos no metabolismo lipídico (CD36, UCP3, CPT1) ou na regulação da glicólise (PDK4), que seriam estimulados pelo exercício, são inibidos pelo uso preferencial de carboidratos (CIVITARESE *et al.*, 2005; DOHM *et al.*, 1983; WAHREN, 1979). Apesar disso, o fluxo de glicose aumentado durante o exercício não é capaz de estimular a expressão de genes relacionados à sinalização insulínica e este efeito é inerente à contração muscular (CIVITARESE *et al.*, 2005).

Apesar do tecido muscular, durante o repouso em estado alimentado, obter a maior parte de sua energia pela oxidação de glicose e do repouso em jejum, ou exercício de baixa intensidade, obter grande parte da energia através da oxidação de ácidos graxos advindos do tecido adiposo, o conteúdo prévio de glicogênio nas fibras musculares é capaz de modificar a utilização de substratos para formação de energia (HOROWITZ, 2001; NEWSHOLME, 1979). Quando a utilização de ácidos graxos como substrato energético é aumentada, há uma concomitante diminuição na utilização de glicose, resultando em uma menor mobilização de glicogênio dos tecidos e vice-versa (DOHM *et al.*, 1983, 1986). Apesar da magnitude das respostas transcricionais deflagradas pelo jejum estar positivamente correlacionada ao seu tempo de duração, o exercício realizado em jejum é capaz de atenuar esta atividade transcricional induzida pelo jejum *per se* (HILDEBRANDT; NEUFER, 2000). Esta atenuação foi comprovada pela diminuição da expressão de enzimas envolvidas no metabolismo lipídico, como a *lipoprotein lipase* (LPL), CPT1, e UCP3 (HILDEBRANDT; NEUFER, 2000).

Embora apresentem diferentes atividades em tecidos adiposos localizados em diferentes regiões corporais, as catecolaminas adrenalina e noradrenalina são hormônios reguladores lipolíticos por excelência (ARNER *et al.*, 1990; MORIGNY *et al.*, 2016). Apesar da lipólise ser inibida pela ação de substâncias como adenosina, prostaglandinas e ainda hormônios como insulina e as próprias catecolaminas por meio da ativação de receptores α 2-adrenérgicos, quando estimulada, esta lipólise apresenta mecanismos de ação diferentes entre o repouso e o exercício (ARNER *et al.*, 1990; HOROWITZ, 2001; MORIGNY *et al.*, 2016). Durante o repouso é a inibição α -adrenérgica que modula a lipólise, enquanto no exercício, são mecanismos de estimulação β -

adrenérgicos que a regulam (ARNER *et al.*, 1990). Estes mecanismos de ativação β -adrenérgica na musculatura vascular lisa são capazes ainda de reduzir o tônus vascular, aumentando assim o fluxo sanguíneo do tecido adiposo, uma das variáveis que controlam a liberação dos ácidos graxos livres por este tecido (HOROWITZ, 2001). Desta maneira, um fluxo sanguíneo aumentado e a deflagração de respostas pelos receptores β -adrenérgicos parecem agir em sinergismo para uma maior mobilização de ácidos graxos do tecido adiposo para sua posterior oxidação durante o exercício. Indivíduos obesos podem desenvolver resistência adrenérgica capaz de prejudicar todo esse sistema de controle de tônus vascular, prejudicando assim o processo de liberação de TAG pelo tecido adiposo branco (GOODPASTER; SPARKS, 2017).

Existem muitos sítios possíveis de controle celular que contribuem para melhor captação, transporte e oxidação de substratos sob condições de contração muscular aumentada, e a elevação da concentração de Ca^{2+} citoplasmático que medeia a contração muscular também ativa a *Ca²⁺/calmodulin-dependent protein kinase IV* (CaMKIV) que, por sua vez, ativa o fator de transcrição *cAMP response element-binding protein* (CREB) (CIVITARESE *et al.*, 2005). Um aumento tanto na atividade da *AMP-activated protein kinase* (AMPK) quanto do CREB são capazes de estimular a expressão de PGC1 α , o principal regulador da transcrição de genes que codificam proteínas responsáveis pela divisão e crescimento mitocondrial e responsável por estimular a utilização de ácidos graxos como fonte energética (VICENTE-SALAR; OTEGUI; COLLADO, 2015; WU *et al.*, 2006).

Apesar da realização de treinamento físico em jejum não aumentar o conteúdo de CD36 e UCP3 no tecido muscular, o conteúdo de FABPpm, *glucose*

transporter 4 (GLUT4) e *hexokinase II* (HKII) são aumentados, caracterizando uma maior captação de ácidos graxos livres plasmáticos, glicose plasmática e fosforilação dessa glicose para posterior metabolização, respectivamente, pelo tecido muscular (DE BOCK *et al.*, 2008). O gasto energético aumentado e a oxidação de lipídeos impostos pelo exercício, em jejum ou não, em indivíduos obesos são capazes de melhorar a relação entre mobilização e oxidação lipídica. Uma proporção considerável dos ácidos graxos oxidados durante o exercício aeróbio de intensidade moderada provém dos TGIM, o que justifica a utilização destes protocolos de exercício com o intuito de melhorar a sensibilidade insulínica de seus praticantes (HOROWITZ, 2001).

Entretanto, parece que os mecanismos de preferência de substratos metabólicos ainda estão longe de serem completamente elucidados, visto que há divergência em resultados que avaliam a oxidação de substratos durante o exercício realizado em jejum. Alguns autores afirmam que o exercício aeróbio de baixa intensidade realizado em jejum não oferece nenhuma vantagem no que concerne à oxidação de ácidos graxos quando comparado ao exercício realizado em estado alimentado, enquanto outros mostram que, no músculo esquelético, o exercício realizado em jejum estimula a utilização de ácidos graxos como fonte primária de energia enquanto suprime o metabolismo glicídico comparado ao estado alimentado (AIRD; DAVIES; CARSON, 2018; IWAYAMA *et al.*, 2015; VAN PROEYEN *et al.*, 2011; VICENTE-SALAR; OTEGUI; COLLADO, 2015; VILAÇA-ALVES *et al.*, 2018; ZOUHAL *et al.*, 2020). Além disso, propriedades morfológicas, fisiológicas e metabólicas que caracterizam os diferentes tipos de músculos devem ser levadas em consideração, já que, músculos glicolíticos apresentam maiores quantidades de enzimas da via glicolítica, menor

vascularização e são conhecidos por fibras de contração rápida, enquanto músculos oxidativos apresentam propriedades de fibras chamadas de contração lenta, caracterizadas por alta densidade mitocondrial, grande conteúdo de mioglobina e aumentada vascularização (SIMONEAU; BOUCHARD, 1989, 1995). Caracteristicamente, o músculo sóleo do rato é classificado como predominantemente oxidativo, enquanto o gastrocnêmio, predominantemente glicolítico (ARMSTRONG; PHELPS, 1984).

Todavia, independente do grupo muscular avaliado, vale ressaltar que todos estes trabalhos avaliaram apenas indivíduos saudáveis. Assim, os efeitos do exercício e do jejum (Figura 4) parecem contribuir para um melhor perfil metabólico geral, sugerindo que sua associação pode potencializar seus efeitos, o que levaria a uma melhora mais rápida na resistência à insulina e inflamação crônica de baixo grau. Os possíveis efeitos do exercício realizado no estado em jejum nos tecidos muscular e adiposo branco são demonstrados na Figura 4 a seguir.

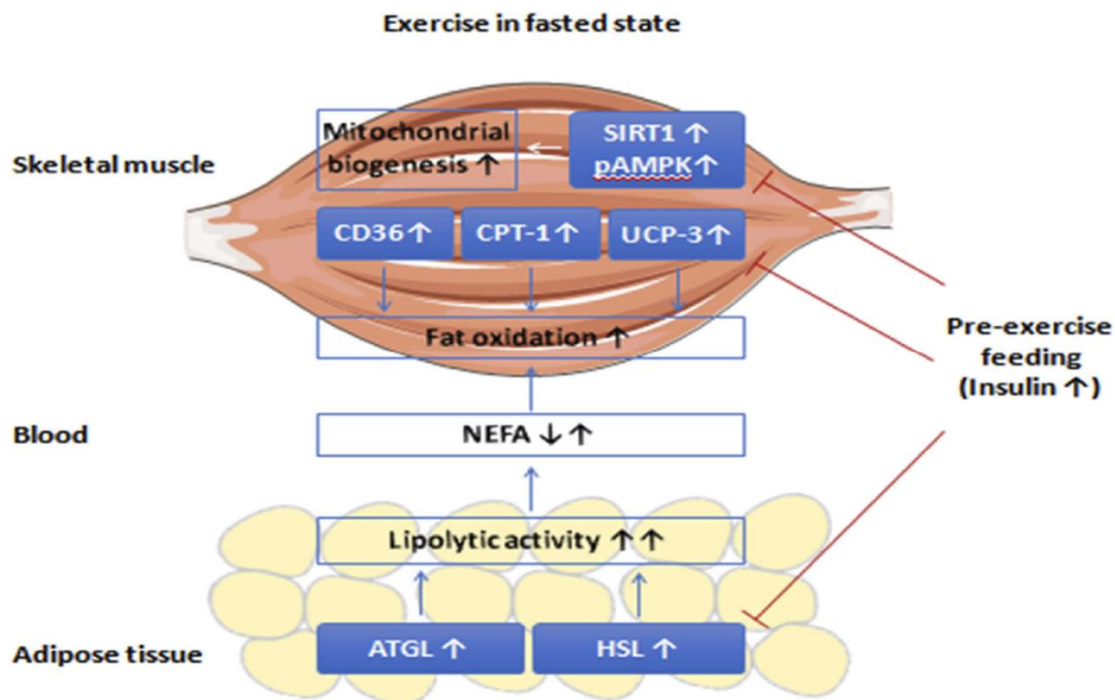


Figura 4. Exercício em jejum em comparação ao estado alimentado. Potencial efeito do exercício aeróbio realizado em jejum no aumento da oxidação de ácidos graxos. Efeitos inibitórios da alimentação pré-exercício sobre a oxidação de ácidos graxos através da estimulação da secreção de insulina e sua consequente inibição da atividade de proteínas como UCP-3, SIRT1, AMPK e HSL. SIRT1, *sirtuin 1*; pAMPK, *phosphorylated AMP-activated protein kinase*; CD36, *cluster of differentiation 36*; CPT-1, *carnitine palmitoyltransferase 1*; UCP-3, *uncoupling protein 3*; NEFA, *non-esterified fatty acids*; ATGL, *adipose triglyceride lipase*; HSL, *hormone-sensitive lipase* (ZOUHAL *et al.*, 2020).

1.4. Via AMPK-SIRT1-PGC1 α

A AMPK é uma molécula heterotrimérica com uma subunidade catalítica α e duas subunidades regulatórias β e γ (CARLING, 2019; FORETZ; EVEN; VIOLLET, 2018; TAMARGO-GÓMEZ; MARIÑO, 2018). A subunidade α apresenta um domínio quinase serina/treonina na região N-terminal onde se encontra um resíduo crítico de treonina (Thr172). Esse resíduo de treonina pode ser fosforilado por inúmeras enzimas quinases, o que constitui o principal mecanismo pela qual a atividade da AMPK é regulada a curto prazo (TAMARGO-GÓMEZ; MARIÑO, 2018).

A AMPK pode ser encontrada em quase todas as células do corpo na forma quiescente, sendo ativada em situações de estresse energético induzido por contração muscular, hipóxia e privação de nutrientes, que basicamente modificam a razão AMP/ATP causando um aumento no consumo de ATP ou reduzindo sua produção (ALLEN; SAHA, 2017; FORETZ; EVEN; VIOLLET, 2018; GALIC *et al.*, 2018; JEON, 2016; SAMOVSKI *et al.*, 2015). Estes e outros fenômenos capazes de ativar a AMPK são mostrados na Figura 5. Além disso, essa proteína é capaz de se apresentar em diferentes frações celulares, como lisossomal, mitocondrial e citosólica, deflagrando assim diferentes respostas (CARLING, 2019). Dessa maneira, as diferentes frações da AMPK celular podem ser ativadas por diferentes vias. A AMPK lisossomal é a mais sensível, sendo ativada por diminuições nas concentrações celulares de *fructose 1,6-bisphosphate* (FBP). Já a AMPK citosólica necessita de modestos aumentos nos níveis de AMP, enquanto a AMPK mitocondrial apresenta baixa sensibilidade, precisando de altas concentrações de AMP. Em contrapartida, a AMPK nuclear não é capaz de ser ativada por nenhum desses estímulos citados (CARLING, 2019).

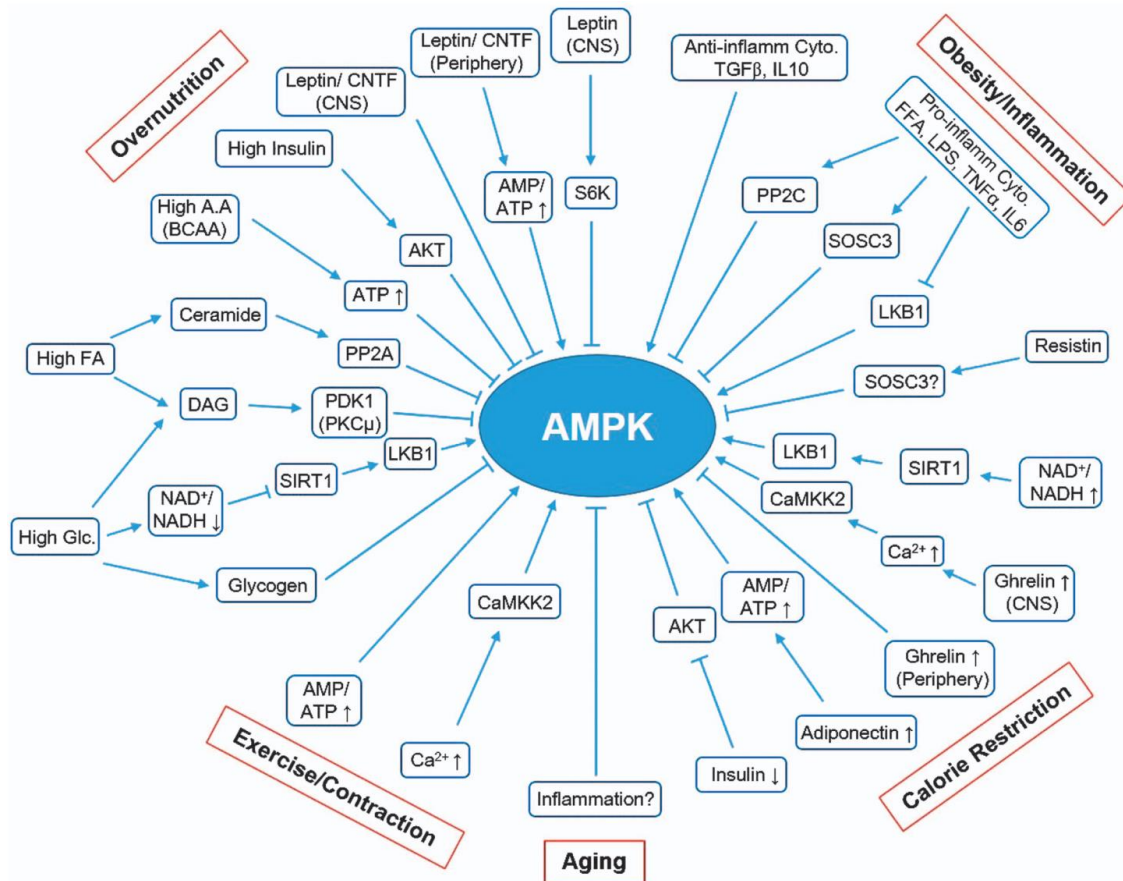


Figura 5. Regulação fisiológica da AMPK. Esquema resumindo os mecanismos subjacentes à regulação da atividade de AMPK sob diversas condições fisiológicas e patológicas. A seta indica ativação e a linha com barra indica inibição (JEON, 2016).

A AMPK é capaz de estimular a oxidação de ácidos graxos através da fosforilação e inativação da enzima *acetyl-CoA carboxylase 2* (ACC2) em um resíduo de serina na posição 222 (Ser222), causando uma redução na concentração de malonil-CoA, importante inibidor da β -oxidação através da diminuição da captação de ácidos graxos pela mitocôndria por seu sistema de transporte CPT1 (BHUTTA; GALLO; BORENSTEIN, 2021; FORETZ; EVEN; VIOLLET, 2018; GALIC *et al.*, 2018; MCGARRY; BROWN, 1997; SAMOVSKI *et al.*, 2015). Além de prevenir um possível acúmulo de malonil-CoA, a AMPK é capaz de inibir diretamente a lipogênese através da inibição da atividade da

SREBP1, proteína chave da via lipogênica (TAMARGO-GÓMEZ; MARIÑO, 2018).

Um aumento na atividade da AMPK ainda está relacionado a um aumento na expressão de PLIN3, demonstrando sua importância na regulação da mobilização também de ácidos graxos (KLEINERT *et al.*, 2016). Além da regulação da mobilização de substratos, a AMPK é indispensável para o catabolismo muscular, provendo assim substratos para o processo de gliconeogênese hepática, prevenindo um quadro de hipoglicemia e hipercetose (BUJAK *et al.*, 2015). Somado a seu papel inibitório na via lipogênica, a AMPK ainda apresenta uma importante função no metabolismo glicídico, onde promove aumento da captação de glicose por sua fosforilação inibitória no *TBC domain family member 1* (TBC1D) e *thiredoxin-interacting protein* (TXNIP), fatores estes que inibem a translocação dos transportadores de glicose *glucose transporter 1* (GLUT1) e GLUT4, respectivamente, à membrana celular (BHUTTA; GALLO; BORENSTEIN, 2021; TAMARGO-GÓMEZ; MARIÑO, 2018). Por último, mas não menos importante, a AMPK pode aumentar a biogênese mitocondrial em situações de déficit energético, com o intuito de aumentar a produção de ATP. Isto se dá tanto pela regulação da AMPK sobre a fosforilação de proteínas como SIRT1 e outras MAPK quanto pela fosforilação de PGC1 α (TAMARGO-GÓMEZ; MARIÑO, 2018; VAN PROEYEN *et al.*, 2011). Fatores como *peroxisome proliferator-activated receptor δ* (PPAR δ) ainda apresentam um papel crucial no metabolismo de ácidos graxos e colesterol, já que sua estimulação pode ser controlada pela AMPK, sendo capaz de aumentar o número de fibras musculares oxidativas (VAN PRAAG *et al.*, 2014).

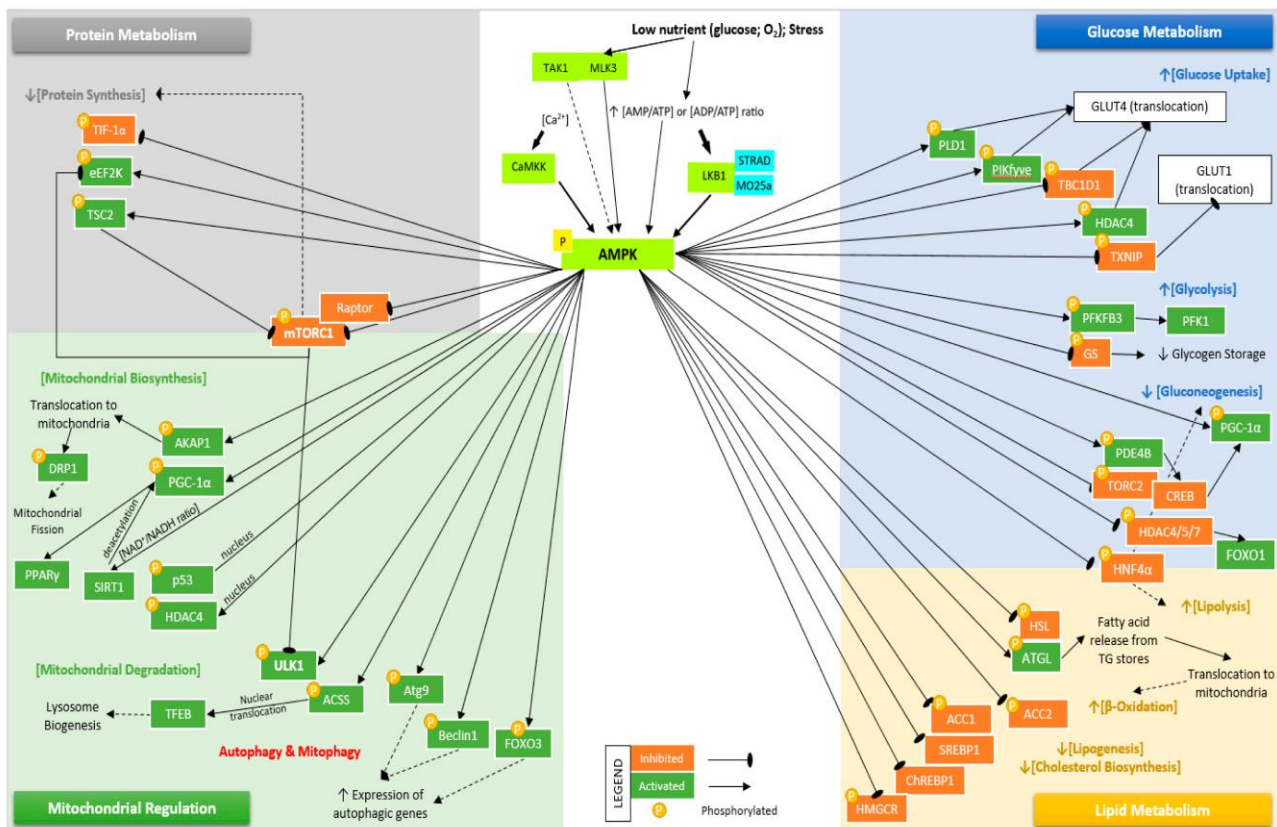


Figura 6. Esquema de proteínas que podem ter suas atividades estimuladas ou inibidas pela ativação da AMPK (BHUTTA; GALLO; BORENSTEIN, 2021).

AMPK, SIRT1 e PGC1 α constituem uma rede de controle energético que controla o metabolismo celular e estão intrinsicamente envolvidas com exercício físico (DRAZNIN *et al.*, 2012). A SIRT1 é ativada por uma modificação na razão NADH/NAD⁺, onde é capaz de aumentar a oxidação de ácidos graxos através da desacetilação de fatores como PGC1 α e deflagrar respostas de resolução de inflamação através da ativação de fatores como *heat shock factor 1* (HSF1) (CANTÓ *et al.*, 2010; NEWSHOLME; DE BITTENCOURT JR, 2014; PARDO; BORIEK, 2011; RODGERS, Joseph T. *et al.*, 2005). O PGC1 α , por sua vez, é capaz de deflagrar respostas como a ativação de receptores nucleares e induzir a transcrição de fatores como PPAR α , PPAR δ , *nuclear respiratory factor 1* (NRF1), *nuclear factor erythroid 2-related factor 2* (NRF2) e novamente HSF1 (MA *et al.*, 2018). Dessa maneira, a modulação da expressão dos genes

codificadores destas proteínas parece de grande valia no combate à obesidade relacionada à inflamação crônica e na potencialização da oxidação de ácidos graxos diminuídas pela obesidade. Na figura 6, é mostrado um esquema resumido das proteínas que podem ter suas atividades moduladas pela ativação da AMPK.

1.5. Resposta de choque térmico

Não obstante, é de fundamental importância o papel da HSP70 no combate à inflamação crônica de baixo grau que, por sua vez, modifica o controle metabólico glicídico, sendo o exercício um dos fatores de estresse capazes de aumentar seu conteúdo e de seu fator de transcrição HSF1 no músculo esquelético (KRAUSE *et al.*, 2015a, 2007; NOBLE; MILNE; MELLING, 2008; SARGE; MURPHY; MORIMOTO, 1993). As HSPs estão envolvidas em processos essenciais para a função celular como apresentação de antígeno, ligação ao receptor de esteroides, tráfego intracelular, ligação ao receptor nuclear e apoptose (YENARI *et al.*, 1999). Contudo, é na biossíntese de proteínas que desempenham sua função mais importante, agindo como chaperonas moleculares, auxiliando polipeptídeos nascentes a assumirem sua conformação tridimensional correta (BECKER; CRAIG, 1994; FEDER; HOFMANN, 1999; MORTON *et al.*, 2006). As HSPs ainda protegem as células da desnaturação e da agregação de polipeptídios induzidos durante o estresse, aumentando as chances de sobrevivência celular (BECKER; CRAIG, 1994; SALO; DONOVAN; DAVIES, 1991; YENARI *et al.*, 1999).

Todos os efeitos da HSP70 são dependentes de seu principal fator de transcrição HSF1, que encontra-se ligado à própria HSP70 de forma quiescente

até que seja imposto algum estresse à célula (KRAUSE *et al.*, 2015b). O HSF1 é ativado em resposta a vários tipos de estresse, fosforilado, trimerizado, translocado ao núcleo celular, mantido desacetilado e ligado à região promotora do gene de interesse pela SIRT1, onde vai regular a transcrição de alguns genes, dentre eles o responsável por codificar a HSP70 (KRAUSE *et al.*, 2015a; SINGH *et al.*, 2002; WU *et al.*, 2013). Sendo assim, os mesmos eventos celulares capazes de aumentar a atividade e expressão do gene da HSP70 são capazes de ativar seu próprio fator de transcrição HSF1, tornando mais rápida e eficiente a resposta contra algum insulto. De maneira geral, indivíduos obesos apresentam aumento de fosforilação de fatores pró-inflamatórios, como *c-Jun N-terminal kinase* (JNK) e *I κ B kinase- β* (IKK- β), inibição do *insulin receptor substrate 1* (IRS1), reduzida translocação de GLUT4 em resposta à insulina e ativação de fatores como *nuclear factor of kappa-light-polypeptide gene enhancer in B-Cells* (NF- κ B) (HOOPER *et al.*, 2014; ZEYDA; STULNIG, 2009). Esse quadro culmina com maior ativação de vias pró-inflamatórias e resistência insulínica, que, por sua vez, pode ser deflagrada pela obesidade, podendo resultar em DM2 e outros desfechos como dislipidemias e hipertensão (ZEYDA; STULNIG, 2009). Os efeitos benéficos da HSP70 sobre a inflamação/resistência insulínica se dão em parte pela ação inibitória tanto sobre IKK- β e JNK, quanto da inibição da translocação nuclear do NF- κ B (Figura 7) (ARCHER; VON SCHULZE; GEIGER, 2017; CHUNG *et al.*, 2008; DE TODA; DE LA FUENTE, 2015; HOOPER *et al.*, 2014). Esses efeitos indicam que a HSP70 apresenta um papel protetor e anti-inflamatório associado à sensibilidade insulínica normal (KRAUSE *et al.*, 2015a).

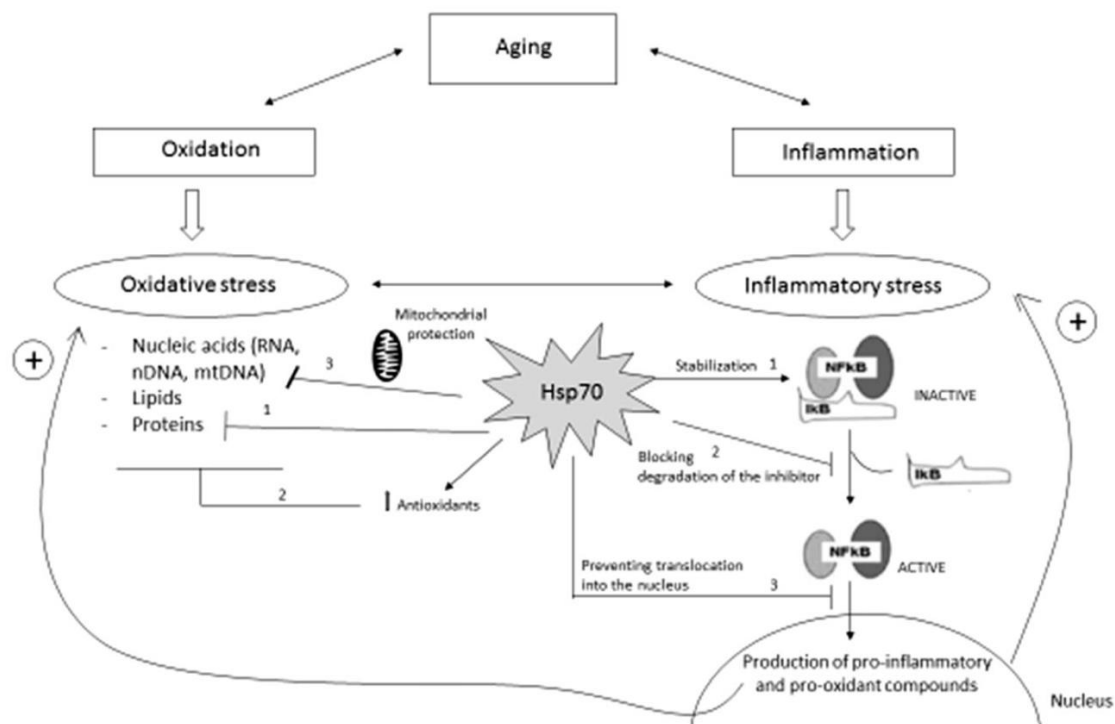


Figura 7. Papel da HSP70 intracelular no combate à inflamação. A HSP70 intracelular atua como um mecanismo de defesa antioxidante por: 1) redobrimento ou direcionamento, por degradação, de proteínas dobradas incorretamente; 2) aumentando a expressão ou atividade de proteínas antioxidantes, como catalase ou superóxido dismutase; e 3) manutenção do potencial de membrana mitocondrial. Além disso, a HSP70 pode combater o estresse inflamatório através do bloqueio da ativação do fator de transcrição NF- κ B por: 1) estabilização do complexo inativo NF- κ B-I κ B; 2) bloqueio da degradação do inibidor I κ B; e 3) impedindo a translocação do complexo NF- κ B ativo ao núcleo celular (DE TODA; DE LA FUENTE, 2015).

Em conclusão, tendo em vista a relevância da prática de exercícios físicos no controle metabólico e molecular, faz-se necessária a investigação do efeito do exercício aeróbio contínuo realizado em jejum sobre estes marcadores para que se possam ser normatizadas variáveis como volume e intensidade desta prática com o intuito da potencialização de seus possíveis resultados. Embora efeitos subagudos sejam caracterizados como os fenômenos observados entre duas sessões seguidas de exercício (DA NOBREGA, 2005), no presente trabalho serão caracterizados como efeitos subagudos os efeitos observados 12h após a realização do exercício, já que apenas uma sessão foi realizada.

2. HIPÓTESE

2.1. O exercício aeróbio contínuo de moderada intensidade realizado em jejum pode potencializar respostas metabólicas tanto agudas (imediatas) quanto subagudas (12 horas após sua realização) já identificadas em condições de jejum ou de exercício separadamente. Podem ser observadas respostas agudas, como modificação no conteúdo de substratos intramusculares, bem como na expressão de genes relacionados ao controle metabólico celular imediatamente após o exercício. Doze horas após a realização do exercício, respostas subagudas também podem ser observadas, como modificações no conteúdo de substratos intramusculares e séricos, e também no conteúdo de proteínas relacionadas à resposta de choque térmico em animais obesos. A figura 8 a seguir mostra a inter-relação entre as vias AMPK-SIRT1-PGC1 α e a via de choque térmico.



Figura 8. Interação entre as vias AMPK-SIRT1-PGC1 α e de choque térmico (HSF1-HSP70).

2.1. Hipótese do artigo 1

Em ratos obesos, o exercício aeróbio contínuo de moderada intensidade em jejum pode potencializar respostas metabólicas e moleculares, tais como alterações nas concentrações de substratos e na expressão da resposta de choque térmico, 12 horas após a sua execução.

2.2. Hipótese do artigo 2

Ratos magros e obesos apresentam respostas positivas agudas (imediatas) à realização do exercício aeróbio contínuo em jejum tanto no que concerne às concentrações séricas de substratos, bem como à mobilização de substratos para oxidação e expressão de genes relacionados ao controle do metabolismo glicídico e lipídico. Ratos magros apresentam diferentes padrões de expressão dos genes relacionados ao metabolismo e diferente mobilização de substratos musculares quando comparados aos obesos. Além disso, tanto ratos magros como obesos, apresentam diferenças metabólicas e moleculares entre músculos glicolíticos e oxidativos.

3. OBJETIVOS

3.1. Objetivo geral

Levando em consideração a importância do exercício físico, de qualquer intensidade, no controle metabólico, pretende-se investigar o efeito de uma sessão de exercício aeróbio contínuo de intensidade moderada realizado em estado alimentado ou em jejum sobre marcadores metabólicos séricos, a oxidação de diferentes substratos, a expressão de genes relacionados ao metabolismo lipídico e quantificar o conteúdo destas proteínas em ratos machos magros e obesos.

3.1.1. Objetivo geral do artigo 1

Avaliar os efeitos subagudos (12h após sua execução) de uma única sessão de exercício de intensidade moderada, realizado no estado alimentado ou em jejum, em parâmetros metabólicos e moleculares em ratos Wistar machos obesos.

3.1.1.1. Objetivos específicos do artigo 1

- Quantificar a concentração de substratos plasmáticos 12h após a intervenção;
- Quantificar o conteúdo de substratos (glicogênio, lactato e triglicerídeos) no fígado, coração e músculos gastrocnêmio e sóleo 12h após a intervenção;
- Avaliar o imunoc conteúdo de proteínas da via de choque térmico como SIRT1 e HSP70 nos músculos gastrocnêmio e sóleo 12h após a intervenção.

3.1.2. Objetivo geral do artigo 2

Investigar a contribuição de diferentes tipos de substratos oxidativos (glicose, alanina e palmitato) durante o exercício em jejum em músculos oxidativos e glicolíticos de ratos sedentários magros e obesos. A expressão de genes envolvidos no controle metabólico e no equilíbrio inflamatório também foi investigada.

3.1.2.1. Objetivos específicos do artigo 2

- Quantificar a concentração de substratos plasmáticos imediatamente após a intervenção;
- Quantificar o conteúdo de glicogênio e de triglicerídeos no fígado, coração e músculos gastrocnêmio e sóleo imediatamente após a intervenção;
- Avaliar a oxidação de substratos como $U\text{-}^{14}\text{C}$ -glicose, ^{14}C -alanina e ^{14}C -palmitato nos músculos gastrocnêmio e sóleo imediatamente após a intervenção; e
- Quantificar a expressão gênica de *AMPK*, *HSP70*, *HSF1*, *SIRT1* e *PGC1 α* nos músculos gastrocnêmio e sóleo imediatamente após a intervenção.

4. MATERIAIS E MÉTODOS

4.1. Considerações éticas

O presente estudo foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal do Rio Grande do Sul (UFRGS) sob o protocolo 34271 (comprovante disponível no Anexo I). O projeto foi desenvolvido no Laboratório de Metabolismo e Endocrinologia Comparada (LaMEC) em conjunto com o Laboratório de Fisiologia Celular (FisCel) no Departamento de Fisiologia do Instituto de Ciências Básicas da Saúde (ICBS) da UFRGS. Todos os procedimentos deste trabalho foram realizados de acordo com as diretrizes do Conselho Nacional de Controle de Experimentação Animal (CONCEA).

4.2. Manutenção dos animais e desenho experimental

Foram utilizados 110 *Rattus norvegicus* linhagem Wistar, machos, adultos, com 8 semanas de idade, obtidos do Centro de Reprodução e Experimentação de Animais em Laboratório (CREAL) da UFRGS, os quais foram alojados no Biotério setorial do Departamento de Farmacologia do ICBS da UFRGS. Os animais foram alojados em caixas/moradias de polipropileno (410mm x 340mm x 160mm) com grades altas que asseguram altura mínima de 17,8cm. A área interna de cada gaiola era de 1110cm². Os animais foram mantidos em temperatura controlada de 21°C, ciclo de 12h claro/12h escuro e umidade mantida entre 40-60% (3 animais por caixa). Foram realizadas três trocas de caixas por semana, com aproximadamente 5cm de cama de maravalha de *Pinnus sp* previamente autoclavada. A higienização das caixas, grades,

bebedouros e bicos foi realizada com detergente neutro e imersas por 2h em solução de hipoclorito a 10%.

Os animais foram distribuídos aleatoriamente em dois grupos principais e alimentados por 12 semanas com dieta padrão Nuvilab (Quimtia®) (DP: 4,48% lipídios; 64,98% carboidratos e 11,75% proteínas) ou dieta hiperlipídica modificada (HFD: 31,07% lipídios, 49,09% carboidratos e 8,18% proteínas) para indução à obesidade. Ao final desse período, os animais magros (n=40) pesavam 502,7g ($\pm 45,1$), enquanto os obesos (n=40) pesavam 643,0g ($\pm 86,0$) (artigo 2). Durante a última semana de protocolo experimental, todos os animais foram aclimatados à esteira, o que consistiu em uma sessão diária de 15 minutos em velocidade muito baixa e inclinação de 0° por cinco dias consecutivos. Ainda neste momento, um teste de tolerância à glicose foi realizado, onde a glicose foi administrada por gavagem (OGTT) ($1\text{g}\cdot\text{kg}^{-1}$) ou injetada intraperitonealmente (IPGTT) ($1\text{g}\cdot\text{kg}^{-1}$) após 8 horas de jejum *overnight*. A glicemia foi quantificada, no sangue da cauda do animal, com a utilização de um glicosímetro (On Call Plus, Acon Laboratories Inc., San Diego, CA, EUA) em 0, 15, 30, 45, 60, 90 e 120 minutos após a administração da solução de glicose. Para ambos os testes, foi calculada a área incremental sob a curva tanto para os animais magros, como para os obesos.

Um período de *washout* de 5 dias entre o último dia de aclimação e a sessão de exercício agudo foi incluído para evitar quaisquer ajustes ou efeitos subagudos da última sessão de aclimação (VOGT *et al.*, 2021). Ao final do tratamento experimental, animais magros e obesos foram alocados aleatoriamente em quatro grupos experimentais: alimentados em repouso (*fed and rested*, FER), alimentados e exercitados (*fed and exercised*, FEE), jejuados

em repouso (*fasted and rested*, FAR) e jejuados e exercitados (*fasted and exercised*, FAE), totalizando oito grupos experimentais com 10 animais em cada grupo. Antes da sessão de exercício, os animais dos grupos FAR e FAE foram submetidos a um período de jejum de 8h.

Para a realização do trabalho onde foram utilizados apenas animais obesos (artigo 1), o mesmo protocolo experimental foi executado, entretanto, foram utilizados 30 animais, pesando 657,2g ($\pm 69,0$). Estes animais realizaram a mesma aclimação à esteira e mesmo protocolo de exercício, mas foram eutanasiados 12 horas após a realização do mesmo, como descrito na metodologia do Artigo 1 (Figura 9).

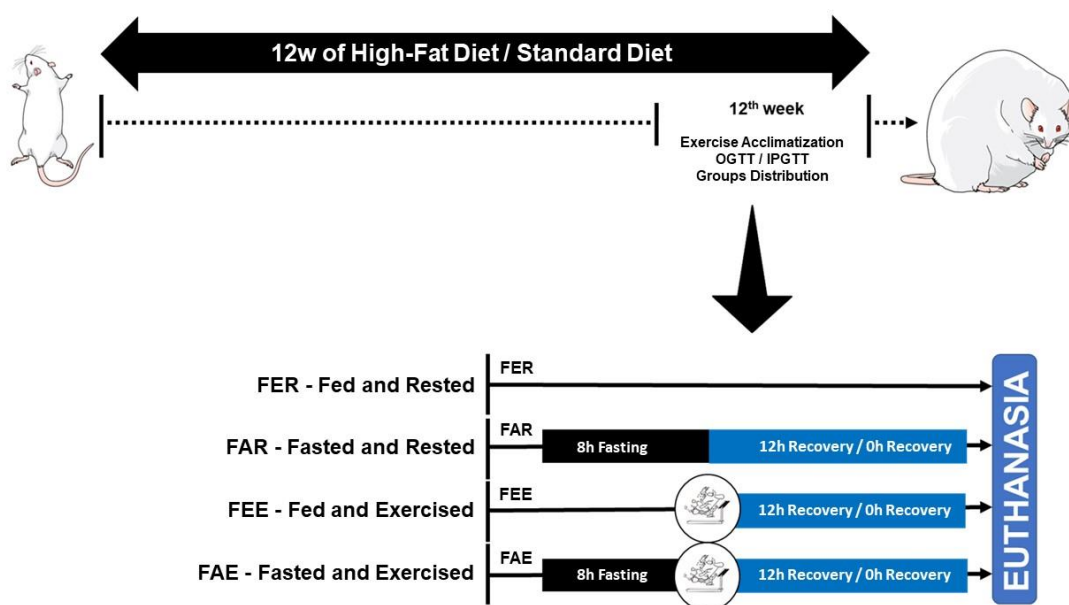


Figura 9. Desenho experimental. Indução à obesidade, alocação dos animais em seus grupos, submissão ao jejum e realização do protocolo de exercício.

4.3. Protocolo de exercício

O protocolo de exercício utilizado foi desenvolvido a partir do descrito em Rodrigues et al. (2007) e consistiu em uma sessão de 30 minutos em esteira com

velocidade e inclinação correspondentes a uma intensidade de 60% do $VO_{2\text{máx}}$ ($10\text{m}\cdot\text{min}^{-1}$, inclinação 0° da esteira). Os animais em repouso foram mantidos em esteira desligada por 30 minutos. Para testar os efeitos agudos da presente intervenção, os animais foram sacrificados imediatamente após a sessão de exercício (artigo 2) ou 12h após a mesma (artigo 1). Os animais foram eutanasiados por decapitação e foram coletadas amostras de sangue, fígado, coração, sóleo e gastrocnêmio. As amostras de sangue foram centrifugadas (10min, $1510g$) e o soro foi utilizado para avaliar as concentrações de glicose, lactato, proteínas totais, colesterol total e triglicerídeos. Os conteúdos de glicogênio e triglicerídeos foram determinados nas amostras de tecido. Tanto a expressão dos genes *HSP70*, *HSF1*, *PGC1a*, *SIRT1* e *AMPK* como o imunocnteúdo de SIRT1 e HSP70 foram determinados em amostras de gastrocnêmio e sóleo.

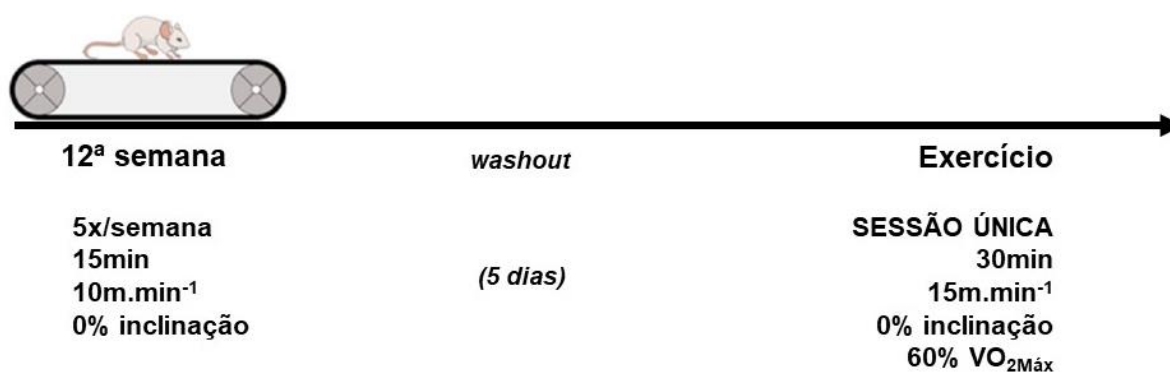


Figura 10. Protocolo de exercício realizado pelos animais (RODRIGUES *et al.*, 2007).

4.4. Análises bioquímicas

Glicose, lactato, proteínas totais, colesterol total e triglicerídeos séricos foram quantificados por meio de *kits* de ensaios enzimáticos (Labtest Diagnóstica SA, Lagoa Santa, Minas Gerais, Brasil) para análise em espectrofotômetro. As concentrações de glicose, triglicerídeos, e colesterol total foram expressas em $\text{mg}\cdot\text{dL}^{-1}$ de soro, lactato em $\text{mmol}\cdot\text{L}^{-1}$ e proteínas totais em $\text{g}\cdot\text{dL}^{-1}$. A concentração de glicogênio nas amostras de tecido foi determinada conforme descrito anteriormente (MODEL *et al.*, 2021a). Resumidamente, as amostras de tecido foram primeiramente homogeneizadas com KOH a 30%, após, lavadas com etanol, hidrolisadas com HCl 4N, neutralizadas com Na_2CO_3 2M e finalmente diluídas com água destilada. As concentrações de glicogênio foram determinadas como equivalentes de glicose utilizando um *kit* de glicose e expressas em mg de glicogênio.g de tecido⁻¹ (peso úmido). Para a extração dos triglicerídeos, as amostras de tecido foram homogeneizadas com solução salina 0,9% na proporção de 10:1 (1mg de tecido para 10 μL de solução salina) e a concentração de triglicerídeos foi determinada com o mesmo *kit* usado para o ensaio de soro (MODEL *et al.*, 2021b). Os resultados foram expressos em mg de triglicerídeo.g de tecido⁻¹ (peso úmido).

4.5. Oxidação de substratos (^{U-14}C-glicose, ¹⁴C-alanina e ¹⁴C-palmitato)

Fatias de tecido ($300 \pm 10\text{mg}$) foram incubadas por 60 minutos em frascos selados com 1mL de tampão Krebs Henseleit pH 7,4 mais 0,1% BSA, 0,1 μCi ^{U-14}C-glicose (55mCi/mmol GE Healthcare) mais 5mM de glicose não marcada, equilibrada com $\text{O}_2:\text{CO}_2$ (95:5, v/v) (COZER *et al.*, 2017). ¹⁴C-alanina e ¹⁴C-

palmitato foram incubados nas mesmas condições, mais 10mM ou 0,05mM de substrato não marcado, respectivamente. Os frascos continham pequenos poços de vidro no interior que foram fixados a tampas de borracha e continham pequenas tiras de papel Whatman 3MM. A incubação foi interrompida pela adição de 0,25mL de TCA a 50% no fundo do frasco através da tampa de borracha e 0,25mL de NaOH 2M nos poços de vidro para reter o $^{14}\text{CO}_2$ produzido. Posteriormente, as tiras de papel Whatman 3MM foram transferidas para frascos contendo líquido de cintilação (MODEL *et al.*, 2021b). Os valores de produção de $^{14}\text{CO}_2$ foram expressos em μmol de ^{14}C -substrato incorporado em $^{14}\text{CO}_2 \cdot \text{g}$ de tecido $^{-1} \cdot \text{h}^{-1}$. A radioatividade foi estimada por um contador de cintilação líquida Beckman.

4.6. Isolamento de RNA, síntese de cDNA e RT-qPCR

Amostras congeladas dos músculos gastrocnêmio e sóleo foram homogeneizadas em TriReagent® (Sigma) e o RNA foi extraído pelo método do clorofórmio-isopropanol. Amostras de RNA (1 μg) foram submetidas à transcrição reversa utilizando o *GoScript Reverse Transcription System* (Promega) em um volume total de 20 μl , conforme protocolo do fabricante. Para análise de mRNA, primers específicos de genes (Tabela 1) foram misturados a GoTaq® qPCR e RT-qPCR Systems (Promega) para uma concentração final das alíquotas de cDNA de 0,2 μM e triplicado de acordo com o protocolo do fabricante. As condições de ciclagem térmica foram de 2 minutos a 95°C, 10 segundos a 95°C, 1 minuto a 60°C, 10 segundos a 95°C, 1 minuto a 60°C, 15 segundos a 95°C (35 ciclos) seguido de análise da curva de *melting* no Stratagene Mx3000P

(Tecnologias Agilent). O método $\Delta\Delta C_t$ foi utilizado para calcular as mudanças relativas nas concentrações de mRNA.

Tabela 1: Sequência dos primers.

Sequências dos primers:

<i>HSP70 forward primer 5'-3'</i>	GTGCGGCCTTAGTAGAGGTG
<i>HSP70 reverse primer 5'-3'</i>	GGTCCACCTGCATCTTCTCT
<i>HSF1 forward primer 5'-3'</i>	TAGCCTGCCTAGACAAGAACG
<i>HSF1 reverse primer 5'-3'</i>	TCACCGAGGGGCTGAACA
<i>PGC1α forward primer 5'-3'</i>	TTGACTGGCGTCATTCAGGA
<i>PGC1α reverse primer 5'-3'</i>	CAGGGCAGCACACTCTATGT
<i>SIRT1 primers</i>	PrimePCR™ Assays BIORAD
<i>AMPK forward primer 5'-3'</i>	TCGGCAAAGTGAAGATTGGAG
<i>AMPK reverse primer 5'-3'</i>	CCAACAACATCTAAACTGCGAA
<i>β-Actin forward primer 5'-3'</i>	ATTGCTGACAGGATGCAGAA
<i>β-Actin reverse primer 5'-3'</i>	TAGAGCCACCAATCCACACAG

4.7. Western blotting

Para a separação das proteínas foi utilizado SDS-PAGE (eletroforese em gel de poliacrilamida com dodecil sulfato de sódio) com uma concentração de poliacrilamida de 10%. Aproximadamente 30 μ g da proteína extraída das amostras foram incubados com solução de Laemmli e adicionadas em cada poço do gel para eletroforese. O equipamento utilizado foi o *mini-PROTEAN® 3 Electrophoresis Cell* (BioRad, EUA). Depois de efetuada a eletroforese, o gel de poliacrilamida foi retirado das placas de vidro e colocado em módulo de

eletrotransferência em contato com a membrana de nitrocelulose (NC) coberto com tampão de transferência. A transferência foi efetuada no sistema *Trans-Blot SD – Semi Dry Electrophoretic Transfer Cell* (BioRad, EUA) por uma hora a uma diferença de potencial de 25V. As membranas de NC contendo as proteínas foram então incubadas durante uma hora em solução bloqueadora. Esta solução satura sítios de ligação inespecíficos na membrana de NC. Após o bloqueio, as membranas de NC foram incubadas por, no mínimo, 16 horas a 4°C sob agitação constante com os anticorpos (números do catálogo SAB4200714: HSP70; AV32386: SIRT1 (Sigma Aldrich)) para as proteínas específicas diluídos em *Tween Tris Buffer Saline* (TTBS). Após a incubação, as membranas foram lavadas com TTBS (1%) e então incubadas com o segundo anticorpo (número do catálogo A9044: *AntiMouse IgG (whole molecule) – rabbit Peroxidase antibody produced IgG fraction of antiserum*, (Sigma Aldrich) por 2 horas em temperatura ambiente. Logo após, as membranas foram lavadas com *Tris Buffer Saline* (TBS) e incubadas, em sala escura, com solução de quimiluminescência por um minuto. A reação de quimiluminescência ocorreu por meio do sistema de detecção baseado em substratos luminescentes (luminol e ácido p-cumárico). Após a incubação com a solução de quimiluminescência, as membranas de NC foram colocadas em contato com o filme fotográfico (GE Healthcare® / Amersham Hypperfilm™ ECL). Realizada a revelação, o filme foi analisado por densitometria óptica e as bandas medidas por um processamento de imagem (ImageMaster VDS, Pharmacia Biotech, EUA). Os resultados foram expressos em unidades arbitrárias (UA).

4.8. Análise estatística

Os resultados obtidos foram primeiramente submetidos ao teste de normalidade de Kolmogorov-Smirnov para verificar a distribuição dos dados e, posteriormente, ao teste de homogeneidade de Levene. Os dados normalmente distribuídos foram analisados usando ANOVA de duas vias para comparar os efeitos do jejum (alimentado x jejum), do exercício (repouso x exercício), bem como a interação entre esses fatores, com *post hoc* de Bonferroni para dados homogêneos ou Games-Howell para dados não homogêneos. Os dados não paramétricos foram examinados com o teste não paramétrico de Kruskal-Wallis, complementado pelo pós-teste de Dunn. Os resultados foram considerados estatisticamente diferentes quando $p < 0,05$. As análises foram realizadas com o *Statistical Package for the Social Sciences* (SPSS versão 25.0, IBM, Armonk, NY, EUA).

5. RESULTADOS

5.1. Primeiro artigo

Título: Metabolic and Molecular Subacute Effects of a Single Moderate-Intensity Exercise Bout, Performed in the Fasted State, in Obese Male Rats

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Article

Metabolic and Molecular Subacute Effects of a Single Moderate-Intensity Exercise Bout, Performed in the Fasted State, in Obese Male Rats

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Abstract: *Introduction and objectives:* Obesity represents a major global public health problem. Its etiology is multifactorial and includes poor dietary habits, such as hypercaloric and hyperlipidic diets (HFDs), physical inactivity, and genetic factors. Regular exercise is, per se, a tool for the treatment and prevention of obesity, and recent studies suggest that the beneficial effects of exercise can be potentiated by the fasting state, thus potentially promoting additional effects. Despite the significant number of studies showing results that corroborate such hypothesis, very few have evaluated the effects of fasted-state exercise in overweight/obese populations. Therefore, the aim of this study was to evaluate the subacute effects (12 h after conclusion) of a single moderate-intensity exercise bout, performed in either a fed or an 8 h fasted state, on serum profile, substrate-content and heat shock pathway-related muscle protein immunocontent in obese male rats. *Methods:* Male Wistar rats received a modified high-fat diet for 12 weeks to induce obesity and insulin resistance. The animals were allocated to four groups: fed rest (FER), fed exercise (FEE), fasted rest (FAR) and fasted exercise (FAE). The exercise protocol was a 30 min session on a treadmill, with an intensity of 60% of VO₂max. The duration of the fasting period was 8 h prior to the exercise session. After a 12 h recovery, the animals were killed and metabolic parameters of blood, liver, heart, gastrocnemius and soleus muscles were evaluated, as well as SIRT1 and HSP70 immunocontent in the muscles. *Results:* HFD induced obesity and insulin resistance. Soleus glycogen concentration decreased in the fasted groups and hepatic glycogen decreased in the fed exercise group. The combination of exercise and fasting promoted a decreased concentration of serum total cholesterol and triglycerides. In the heart, combination fasting plus exercise was able to decrease triglycerides to control levels. In the soleus muscle, both fasting and fasting plus exercise were able to decrease triglyceride concentrations. In addition, heat shock protein 70 and sirtuin 1 immunocontent increased after exercise in the gastrocnemius and soleus muscles. *Conclusions:* An acute bout of moderate intensity aerobic exercise, when realized in fasting, may induce, in obese rats with metabolic dysfunctions, beneficial adaptations to their health, such as better biochemical and molecular adaptations that last for at least 12 h. Considering the fact that overweight/obese populations present an increased risk of cardiovascular events/diseases, significant reductions in such plasma markers of lipid metabolism are an important achievement for these populations.

Keywords: fasting; aerobic exercise; obesity; metabolism; inflammation

1. Introduction

Resulting from an imbalance between acquired and expended calories, overweight and obesity have replaced tobacco consumption as the number one lifestyle-related major health problem worldwide [1–3]. According to the World Health Organization (WHO), overweight and obesity are the fifth highest risk factor for global deaths [4]. At least 2.8 million adults die each year because of being overweight or obese. Dietary fat plays a major role in obesity and is linked with the development of several chronic health problems, such as hyperglycemia, dyslipidemia, hypertension, type 2 diabetes mellitus (T2DM) and certain types of cancer [3,5].

The low-grade inflammation induced by adipose tissue expansion is a hallmark of obesity and metabolic diseases [6], leading to the chronic release of cytokines, such as TNF α , IL-6 and IL-1 β , which leads to the activation of serine threonine kinases such as cJun N-terminal (JNK) and I κ B kinase kinase (IKK) [7]. As a result, both JNK and IKK phosphorylate insulin receptor (IR) substrate-1 (IRS-1) on Ser-307, leading to the inactivation of the insulin receptor downstream response [7] and thus causing insulin resistance. Lipid oversupply and hyperglycemia can lead to increased deposition of lipid species such as diacylglycerols and ceramides, which can also activate JNK and IKK in liver and/or skeletal muscle, leading to insulin resistance and sustained hyperglycemia and hyperlipemia [8]. In addition to cytokines, the chronic presence of saturated fatty acids in the diet can directly induce chronic inflammation and its complications [9–11]. This inflammatory effect can be partially mediated through their binding to Toll-like receptors (TLRs), particularly 2 and 4, inducing the activation of pro-inflammatory pathways that can activate JNK and protein kinase C (PKC), both inhibitors of the insulin signaling, thus promoting insulin resistance and cellular dysfunction in several tissues, such as smooth muscle cells, cardiac tissue, skeletal muscle, hepatocytes, endothelial cells and others [12–14].

The economic and social consequences of obesity and inflammation-associated diseases represent a challenge in terms of assuring sufficient resources and effective health services.

Therefore, strategies to prevent and treat obesity are essential, involving a series of lifestyle modifications that include diet and exercise interventions, pharmacological therapy and bariatric surgery [15–17]. Physical exercise, an important component of obesity treatment and prevention, can alter the biochemical and molecular machinery to modify the mobilization of energetic substrates [17,18]. Recent studies suggest that the beneficial effects of exercise can be potentiated if performed while fasting, thus promoting additional effects in comparison to the fed state, such as higher lipid mobilization and oxidation as well as superior metabolic adaptations [19].

Fasting is characterized by the absence of food intake for a period of time that lasts from several hours to a few weeks [20], resulting in a metabolic switch in the use of energy substrates in which non-esterified fatty acids (NEFAs), ketone bodies and glucose derived from liver glycogen and gluconeogenesis are the predominant energy sources for ATP production [21]. Fasting is capable of activating a series of transcription factors responsible for stimulating the transcription of enzyme genes with a crucial role in metabolic pathways, such as lipoprotein lipase (LPL), muscle carnitine palmitoyltransferase I (CPT I) and long-chain acyl-CoA dehydrogenase (LCAD), changing the rate of gluconeogenesis, fatty acid oxidation and ketogenesis [22]. Similarly to fasting, aerobic exercise, particularly that of low-to-moderate intensity, induces an increased lipolytic activity that results in higher plasma NEFA availability, causing an important metabolic shift that leads to the increased utilization/oxidation of fat rather than carbohydrates [23]. In fact, conditions that lead to increased supply of free fatty acids to skeletal or cardiac muscle reduce utilization and oxidation of glucose [24]. In both conditions (fasting and exercise), these changes are attributed to several hormonal modifications, including increased adrenaline and decreased insulin secretion in the blood [25].

In addition to the metabolic changes, both fasting and exercise can induce positive adaptations in terms of inflammatory control. Resolution of inflammation is key to improving insulin sensitivity and metabolism [26]. The heat shock response (HSR) is one of the molecular pathways capable of promoting insulin sensitivity and decreasing inflammation [27]. The nicotinamide adenine dinucleotide (NAD⁺) dependent deacetylase sirtuin 1 (SIRT1) is activated by the increase in the NAD⁺/NADH ratio, induced by metabolic stress/demand (fasting and exercise) [28]. SIRT1 prolongs the heat shock factor 1 (HSF1) binding to the promoter regions of heat shock genes by maintaining HSF1 in a deacetylated and DNA-binding competent state, enhancing the transcription of molecular

chaperones such as heat shock proteins 70 (HSP70) and 25 (HSP25) [29,30]. HSP70 is a classical molecular chaperone that interacts with other proteins (unfolded, in non-native state and/or stress-denatured conformations), avoiding inappropriate interactions, formation of protein aggregates and degradation of damaged proteins as well as helping the correct refolding of nascent proteins [31]. In addition to its several functions (antiapoptosis, protein translocation, metabolism and others) [27], this protein exerts, intracellularly, a potent anti-inflammatory effect [32]. The anti-inflammatory effect of HSP70 is attributed mainly to its interaction with NF- κ B, which is increased together with IKK in different obese experimental models [27]. The role of HSR in insulin sensitivity has been recently demonstrated, since HSP70 was shown to protect against high-fat-diet- and obesity-induced hyperglycemia, hyperinsulinemia, glucose intolerance and insulin resistance [7,33,34].

Recent evidence has shown that, when compared with exercise performed in the fed state, fasting aerobic exercise increases the fat contribution as an energy substrate after exercise for up to 24 h [35–40]. Some authors [41,42] claim that low-to-moderate intensity aerobic exercise performed on an empty stomach offers no advantage with regard to fatty acid oxidation when compared to exercise performed in a fed state, while others [23,43] show that, in skeletal muscle, exercising while fasting stimulates the use of fatty acids as the primary source of energy while suppressing glucose metabolism, as compared to the fed state. However, it is worth mentioning that these studies evaluated only healthy and lean individuals. Since higher fat oxidation capacity during exercise seems to be related to a decrease in the number of metabolic risk factors, aerobic exercise performed in the fasted state could be considered a strategy to increase fat oxidation and to promote adaptations that may be beneficial to health, particularly with regard to obesity [44]. Therefore, it can be hypothesized that the use of simultaneous metabolic challenges (exercise and fasting) would induce several changes in skeletal muscle cellular signaling and in lipid, protein, and carbohydrate metabolism.

Despite the significant number of studies showing results that corroborate such hypotheses, only a few research studies have evaluated the effects of exercise in the fasted state in overweight/obese populations. Therefore, the aim of this study was to evaluate the subacute effects (12 h after its execution) of a single moderate-intensity exercise bout, performed in the fed or fasted state, on biochemical and molecular parameters in HFD obese male Wistar rats.

2. Materials and Methods

2.1. Animals and Ethics

Male Wistar rats *Rattus norvegicus* ($n = 30$), 60 days old, were obtained from Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL) of Universidade Federal do Rio Grande do Sul (UFRGS). The animals were maintained in standard biotarium conditions: 12 h light/dark cycle, controlled temperature (21 ± 2 °C), 70% relative humidity, with food and water ad libitum. This project was approved by the Ethics Committee on the Use of Animals (CEUA) of UFRGS (protocol 34271).

2.2. High-Fat Diet and Experimental Groups

The animals received a modified high-fat diet (HFD: 31.07% lipids, 49.09% carbohydrates and 8.18% proteins) for 12 weeks to induce obesity. Body mass was recorded weekly. To assess glucose homeostasis and to test the efficiency of the HFD to induce insulin resistance, an oral glucose tolerance test (OGTT) and an injected (intraperitoneally) glucose tolerance test (ipGTT) were performed at the end of the treatment, and the results were compared with an additional control group (fed a standard diet). This lean control animals were used only in this test because we were interested in evaluating the effects of fasting and exercise in individuals with diet-induced obesity. In the last week of treatment, all animals performed one week of acclimatization to the treadmill, which consisted of five daily 15 min sessions at very low speed and 0° treadmill grade. A washout period of 5 days, between the last day of acclimatization and the acute exercise session, was included to avoid any adaptations or subacute effects of the last acclimation session. At the end of the experimental treatment, the animals were randomly allocated to one of the four experimental groups: fed rest (FER), fed exercise (FEE), fasted rest (FAR) and fasted exercise (FAE).

2.3. Glucose Tolerance Tests

Glucose was given by gavage (OGTT) (1 g/kg) or injected intraperitoneally (IPGTT) (1 g/kg) to overnight fasted mice, and glycemia was measured using a glucometer (On Call Plus, Acon Laboratories Inc., San Diego, CA, USA) at 0, 30, 60, 90 and 120 min after glucose administration. For both tests, the area under the curve (AUC) was calculated.

2.4. Exercise Protocol and Experimental Design

Prior to the exercise session, animals from the FAR and FAE groups were subjected to a fasting period of 8 h [45]. The acute

exercise consisted of a 30 min session on the treadmill, with speed and inclination corresponding to an intensity of 60% of VO_2 max (10 m/min, 0° treadmill grade), according to Rodrigues et al. [24]. Since the VO_2 was not directly tested, we measured the lactate concentration before and after the exercise. Confirming that our exercise protocol was aerobic (moderate), no changes in lactate were found between rest and immediately post-exercise (Figure 1, Supplemental Material). To test the subacute effects of our intervention, the animals were euthanized 12 h after the exercise session. Control animals were kept in rest and killed at the same time. During this recovery period, the animals were resubmitted to their high-fat diet. The animals were killed by decapitation, and the blood, liver, heart, soleus, and gastrocnemius samples were collected. Blood samples were centrifuged (10 min, 1510 g), and the serum was used to evaluate glucose, total proteins, total cholesterol, and triglyceride (TGL) levels. Glycogen, lactate and triglyceride concentrations were determined for the tissue samples. HSP70 and sirtuin 1 immunocontent were determined for skeletal muscle samples. From the 32 animals, two refused to perform the exercise bout (1 from each exercise group). For this reason, they were excluded from the analysis.

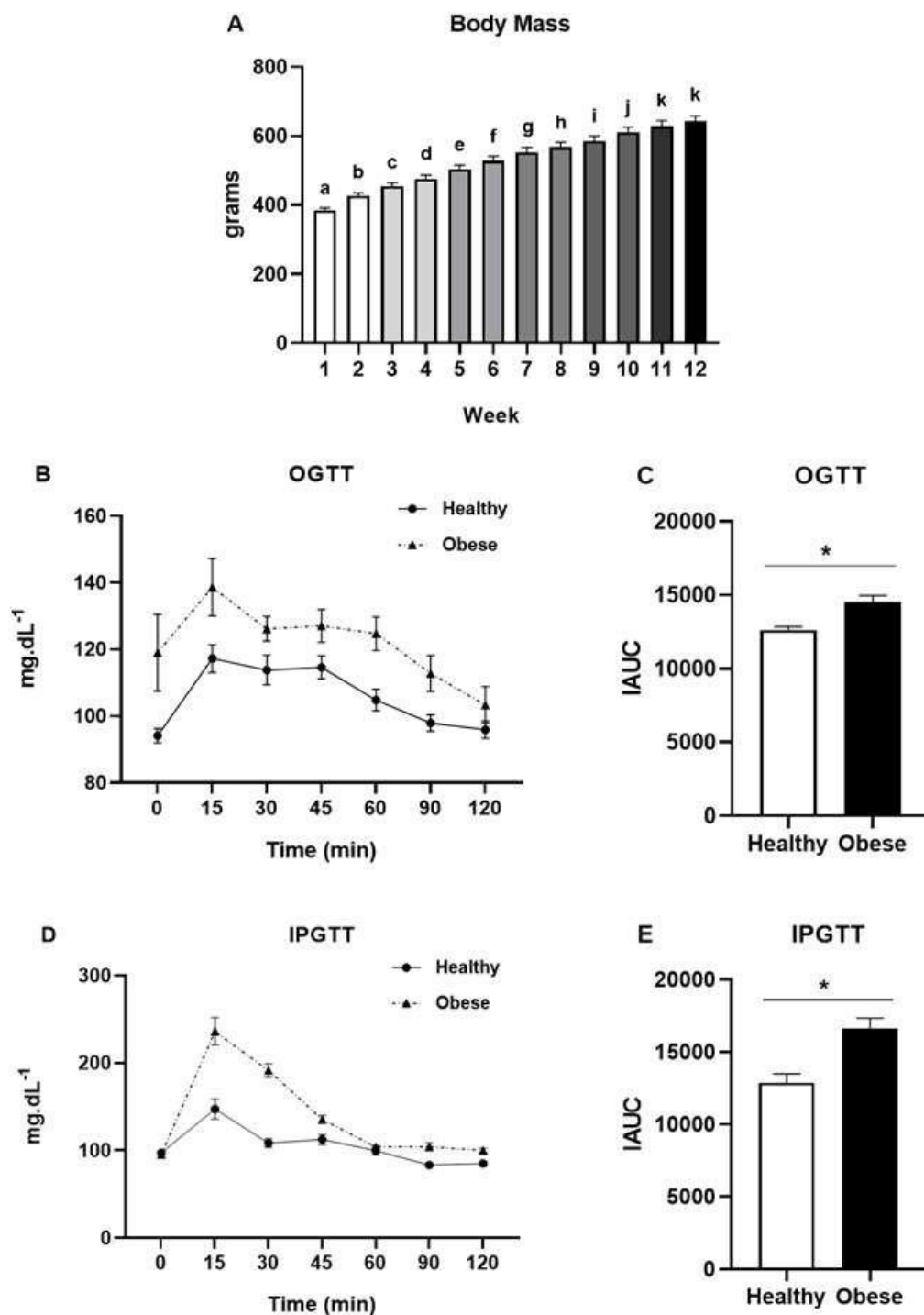


Figure 1. Total body mass (A) of the rats during the treatment with high-fat diet. Oral glucose tolerance test (OGTT) (B) and OGTT incremental area under the curve (IAUC) (C) on the 11th week of treatment. Intraperitoneal glucose tolerance test (IPGTT) (D) and IPGTT incremental area under the curve (IAUC) (E) on the 11th week of treatment. Data were analyzed by two-way ANOVA (Bonferroni post hoc), one-way ANOVA (Bonferroni post hoc) or Student's *t* test. Results were considered different when $p < 0.05$. Letters represent significant differences between weeks. * significant difference between experimental groups.

2.5. Biochemical Analyses

Glucose, lactate, total proteins, total cholesterol, and triglycerides in the serum were measured using enzymatic assay kits (Labtest Diagnóstica SA, Lagoa Santa, Minas Gerais, Brazil) for spectrophotometer analysis. The concentrations of glucose, triglycerides, lactate, and cholesterol were expressed as $\text{mg}\cdot\text{dL}^{-1}$ of plasma and total proteins as $\text{g}\cdot\text{dL}^{-1}$. The concentration of glycogen in the tissue samples was determined as previously described [46]. Briefly, the tissue samples were first homogenized with 30% KOH, then washed with ethanol, hydrolyzed with 4N HCl, neutralized with 2 M Na_2CO_3 and finally diluted with water. The levels of glycogen were determined as glucose equivalents using a glucose kit and expressed as $\text{mg glycogen}\cdot\text{g tissue}^{-1}$ (wet weight). To determine lactate concentration, tissue samples were homogenized with 0.9% saline solution in tubes previously treated with 0.1 M NaF. Lactate was measured using the same assay kit as used for plasma. Results were expressed as $\text{mg lactate}\cdot\text{g muscle}^{-1}$ [47]. To extract triglycerides, tissue samples were homogenized with 0.9% saline in a ratio of 10:1 (1 mg of tissue to 10 μL of saline), and the concentration of triglycerides was determined with the same kit as used for the plasma assay [46]. The results were expressed as $\text{mg}\cdot\text{g}^{-1}$ (wet weight).

2.6. SDS-PAGE and Immunoblotting Analysis

For protein separation, SDS-PAGE (polyacrylamide gel electrophoresis with sodium dodecyl sulfate, mini-PROTEAN® 3 Electrophoresis Cell, BioRad, Hercules, CA, USA) was used with a polyacrylamide concentration of 10%. Approximately 30 μg of the protein extracted from the samples was incubated with Laemmli solution and added to each well of the gel for electrophoresis. After the electrophoresis, the polyacrylamide gel was removed from the glass plates and placed in the electrotransfer module in contact with the nitrocellulose (NC) membrane and covered with transfer buffer. The transfer was made in the Trans-Blot SD system (Semi Dry Electrophoretic Transfer Cell, BioRad, USA) for one hour, at a potential difference of 25 V. The NC membranes containing the proteins were then incubated for one hour in blocking solution. After blocking, the NC membranes were incubated with antibodies (catalog numbers SAB4200714: HSP70; AV32386: SIRT1; Sigma Aldrich, Saint Louis, MO, USA) and diluted in Tween Tris Buffer Saline (TTBS) for at least sixteen hours at 4 °C, under constant agitation. After incubation, the membranes were washed with TTBS (1%) and then incubated with the second antibody (catalog number A9044: Anti-Mouse IgG (whole molecule)—Peroxidase antibody produced in

rabbit— IgG fraction of antiserum, buffered aqueous solution, Sigma Aldrich) for two hours at room temperature. Soon after, the membranes were washed with Tris Buffer Saline (TBS) and incubated in a dark room with chemiluminescence solution for one minute. The chemiluminescence reaction was performed using a detection system based on luminescent substrates (luminol and p-cumaric acid). After incubation with the chemiluminescence solution, the NC membranes were placed in contact with a photographic film (GE Healthcare®/Amersham Hypperfilm™ ECL, Darmstadt, Germany). After developing, the film was analyzed by optical densitometry, and the bands were measured by image processing (ImageMaster VDS, Pharmacia Biotech, San Diego, CA, EUA). The results were expressed in arbitrary units (AUs).

2.7. Statistical Analysis

The oral (OGTT) and injected (ipGTT) glucose tolerance tests of lean and HFD obese animals were analyzed by the area under the curve (AUC). The results obtained in the diet-induced obese animals submitted to fasting and exercise were first analyzed with the Kolmogorov-Smirnov normality test to check the distribution of the data and, subsequently, by the Levene's homogeneity test. Normally distributed data were analyzed using two-way ANOVA to compare the effects of fasting (fed × fasting) and exercise (rest × exercise), as well as the interaction between these factors, with Bonferroni's post hoc for homogeneous data or Games-Howell for non-homogeneous data. Non-parametric data were examined with the Kruskal-Wallis non-parametric test, complemented by Dunn's post-test. Results were considered statistically different when $p < 0.05$. Analyses were performed with the Statistical Package for Social Sciences (SPSS version 25.0, IBM, Armonk, NY, USA).

3. Results

The animals' total body mass increased progressively ($F(2.34,72.62) = 317.212$; $p < 0.01$) along the treatment (Figure 1A). A high-fat diet, when compared to a usual diet, increased insulin resistance, as depicted in Figure 1B–D.

Blood glucose levels were not affected by fasting or by exercise, while total protein levels decreased only in the group submitted to fasting and exercise ($\chi^2(3) = 12.075$; $p < 0.01$) when compared to the other groups (Figure 2A,B, respectively). Gastrocnemius glycogen concentration (Figure 3A) was not affected by fasting or exercise, while soleus glycogen concentration decreased in the fasted groups (2-way ANOVA, $F(1,24) = 18.310$, $p < 0.01$) (Figure 3B). Hepatic glycogen decreased in the fed exercise group when compared to the

rest group ($\chi^2(3) = 9.294$; $p = 0.03$) (Figure 3C). There were no differences in the glycogen content in the heart (Figure 3D). Lactate concentrations were not different between the groups in any of the analyzed samples, including serum, liver, gastrocnemius, and soleus muscles (Figure 4A–D).

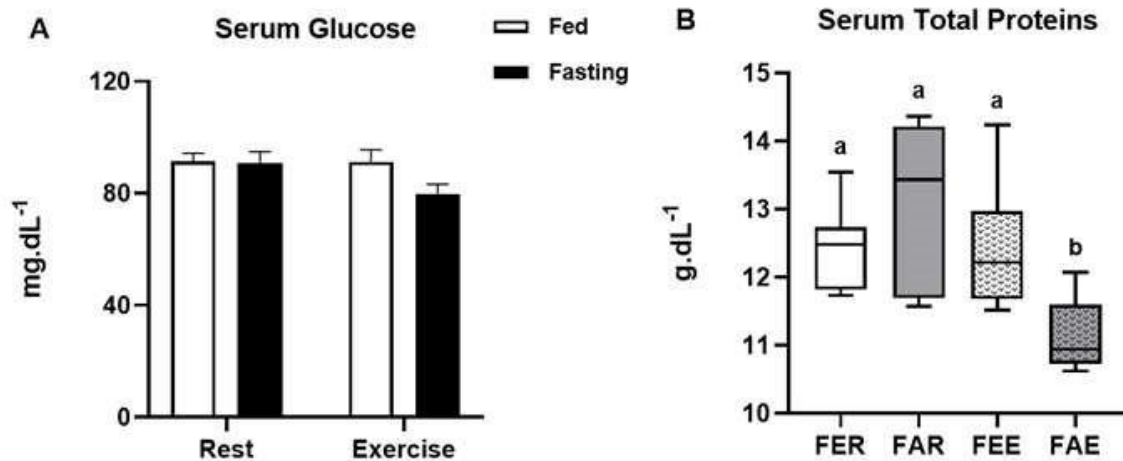


Figure 2. Concentration of glucose (A) and total proteins (B) in the serum at the end of the experimental treatment. Animal groups: fed rest (FER), fed exercise (FEE), fasted rest (FAR) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc) or Kruskal-Wallis test. Results were considered different when $p < 0.05$. Letters represent significant differences between each experimental group.

Although fasting alone was not able to alter the concentrations of serum total cholesterol, there was a significant decrease caused by exercise (2-way ANOVA, $F(1,25) = 9.168$, $p = 0.01$) and by the interaction between exercise and fasting (2-way ANOVA, $F(1,25) = 10.991$, $p < 0.01$) (Figure 5A). Although fasting did not change serum triglycerides, both exercise (2-way ANOVA, $F(1,25) = 10.799$, $p < 0.01$) and the interaction between exercise and fasting (2-way ANOVA, $F(1,25) = 6.894$, $p = 0.02$) were able to significantly decrease these concentrations (Figure 5B). Liver triglyceride concentration did not change between the treatments (Figure 5C). In the heart, there was an interaction between fasting and exercise, and the combination of fasting plus exercise was able to decrease triglycerides to control levels (2-way ANOVA, $F(1,25) = 17.169$, $p < 0.01$) (Figure 5D). In the gastrocnemius muscle, the triglyceride concentration did not change between treatments, while in the soleus muscle, both fasting and fasting plus exercise were able to decrease triglyceride concentrations compared to the control group ($\chi^2(3) = 8.109$; $p = 0.04$) (Figure 5E, F).

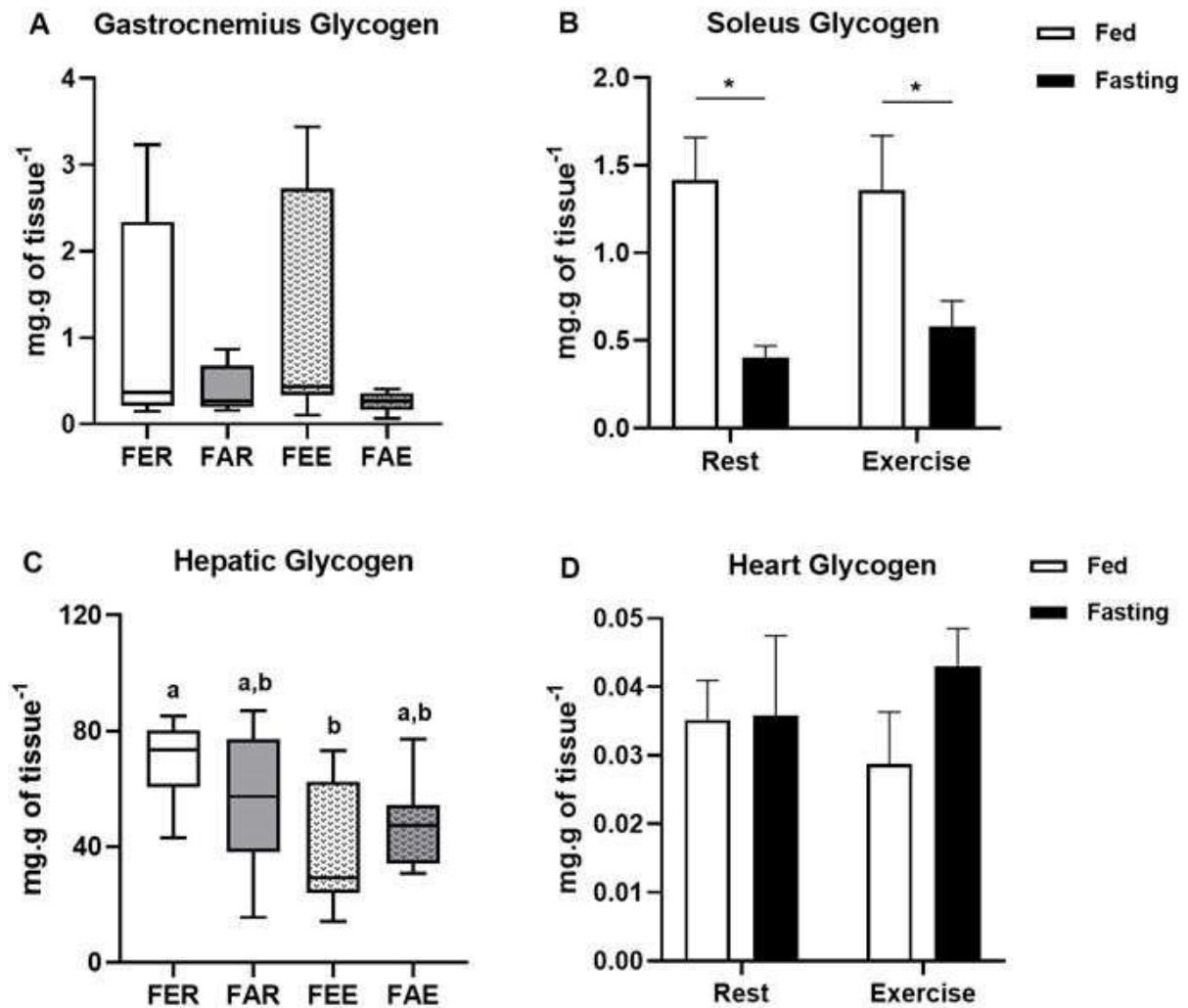


Figure 3. Glycogen concentration in the gastrocnemius (A), soleus (B), liver (C) and heart (D) of the rats at the end of the experimental treatment. Animal groups: fed rest (FER), fed exercise (FEE), fasted rest (FAR) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc) or Kruskal-Wallis test. Results were considered different when $p < 0.05$. * significant difference between fed and fasted groups. Letters represent significant differences between each experimental group.

Heat shock protein 70 (HSP70) immunocontent increased after exercise in the gastrocnemius (2-way ANOVA, $F(1,26) = 13.301$, $p < 0.01$) and soleus muscles (2-way ANOVA, $F(1,24) = 19.861$, $p < 0.01$) (Figure 6A, B). In both muscles, fasting was not able to modify HSP70 content, although exercise was able to increase it. Sirtuin 1 (SIRT1) deacetylase immunocontent behaved similarly to HSP70 in the gastrocnemius (2-way ANOVA, $F(1,26) = 7.331$, $p = 0.01$) and soleus (2-way ANOVA, $F(1,26) = 6.020$, $p = 0.02$) muscles (Figure 6C,D, respectively). Again, fasting did not change the protein content in the muscles, but exercise increased it significantly. Once again, there was no interaction between exercise and fasting.

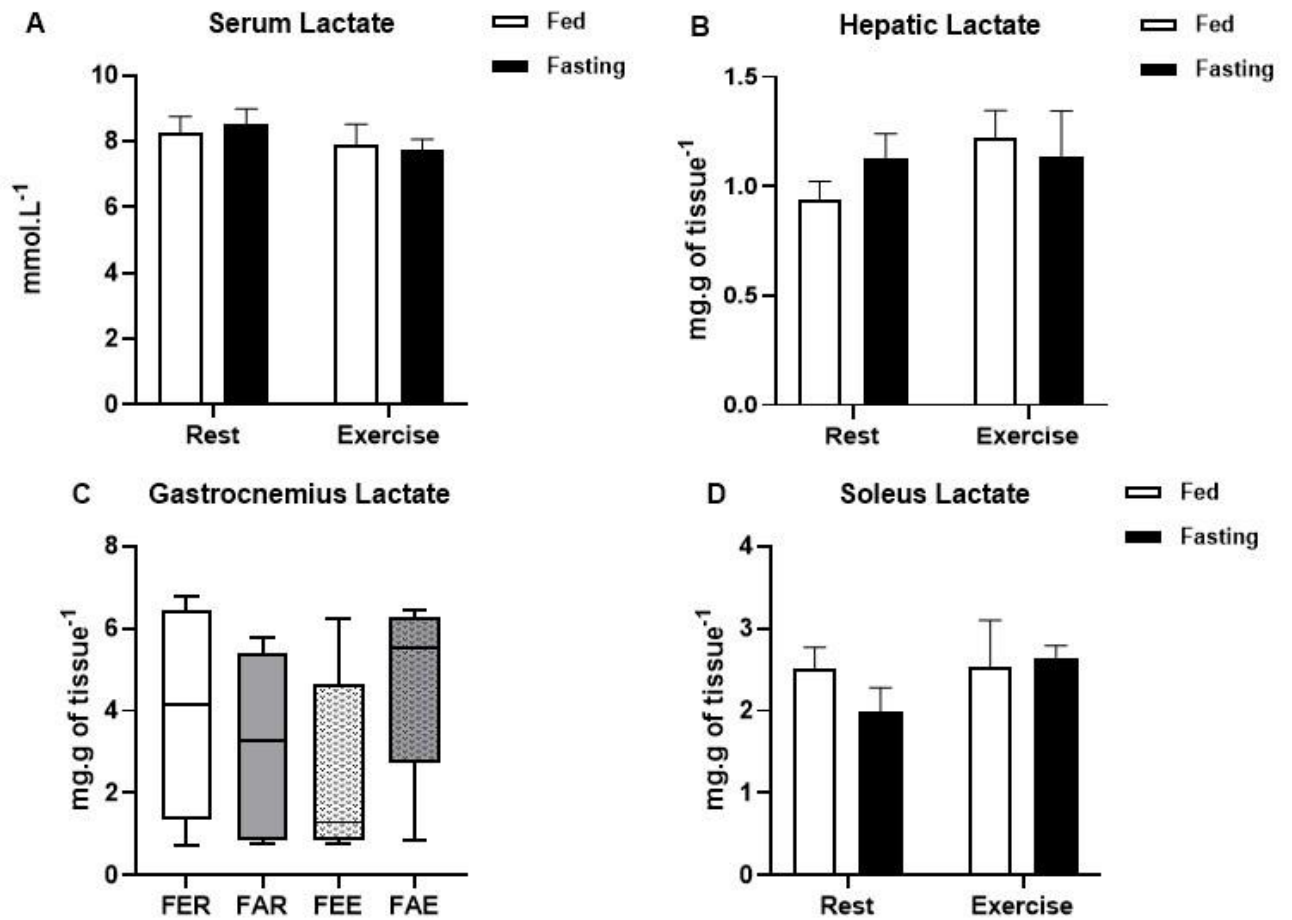


Figure 4. Lactate concentration in the serum (A), liver (B), gastrocnemius (C) and soleus (D) muscles at the end of the experimental treatment. Animal groups: fed rest (FER), fed exercise (FEE), fasted rest (FAR) and fasted exercise (FAE).

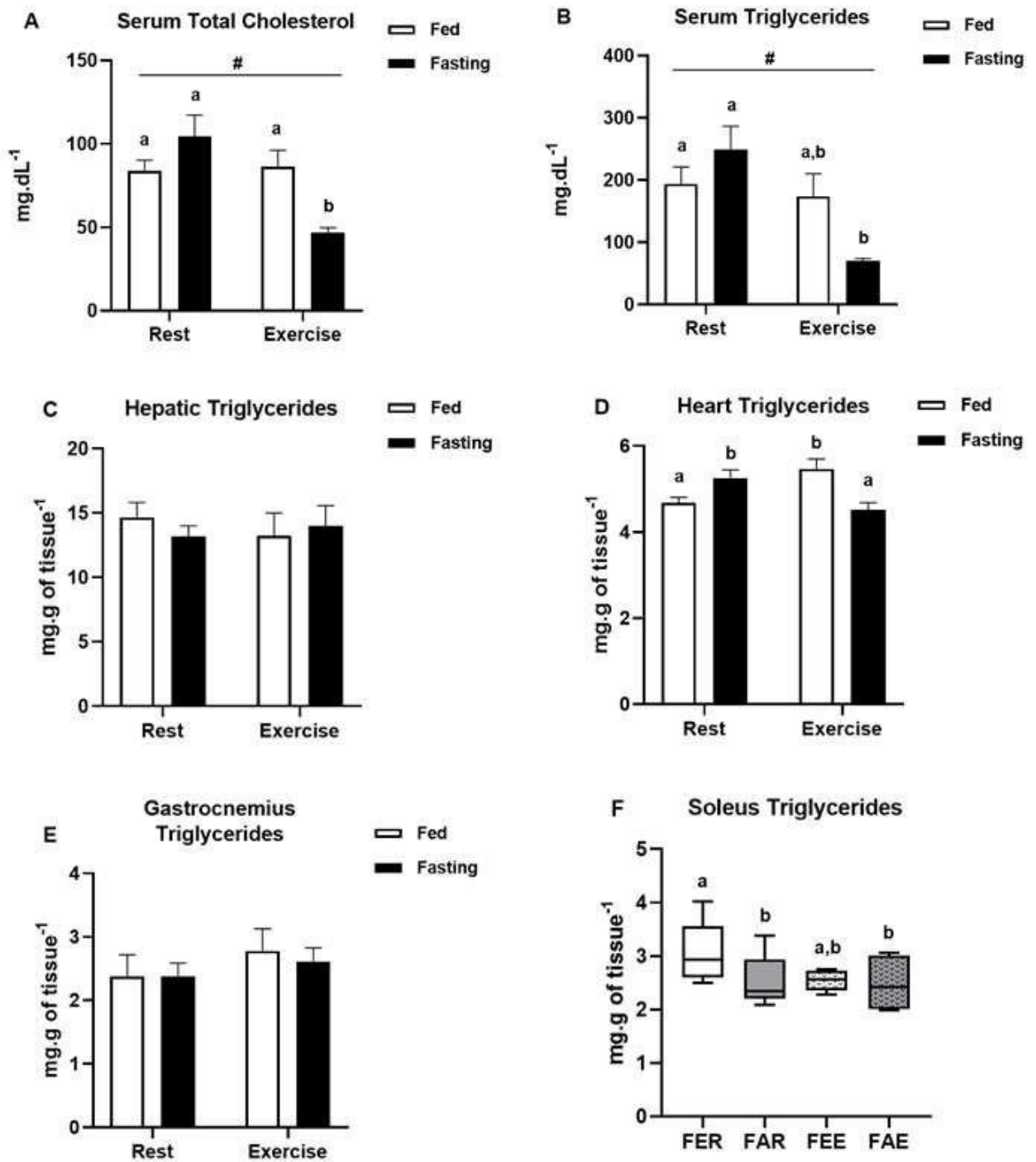


Figure 5. Concentration of lipids in the tissues. Serum total cholesterol (A). Triglycerides in the serum (B), liver (C), heart (D), gastrocnemius (E) and soleus (F) muscles at the end of the experimental treatment. Animal groups: fed rest (FER), fed exercise (FEE), fasted rest (FAR) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc) or Kruskal-Wallis test. Results were considered different when $p < 0.05$. # Represents significant difference between rest and exercised groups. Letters represent significant differences between each experimental group.

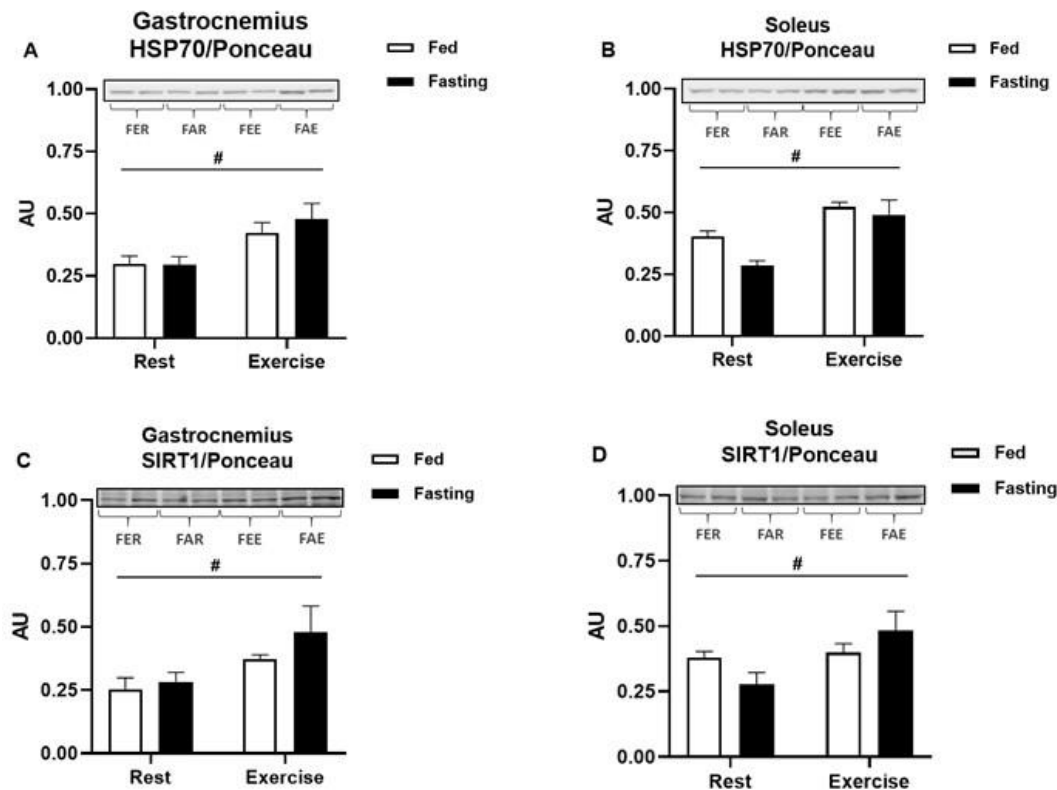


Figure 6. Protein immunocontent at the end of the experimental treatment. Heat shock protein 70 (HSP70) in the gastrocnemius (A) and soleus (B) muscles. Sirtuin 1 (SIRT1) in the gastrocnemius (C) and soleus (D) muscles. Data were analyzed by two-way ANOVA followed by Bonferroni post hoc. Results were considered different when $p < 0.05$. # Represents significant difference between rest and exercised groups.

4. Discussion

Exercise performed in a fasted state induced healthy metabolic effects on carbohydrate and lipid metabolism, such as reduced levels of serum cholesterol and triglycerides in the serum, heart, and soleus muscle, which lasted for up to 12 h after the exercise bout, in an obese-induced insulin-resistant rat model. The benefits of the combination fasting plus exercise were previously assessed in other animal and human studies [19]. However, different from our work, most studies investigated the effects of fasting and exercise in healthy and physically active individuals/animals, which were evaluated immediately after the exercise (i.e., acute effects, for up to 3 h). To our knowledge, this is the first study looking at subacute effects of exercise in the fasted state (12 h after the exercise intervention) in HFD obese and insulin-resistant rats.

Considering that the soleus and gastrocnemius exhibit different metabolic profiles, we evaluated triglycerides and glycogen in both muscles. Like previous studies [48,49], a decreased glycogen content was found in the skeletal muscles from fasted animals (statistically significant only in the soleus), with no differences between non-exercised and exercised animals. The same scenario occurred in the

levels of triglycerides. This is an intriguing finding, since these animals were fed an HFD during the recovery period, prior to euthanasia (12 h). The inability to restore muscle glycogen levels during the fed state may be explained by the increased insulin-resistance state promoted by the HFD, which can be caused by low-grade inflammation, accumulation of intramuscular lipid metabolites, fatty-acid-induced toll-like receptor (TLR) activation and the higher rates of fatty acid uptake [6]. In fact, other models of obese insulin-resistant rats confirm that skeletal muscle glycogen remains reduced during the recovery period [50]. Acute exercise is known to improve glucose uptake and glycogen synthesis, even in insulin-resistant individuals [51]. However, this was not the case for our intervention, since exercised animals had low levels of muscle glycogen, even after a 12 h recovery period with free access to food. The increased lipolytic rate induced by fasting and exercise, along with increased fatty acid uptake and use in the exercise group (where an enhanced rate of beta-oxidation is expected) may have led to reduced glucose uptake (Randle's cycle, [24]), reducing muscle capacity to use and store glucose as glycogen [24]. In addition, during the recovery time, the animals returned to their HFD, increasing the offer of lipids to the muscles, and resulting in diminished glucose metabolism. This increased fat metabolism (increased lipolytic activity, fatty acid uptake and use), may be responsible for the lower serum triglycerides and total cholesterol found in fasted exercised animals (Figure 5). However, we cannot exclude that our intervention changed appetite and food intake in the subsequent hours following exercise, thus inducing modifications in plasma metabolite concentration [52].

On the other hand, hepatic glycogen followed a different pattern, in which glycogen levels of fed and rested animals reduced 12 h after the time in which the other groups exercised (Figure 3C). This indicates that fed animals rely more on glucose metabolism during exercise, and therefore liver glycogenolysis is increased to maintain their glycaemia. In contrast, the animals that exercised during fasting were able to maintain their liver glycogen levels, probably because the increased availability of fatty acids induced a glycogen sparing effect. Higher rates of lipolysis during exercise in the fasted state (thus a greater release of glycerol) may result in increased gluconeogenesis during the recovery period (by using glycerol as a source to synthesize new glucose). In addition to the use of glycerol, amino acids and proteins could also be used for this purpose. This may explain why only this group of animals was able to recover their levels of glycogen in the hours after exercise. Apparently, the unchanged levels of lactate in all tissues indicates

that this metabolite did not play an important role during the experimental intervention and confirms that the level of exercise was not intense.

Curiously, the content of total serum proteins was lower in the fasted-exercised group (Figure 2B). It is expected the fasting induces reductions in plasma protein concentration. However, this response usually occurs after at least 48 h of fasting [53,54]. Since all analyses were conducted 12 h after the intervention, and the animals were resubmitted to their high-fat diet during the recovery period, we suggest that exercise performed in the fasted state may accelerate the effect of fasting on plasma proteins. This effect may need to be considered for longer interventions (exercise training in fasted state), since lower levels of plasma protein can induce changes in oncotic pressure and thus water homeostasis (osmolality/volume/pressure).

In obese male rats that were submitted to exercise (in the fasted or fed state), SIRT1 and HSP70 expression remained increased in both studied skeletal muscles (gastrocnemius and soleus, Figure 6) for up to 12 h after the exercise bout. However, no additional effects of fasting were seen at 12 h after exercise. The increased HSP70 expression induced by exercise is important not only for metabolism but also for its implication in proteostasis and resolution of inflammation, particularly in a model of low-grade inflammation, such as the one in this study.

The metabolic switch that occurs in the fasting state and exercise depends on the activation of several proteins and transcription factors, considered as “master regulators” of metabolism, such as 5' AMP-activated protein kinase (AMPK), SIRT1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) ([24]. The activation of these factors depends on the energetic status of the cells; thus, changing the levels of AMP/ATP and NAD⁺/NADH will lead to metabolic changes favoring catabolism and oxidative metabolism. In fact, endurance exercise in the fasted state results in the activation of AMPK and acetyl-CoA carboxylase- α [55]. Other molecular pathways that are sensitive to the changes in cellular energetic status that occur during metabolic challenges are NAD⁺-dependent deacetylase sirtuin 1 (SIRT1) and heat shock response (HSR) [56]. SIRT1 is a deacetylase that is activated by the increase in the NAD⁺/NADH ratio, induced by metabolic stress/demand. Interestingly, a direct crosstalk between SIRT1, AMPK, PGC-1 α and HSR occurs in different cells [28]. For instance, SIRT1 can activate AMPK by deacetylation, inducing direct metabolic effects [57].

Other studies have shown that exercise is able to increase the expression and synthesis of HSP70 [33,58,59]. Its decreased intracellular concentration is correlated with insulin resistance [7,60], while the induction of its expression, by whatever route, is related to cell protection against inflammatory damage [61,62]. The anti-inflammatory role is due to its ability to inhibit the translocation of factors such as NF- κ B (Nuclear Factor-kappa B) to the cell nucleus [32]; NF- κ B activity is increased in obese individuals [60]. An increase in the intracellular HSP70 immunoccontent is also able to oppose the phosphorylation and activation of pro-inflammatory proteins such as JNK (c-Jun N-terminal Kinase), induced by hyperlipidic diets [7], thus reducing its associated harmful effects.

During fasting, proteins such as SIRT1 are activated in the metabolic management of glycogenolysis for ketogenesis, which leads to the activation of HSF1 (Heat Shock Factor 1), a HSP70 transcription factor [32], the deacetylation and degradation of CRTC2 (CREB Regulated Transcription Coactivator 2), the main mediator of hepatic gluconeogenesis [63,64], and to indirect increase in fatty acid oxidation through the deacetylation of factors such as PGC1 α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1- α) [65,66] and activation of PPAR α [67]. Thus, the effects of both exercise and fasting seem to contribute to a better overall metabolic profile, suggesting that their association can potentiate their effects, which would lead to a faster improvement in insulin resistance and low-grade chronic inflammation.

Most of the significant changes found in our intervention were induced when exercise was performed in the fasted state. The combination of these two metabolic challenges resulted in acute and subacute metabolic changes (particularly in fat metabolism) and in skeletal muscle signaling. Previous studies have shown that, despite the performance of fasting, physical training does not increase the content of FATCD36 (Fatty Acid Translocase) and UCP3 (Uncoupling Protein 3) in muscle tissue; the content of FABPpm (membrane-associated Fatty Acid Binding Protein), GLUT4 (Glucose Transporter 4) and HKII (Hexokinase II) are increased, characterizing a greater uptake of free fatty acids, glucose and phosphorylation of this glucose for further metabolization by muscle tissue, respectively [68]. The increased energy expenditure and the oxidation of lipids imposed by exercise, fasting or not, in obese individuals are able to improve the relationship between mobilization and lipid oxidation. A considerable proportion of the oxidized fatty acids during aerobic exercise of low-to-moderate intensity comes from intramuscular triglycerides, which justifies the use of these exercise protocols in order to improve the insulin sensitivity of its practitioners [36].

Although the present work has not quantified the oxidation of fatty acids per se, it seems that an increased uptake of substrates culminates in the respective increase in their oxidation.

Finally, it must be emphasized that the combination of these two metabolic challenges resulted in an important reduction of blood lipids (triglycerides and total cholesterol). This is of particular interest for the population with obesity and diabetes, since these lipids are known inducers/markers of the development of cardiovascular complications [69].

5. Conclusions

We conclude that exercise in the fasted state induces additional positive metabolic changes in fat metabolism that last for up to 12 h after the exercise bout. The reduction of blood triglycerides and total cholesterol (both markers of cardiovascular risk) observed when the two metabolic challenges are associated indicates that this intervention may be particularly important for obese and diabetic subjects. Although no interaction between exercise and fasting was seen, for 12 h after the intervention, in the immunocontent of HSP70 and SIRT1, metabolic differences were found in glycogen and lipid metabolism. Despite the lack of visible interactions between exercise and fasting on HSR and SIRT1, the potential importance of exercise in managing comorbidities of inflammatory origin is evident. In addition, the immunocontent of these proteins might have displayed a different pattern if the exercise recovery intervals were different. It is important to highlight the main limitations of this study: the absence of a non-obese control group and the fact that exercise intensity was not directly measured. Finally, in terms of human health, it is difficult to extrapolate our findings in rats to the human obese population. However, the induced metabolic changes (especially with regard to lower triglycerides and total cholesterol) and the fact that they can last for up to 12 h after the exercise bout are promising findings that need to be investigated in humans.

Supplementary Materials: The following are available online at www.mdpi.com/16604601/18/14/7543/s1, Figure S1: Blood lactate concentration in fasting (A) and fed (B) conditions of the rats before and after the exercise protocol., Table S1: Effects of fasting exercise on plasma biochemical profile, glycogen, triglycerides storage, and muscle immunocontent of HSP70 and SIRT1.

Author Contributions: Conceptualization, M.K., É.L.V. and A.S.V.; methodology, É.L.V., M.C.V.D., D.S.R., J.F.A.M., L.S.K., S.K.d.S., V.d.O.G., A.S.V. and M.K.; formal analysis, É.L.V., M.C.V.D., D.S.R., J.F.A.M., L.S.K., S.K.d.S., V.d.O.G.; investigation, É.L.V., M.C.V.D., D.S.R., J.F.A.M., L.S.K., S.K.d.S., V.d.O.G., A.S.V., P.I.H.d.B.J. and M.K.; resources, P.I.H.d.B.J., A.S.V. and M.K.; data curation, M.K., É.L.V., and A.S.V.; writing—original draft preparation, M.K., É.L.V. and A.S.V.; writing—review and editing, M.K., É.L.V., R.F. and A.S.V.; supervision, A.S.V. and M.K.; project administration, A.S.V. and M.K.; funding acquisition, A.S.V. and M.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee on the Use of Animals (CEUA) of UFRGS (protocol 34271).

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References

1. Bluher, M. Obesity: Global epidemiology and pathogenesis. *Nat. Rev. Endocrinol.* **2019**, *15*, 288–298, doi:10.1038/s41574-019-0176-8.
2. Murtagh, E.; NCD Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet* **2017**, *390*, 2627–2642, doi:10.1016/S0140-6736(17)32129-3.
3. Oussaada, S.M.; van Galen, K.A.; Cooman, M.I.; Kleinendorst, L.; Hazebroek, E.J.; van Haelst, M.M.; Ter Horst, K.W.; Serlie, M.J. The pathogenesis of obesity. *Metabolism* **2019**, *92*, 26–36, doi:10.1016/j.metabol.2018.12.012.
4. James, W.P. WHO recognition of the global obesity epidemic. *Int. J. Obes.* **2008**, *32* (Suppl. S7), S120–S126, doi:10.1038/ijo.2008.247.
5. Cheung, W.W.; Mao, P. Recent advances in obesity: Genetics and beyond. *ISRN Endocrinol.* **2012**, *2012*, 536905, doi:10.5402/2012/536905.
6. Muller, C.H.L.M.; de Matos, J.R.; Grigolo, G.B.; Schroeder, H.T.; Rodrigues-Krause, J.C.; Krause, M. Exercise Training for the Elderly: Inflammaging and the Central Role for HSP70. *J. Sci. Sport Exerc.* **2019**, *1*, 10–25.
7. Chung, J.; Nguyen, A.K.; Henstridge, D.C.; Holmes, A.G.; Chan, M.H.; Mesa, J.L.; Lancaster, G.I.; Southgate, R.J.; Bruce, C.R.; Duffy, S.J.; et al. HSP72 protects against obesity-induced insulin resistance. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1739–1744, doi:10.1073/pnas.0705799105.
8. Watt, M.J.; Hevener, A.; Lancaster, G.I.; Febbraio, M.A. Ciliary neurotrophic factor prevents acute lipid-induced insulin resistance by attenuating ceramide accumulation and phosphorylation of c-Jun N-terminal kinase in peripheral tissues. *Endocrinology* **2006**, *147*, 2077–2085, doi:10.1210/en.2005-1074.
9. Newsholme, P.a.M.K. Diet, Obesity, and Reactive Oxygen Species—Implications for Diabetes and Aging. *Syst. Biol. Free Radic. Antioxid.* **2014**, *1*, 3361–3374.
10. Rahati, S.; Shahraki, M.; Arjomand, G.; Shahraki, T. Food pattern, lifestyle and diabetes mellitus. *Int. J. High Risk Behav. Addict.* **2014**, *3*, e8725, doi:10.5812/ijhrba.8725.
11. Schwab, U.; Lauritzen, L.; Tholstrup, T.; Haldorssoni, T.; Riserus, U.; Uusitupa, M.; Becker, W. Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: A systematic review. *Food Nutr. Res.* **2014**, *58*, doi:10.3402/fnr.v58.25145.
12. Hameed, I.; Masoodi, S.R.; Mir, S.A.; Nabi, M.; Ghazanfar, K.; Ganai, B.A. Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. *World J. Diabetes* **2015**, *6*, 598–612, doi:10.4239/wjd.v6.i4.598.
13. Jin, C.; Flavell, R.A. Innate sensors of pathogen and stress: Linking inflammation to obesity. *J. Allergy Clin. Immunol.* **2013**, *132*, 287–294, doi:10.1016/j.jaci.2013.06.022.

14. Li, H.; Bao, Y.; Zhang, X.; Yu, Y. Free fatty acids induce endothelial dysfunction and activate protein kinase C and nuclear factor- κ B pathway in rat aorta. *Int. J. Cardiol.* **2015**, *152*, 218–224, doi:10.1016/j.ijcard.2010.07.019.
15. Bessesen, D.H.; Van Gaal, L.F. Progress and challenges in anti-obesity pharmacotherapy. *Lancet Diabetes Endocrinol.* **2018**, *6*, 237–248, doi:10.1016/S2213-8587(17)30236-X.
16. Montegut, L.; Lopez-Otin, C.; Magnan, C.; Kroemer, G. Old Paradoxes and New Opportunities for Appetite Control in Obesity. *Trends Endocrinol. Metab.* **2021**, *32*, 264–294, doi:10.1016/j.tem.2021.02.005.
17. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. *Metabolism* **2019**, *92*, 163–169, doi:10.1016/j.metabol.2018.10.009.
18. Goodpaster, B.H.; Sparks, L.M. Metabolic Flexibility in Health and Disease. *Cell Metab.* **2017**, *25*, 1027–1036, doi:10.1016/j.cmet.2017.04.015.
19. Vieira, A.F.; Costa, R.R.; Macedo, R.C.; Coconcelli, L.; Krueel, L.F. Effects of aerobic exercise performed in fasted v. fed state on fat and carbohydrate metabolism in adults: A systematic review and meta-analysis. *Br. J. Nutr.* **2016**, *116*, 1153–1164, doi:10.1017/S0007114516003160.
20. Longo, V.D.; Mattson, M.P. Fasting: Molecular mechanisms and clinical applications. *Cell Metab.* **2014**, *19*, 181–192, doi:10.1016/j.cmet.2013.12.008.
21. Cahill, G.F., Jr. Fuel metabolism in starvation. *Annu. Rev. Nutr.* **2006**, *26*, 1–22, doi:10.1146/annurev.nutr.26.061505.111258.
22. Bertholdt, L.; Gudiksen, A.; Stankiewicz, T.; Villesen, I.; Tybirk, J.; van Hall, G.; Bangsbo, J.; Plomgaard, P.; Pilegaard, H. Impact of training state on fasting-induced regulation of adipose tissue metabolism in humans. *J. Appl. Physiol.* **2018**, *124*, 729–740, doi:10.1152/jappphysiol.00664.2017.
23. Van Proeyen, K.; Szlufcik, K.; Nielens, H.; Ramaekers, M.; Hespel, P. Beneficial metabolic adaptations due to endurance exercise training in the fasted state. *J. Appl. Physiol.* **2010**, *110*, 236–245, doi:10.1152/jappphysiol.00907.2010.
24. Curi, R.; Newsholme, P.; Marzuca-Nassr, G.N.; Takahashi, H.K.; Hirabara, S.M.; Cruzat, V.; Krause, M.; de Bittencourt, P.I., Jr. Regulatory principles in metabolism-then and now. *Biochem. J.* **2016**, *473*, 1845–1857, doi:10.1042/BCJ20160103.
25. Jeukendrup, A.E. Modulation of carbohydrate and fat utilization by diet, exercise and environment. *Biochem. Soc. Trans.* **2003**, *31 Pt 6*, 1270–1273, doi:10.1042/bst0311270.
26. Krause, M.; Gerchman, F.; Friedman, R. Coronavirus infection (SARS-CoV-2) in obesity and diabetes comorbidities: Is heat shock response determinant for the disease complications? *Diabetol. Metab. Syndr.* **2020**, *12*, 63, doi:10.1186/s13098-020-00572-w.
27. Krause, M.; Heck, T.G.; Bittencourt, A.; Scorzazon, S.P.; Newsholme, P.; Curi, R.; Homem de Bittencourt, P.I., Jr. The chaperone balance hypothesis: The importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. *Mediat. Inflamm.* **2015**, *2015*, 249205, doi:10.1155/2015/249205.
28. Newsholme, P.; de Bittencourt, P.I., Jr. The fat cell senescence hypothesis: A mechanism responsible for abrogating the resolution of inflammation in chronic disease. *Curr. Opin. Clin. Nutr. Metab. Care* **2014**, *17*, 295–305.
29. Singh, I.S.; He, J.R.; Calderwood, S.; Hasday, J.D. A high affinity HSF-1 binding site in the 5'-untranslated region of the murine tumor necrosis factor- α gene is a transcriptional repressor. *J. Biol. Chem.* **2002**, *277*, 4981–4988, doi:10.1074/jbc.M108154200.
30. Wu, L.; Hu, C.; Huang, M.; Jiang, M.; Lu, L.; Tang, J. Heat shock transcription factor 1 attenuates TNF α -induced cardiomyocyte death through suppression of NF κ B pathway. *Gene* **2013**, *527*, 89–94, doi:10.1016/j.gene.2013.05.024.
31. Madden, L.A.; Sandstrom, M.E.; Lovell, R.J.; McNaughton, L. Inducible heat shock protein 70 and its role in preconditioning and exercise. *Amino Acids* **2008**, *34*, 511–516, doi:10.1007/s00726-007-0004-7.
32. Krause, M.; Bock, P.M.; Takahashi, H.K.; Homem De Bittencourt, P.I., Jr.; Newsholme, P. The regulatory roles of NADPH oxidase, intra- and extra-cellular HSP70 in pancreatic islet function, dysfunction and diabetes. *Clin. Sci.* **2015**, *128*, 789–803, doi:10.1042/CS20140695.

33. Atalay, M.; Oksala, N.K.; Laaksonen, D.E.; Khanna, S.; Nakao, C.; Lappalainen, J.; Roy, S.; Hanninen, O.; Sen, C.K. Exercise training modulates heat shock protein response in diabetic rats. *J. Appl. Physiol.* **2004**, *97*, 605–611.
34. Kurucz, I.; Morva, A.; Vaag, A.; Eriksson, K.F.; Huang, X.; Groop, L.; Koranyi, L. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. *Diabetes* **2002**, *51*, 1102–1109, doi:10.2337/diabetes.51.4.1102.
35. De Bock, K.; Richter, E.A.; Russell, A.P.; Eijnde, B.O.; Derave, W.; Ramaekers, M.; Koninckx, E.; Leger, B.; Verhaeghe, J.; Hespel, P. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *J. Physiol.* **2005**, *564 Pt 2*, 649–660, doi:10.1113/jphysiol.2005.083170.
36. Horowitz, J.F. Regulation of lipid mobilization and oxidation during exercise in obesity. *Exerc. Sport Sci. Rev.* **2001**, *29*, 42–46, doi:10.1097/00003677-200101000-00009.
37. Iwayama, K.; Kawabuchi, R.; Nabekura, Y.; Kurihara, R.; Park, I.; Kobayashi, M.; Ogata, H.; Kayaba, M.; Omi, N.; Satoh, M.; et al. Exercise before breakfast increases 24-h fat oxidation in female subjects. *PLoS ONE* **2017**, *12*, e0180472, doi:10.1371/journal.pone.0180472.
38. Iwayama, K.; Kawabuchi, R.; Park, I.; Kurihara, R.; Kobayashi, M.; Hibi, M.; Oishi, S.; Yasunaga, K.; Ogata, H.; Nabekura, Y.; et al. Transient energy deficit induced by exercise increases 24-h fat oxidation in young trained men. *J. Appl. Physiol.* **2015**, *118*, 80–85, doi:10.1152/jappphysiol.00697.2014.
39. Iwayama, K.; Kurihara, R.; Nabekura, Y.; Kawabuchi, R.; Park, I.; Kobayashi, M.; Ogata, H.; Kayaba, M.; Satoh, M.; Tokuyama, K. Exercise Increases 24-h Fat Oxidation Only When It Is Performed Before Breakfast. *EBioMedicine* **2016**, *2*, 2003–2009, doi:10.1016/j.ebiom.2015.10.029.
40. Shimada, K.; Yamamoto, Y.; Iwayama, K.; Nakamura, K.; Yamaguchi, S.; Hibi, M.; Nabekura, Y.; Tokuyama, K. Effects of postabsorptive and postprandial exercise on 24 h fat oxidation. *Metabolism* **2013**, *62*, 793–800, doi:10.1016/j.metabol.2012.12.008.
41. Vicente-Salar, N.; Urdampilleta Otegui, A.; Roche Collado, E. Endurance Training in Fasting Conditions: Biological Adaptations and Body Weight Management. *Nutr. Hosp.* **2015**, *32*, 2409–2420, doi:10.3305/nh.2015.32.6.9488.
42. Vilaca-Alves, J.; Muller, F.; Rosa, C.; Payan-Carreira, R.; Lund, R.; Matos, F.; Garrido, N.; Saavedra, F.J.; Machado Reis, V. Cardiorespiratory, enzymatic and hormonal responses during and after walking while fasting. *PLoS ONE* **2018**, *13*, e0193702, doi:10.1371/journal.pone.0193702.
43. Aird, T.P.; Davies, R.W.; Carson, B.P. Effects of fasted vs fed-state exercise on performance and post-exercise metabolism: A systematic review and meta-analysis. *Scand J. Med. Sci. Sports* **2018**, *28*, 1476–1493, doi:10.1111/sms.13054.
44. Rosenkilde, M.; Nordby, P.; Nielsen, L.B.; Stallknecht, B.M.; Helge, J.W. Fat oxidation at rest predicts peak fat oxidation during exercise and metabolic phenotype in overweight men. *Int. J. Obes.* **2010**, *34*, 871–877, doi:10.1038/ijo.2010.11.
45. Andrade, M.C.D. Metabolism during Fasting and Starvation: Understanding the Basics to Glimpse New Boundaries. *J. Nutr. Diet.* **2017**, *1*, e02.
46. Model, J.F.A.; Lima, M.V.; Ohlweiler, R.; Lopes Vogt, E.; Rocha, D.S.; Souza, S.K.; Turck, P.; Araujo, A.; Vinagre, A.S. Liraglutide improves lipid and carbohydrate metabolism of ovariectomized rats. *Mol. Cell Endocrinol.* **2021**, *524*, 111158, doi:10.1016/j.mce.2021.111158.
47. Souza, S.K.; Martins, T.L.; Ferreira, G.D.; Vinagre, A.S.; Silva, R.S.; Frizzo, M.E. Metabolic effects of perinatal asphyxia in the rat cerebral cortex. *Metab. Brain Dis.* **2012**, *28*, 25–32, doi:10.1007/s11011-012-9367-x.
48. Dohm, G.L.; Tapscott, E.B.; Barakat, H.A.; Kasperek, G.J. Influence of fasting on glycogen depletion in rats during exercise. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1983**, *55*, 830–833, doi:10.1152/jappphysiol.1983.55.3.830.
49. Koubi, H.E.; Desplanches, D.; Gabrielle, C.; Cottet-Emard, J.M.; Sempore, B.; Favier, R.J. Exercise endurance and fuel utilization: A reevaluation of the effects of fasting. *J. Appl. Physiol.* **1991**, *70*, 1337–1343, doi:10.1152/jappphysiol.1991.70.3.1337.
50. Bruce, C.R.; Lee, J.S.; Kiens, B.; Hawley, J.A. Postexercise muscle triacylglycerol and glycogen metabolism in obese insulinresistant zucker rats. *Obes. Res.* **2004**, *12*, 1158–1165, doi:10.1038/oby.2004.145.

51. Newsholme, P.; Homem De Bittencourt, P.I.; O'Hagan, C.; De Vito, G.; Murphy, C.; Krause, M.S. Exercise and possible molecular mechanisms of protection from vascular disease and diabetes: The central role of ROS and nitric oxide. *Clin. Sci.* **2009**, *118*, 341–349, doi:10.1042/CS20090433.
52. Deighton, K.; Batterham, R.L.; Stensel, D.J. Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite* **2014**, *81*, 52–59, doi:10.1016/j.appet.2014.06.003.
53. Felding, P.; Fex, G. Factors responsible for the decreased plasma concentration of prealbumin during acute inflammation and fasting in the rat. *Acta Physiol. Scand* **1983**, *117*, 377–383, doi:10.1111/j.1748-1716.1983.tb00009.x.
54. Torbert, H.C. The Effect of Fasting on the Serum Protein Concentration of the Rat: With Special Reference to the Question of the Existence of an Immediately Utilizable Circulating Protein Fraction. *J. Exp. Med.* **1935**, *62*, 1–10, doi:10.1084/jem.62.1.1.
55. Jamart, C.; Naslain, D.; Gilson, H.; Francaux, M. Higher activation of autophagy in skeletal muscle of mice during endurance exercise in the fasted state. *Am. J. Physiol. Endocrinol. Metab.* **2013**, *305*, E964–E974, doi:10.1152/ajpendo.00270.2013.
56. Krause, M.; Ludwig, M.S.; Heck, T.G.; Takahashi, H.K. Heat shock proteins and heat therapy for type 2 diabetes: Pros and cons. *Curr. Opin. Clin. Nutr. Metab. Care* **2015**, *18*, 374–380, doi:10.1097/MCO.0000000000000183.
57. Moreno, M.; Lombardi, A.; Silvestri, E.; Senese, R.; Cioffi, F.; Goglia, F.; Lanni, A.; de Lange, P. PPARs: Nuclear Receptors Controlled by, and Controlling, Nutrient Handling through Nuclear and Cytosolic Signaling. *PPAR Res.* **2010**, *2010*, doi:10.1155/2010/435689.
58. de Matos, M.A.; Ottone Vde, O.; Duarte, T.C.; Sampaio, P.F.; Costa, K.B.; Fonseca, C.A.; Neves, M.P.; Schneider, S.M.; Moseley, P.; Coimbra, C.C.; et al. Exercise reduces cellular stress related to skeletal muscle insulin resistance. *Cell Stress Chaperones* **2013**, *19*, 263–270, doi:10.1007/s12192-013-0453-8.
59. Liu, Y.; Lormes, W.; Baur, C.; Opitz-Gress, A.; Altenburg, D.; Lehmann, M.; Steinacker, J.M. Human skeletal muscle HSP70 response to physical training depends on exercise intensity. *Int. J. Sports Med.* **2000**, *21*, 351–355, doi:10.1055/s-2000-3784.
60. Rodrigues-Krause, J.; Krause, M.; O'Hagan, C.; De Vito, G.; Boreham, C.; Murphy, C.; Newsholme, P.; Colleran, G. Divergence of intracellular and extracellular HSP72 in type 2 diabetes: Does fat matter? *Cell Stress Chaperones* **2012**, *17*, 293–302, doi:10.1007/s12192-011-0319-x.
61. Henstridge, D.C.; Whitham, M.; Febbraio, M.A. Chaperoning to the metabolic party: The emerging therapeutic role of heatshock proteins in obesity and type 2 diabetes. *Mol. Metab.* **2014**, *3*, 781–793, doi:10.1016/j.molmet.2014.08.003.
62. Krause, M.; Crognale, D.; Cogan, K.; Contarelli, S.; Egan, B.; Newsholme, P.; De Vito, G. The effects of a combined bodyweightbased and elastic bands resistance training, with or without protein supplementation, on muscle mass, signaling and heat shock response in healthy older people. *Exp. Gerontol.* **2019**, *115*, 104–113, doi:10.1016/j.exger.2018.12.004.
63. Walker, A.K.; Yang, F.; Jiang, K.; Ji, J.Y.; Watts, J.L.; Purushotham, A.; Boss, O.; Hirsch, M.L.; Ribich, S.; Smith, J.J.; et al. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev.* **2010**, *24*, 1403–1417, doi:10.1101/gad.1901210.
64. Wang, Y.; Vera, L.; Fischer, W.H.; Montminy, M. The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. *Nature* **2009**, *460*, 534–537, doi:10.1038/nature08111.
65. Canto, C.; Gerhart-Hines, Z.; Feige, J.N.; Lagouge, M.; Noriega, L.; Milne, J.C.; Elliott, P.J.; Puigserver, P.; Auwerx, J. AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity. *Nature* **2009**, *458*, 1056–1060, doi:10.1038/nature07813.
66. Canto, C.; Jiang, L.Q.; Deshmukh, A.S.; Matak, C.; Coste, A.; Lagouge, M.; Zierath, J.R.; Auwerx, J. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab.* **2010**, *11*, 213–219, doi:10.1016/j.cmet.2010.02.006.
67. Purushotham, A.; Schug, T.T.; Xu, Q.; Surapureddi, S.; Guo, X.; Li, X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.* **2009**, *9*, 327–338, doi:10.1016/j.cmet.2009.02.006.

68. De Bock, K.; Derave, W.; Eijnde, B.O.; Hesselink, M.K.; Koninckx, E.; Rose, A.J.; Schrauwen, P.; Bonen, A.; Richter, E.A.; Hespel, P. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. *J. Appl. Physiol.* **2008**, *104*, 1045–1055, doi:10.1152/jappphysiol.01195.2007.
69. Nordestgaard, B.G. Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. *Circ. Res.* **2016**, *118*, 547–563, doi:10.1161/CIRCRESAHA.115.306249.

5.2. Segundo artigo

Título: Acute Effects of a Single Moderate-Intensity Exercise Bout Performed in Fast or Fed States on Cell Metabolism and Signaling: Comparison between Lean and Obese Rats

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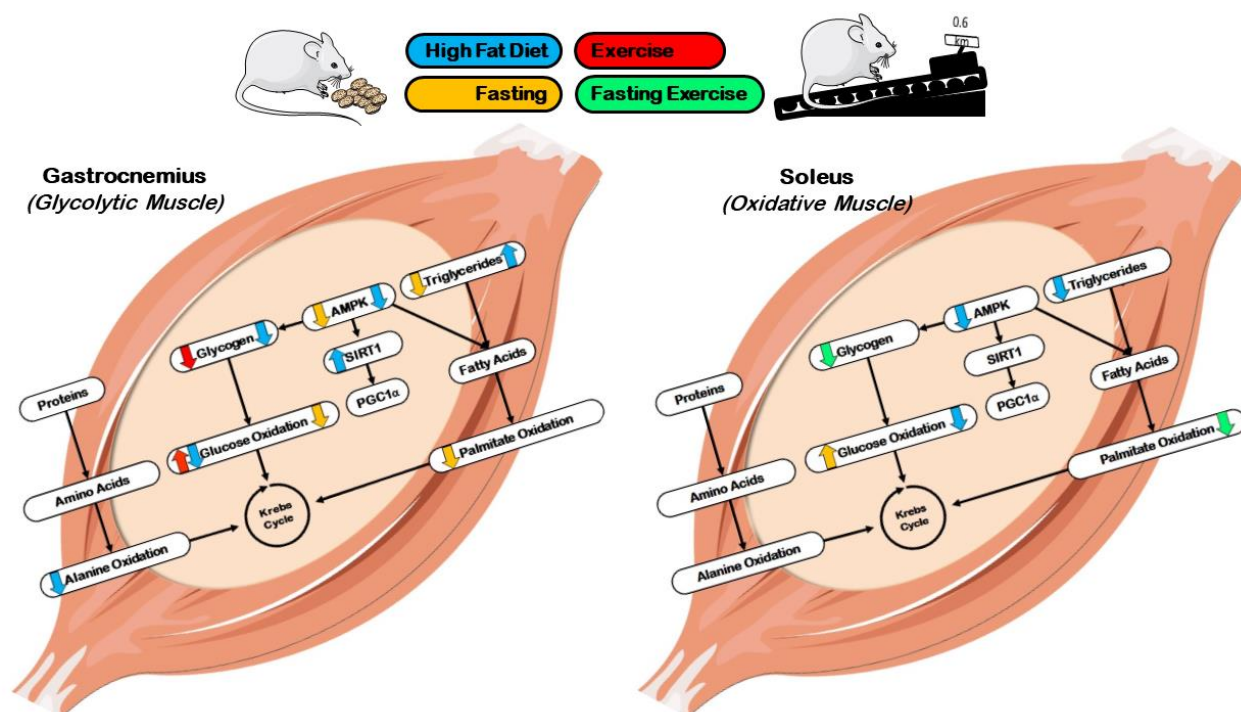
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Abstract:	<p>Aims: Although it is already known that the benefits of exercise can be potentiated by the fasting state in healthy subjects, only few studies evaluated the effects of this intervention on the metabolism of obese subjects. Therefore, this study aimed to investigate the immediate effects of a single moderate-intensity exercise bout performed in fast or fed states on the metabolism of sedentary lean and obese rats. Main methods: Male rats received a high-fat diet (HFD) for twelve weeks to induce obesity. After this period, the animals were allocated in four groups: fed and rest (FER), fed and exercise (30 min treadmill session, FEE), 8h fasted and rest (FAR) and fasted and exercise (FAE). The oxidative capacity of muscles was investigated using ¹⁴Cglucose, ¹⁴C-palmitate, and ¹⁴C-alanine as substrates. The gene expression of AMPK, PGC1α, SIRT1, HSF1 and HSP70 were analyzed in muscle samples. Key findings: The HFD induced obesity, increased serum lactate and liver glycogen and decreased AMPK expression and glucose oxidation in soleus and gastrocnemius. The association of fasting and exercise had different effects in gastrocnemius, a glycolytic muscle, in relation to soleus, an oxidative muscle of obese animals. The combination of fasting and exercise had a glycogen-sparing effect and increased palmitate oxidation in soleus, while in gastrocnemius, it increased glucose oxidation. Significance: The AMPK response is more sensitive to exercise and fasting in lean than obese animals and is different between oxidative and glycolytic muscles. Therefore, exercise while fasting may promote different healthy benefits for sedentary and obese subjects.</p>
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Graphical Abstract



Manuscript File

Title: Acute Effects of a Single Moderate-Intensity Exercise Bout Performed in Fast or Fed States on Cell Metabolism and Signaling: Comparison between Lean and Obese Rats

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Abstract:

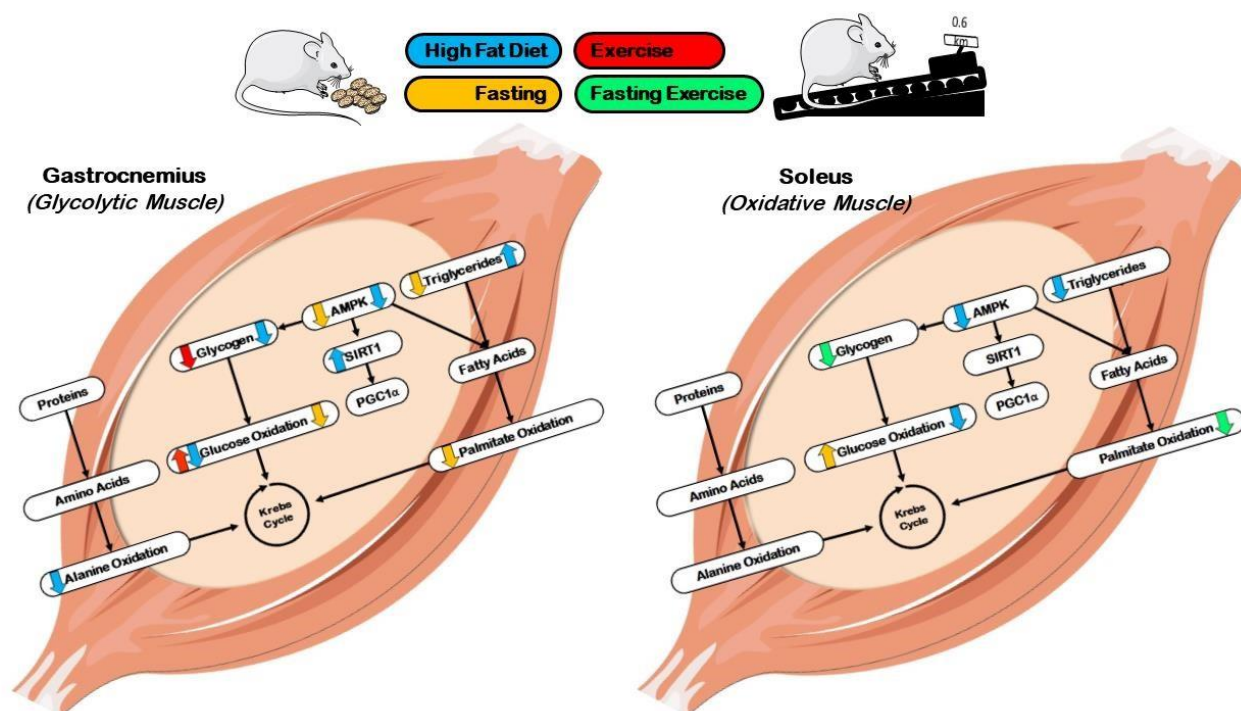
Aims: Although it is already known that the benefits of exercise can be potentiated by the fasting state in healthy subjects, only few studies evaluated the effects of this intervention on the metabolism of obese subjects. Therefore, this study aimed to investigate the immediate effects of a single moderate-intensity exercise bout performed in fast or fed states on the metabolism of sedentary lean and obese rats. **Main methods:** Male rats received a high-fat diet (HFD) for twelve weeks to induce obesity. After this period, the animals were allocated in four groups: fed and rest (FER), fed and exercise (30 min treadmill session, FEE), 8h fasted and rest (FAR) and fasted and exercise (FAE). The oxidative capacity of muscles was investigated using ^{14}C -glucose, ^{14}C -palmitate, and ^{14}C -alanine as substrates. The gene expression of AMPK, PGC1 α , SIRT1, HSF1 and HSP70 were analyzed in muscle samples. **Key findings:** The HFD induced obesity, increased serum lactate and liver glycogen and decreased *AMPK* expression and glucose oxidation in soleus and gastrocnemius. The association of fasting and exercise had different effects in gastrocnemius, a glycolytic muscle, in relation to soleus, an oxidative muscle of obese animals. The combination of fasting and exercise had a glycogen-sparing effect and increased palmitate oxidation in soleus, while in gastrocnemius, it increased glucose oxidation. **Significance:** The AMPK response is more sensitive to exercise and fasting in lean than obese animals and is different between oxidative and glycolytic muscles. Therefore, exercise while fasting may promote different healthy benefits for sedentary and obese subjects.

Keywords: muscle metabolism, obesity, fasting, exercise, glycogen, AMPK

Highlights:

- Exercise and fasting induced different effects in glycolytic and oxidative muscles of obese rats
- In oxidative muscles, fasting and exercise spared glycogen and increased palmitate oxidation
- Fasting and exercise increased glucose oxidation in glycolytic muscles of obese animals
- AMPK response is more sensitive to exercise and fasting in lean than obese rats
- AMPK response is different between oxidative and glycolytic muscles

Graphical abstract:



Introduction

Obesity results from an imbalance between energy acquisition and consumption, is characterized by an expanding adipose tissue, and can be classified by a body mass index (BMI) higher than $\geq 30\text{kg/m}^2$ [1,2]. The adipose tissue is an endocrine-active gland with important role in the regulation of the intermediary metabolism [2]. Therefore, obesity is associated with various types of health issues, such as dyslipidemia, hyperglycemia, type 2 diabetes mellitus (DM2), hypertension, cardiovascular diseases, inflammation, and musculoskeletal disorders leading to a poor life quality [1–5].

A regular routine of exercise is one of the main non-pharmacological approaches to treat obesity, improving insulin and triglycerides profiles. [2,6]. Physical exercise can switch biochemical and molecular pathways increasing energetic substrates availability

to meet the energetic requirements and preventing insulin resistance (IR) [7,8]. One of the gene pathways involved in this metabolic switch during exercise is the 5' AMP-activated Protein Kinase (AMPK), Sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) pathway [9–12]. Exercise also activates the heat shock factor 1 (HSF1) that triggers the heat shock response (HSR), leading to increased expression of the heat shock proteins (HSPs), specially HSP70, which has a key role in the control of carbohydrate metabolism and low grade inflammation resolution [13–16].

The metabolic switches that occur during the rest to exercise transition, like activation of glycogenolysis, lipolysis, ketogenesis and gluconeogenesis, can also be found during the fed to fasting states transition [7,8,17]. Based on these metabolic similarities, and to potentiate this metabolic profile, exercise while fasting has been proposed as a new approach in obesity treatment. Some authors found that exercise during fasting increase fatty acid oxidation (FAO) as the primary source of energy [18–20], while other studies show that low-intensity aerobic exercise during fasting does not increase FAO in relation to exercise in fed state [21,22]. It is important to emphasize that most of these studies were developed in healthy humans or rodents. Only few studies with obese subjects and insulin resistant states were reported and their results indicate that skeletal muscle have decreased oxidative capacity during rest and during exercise after feeding [6,23–26].

In a previous study, we evaluated the subacute effects (12 hours after) of a single moderate-intensity exercise bout on male obese rats that were fed or in an 8-hour fasted-state [23]. The combination of exercise and fasting promoted an important decrease in serum total cholesterol and triglycerides concentrations in obese animals. Considering that there are still some important gaps in the comprehension of the effects of exercise while fasting in obese subjects, this study aimed to investigate the contribution of different types of oxidative substrates (glucose, alanine, and palmitate) during exercise in the fasting state in oxidative and glycolytic muscles of sedentary lean and obese rats. The expression of genes involved in metabolic control and inflammatory balance, were also investigated.

Material and Methods

Animal husbandry

Male Wistar rats *Rattus norvegicus* ($n = 80$), 60 days old, were obtained from Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL) of Universidade Federal do Rio Grande do Sul (UFRGS). This project was approved by the Ethics Committee on the Use of Animals (CEUA) of UFRGS (protocol 34271). The animals were maintained in standard *bioterium* conditions: 12h light/dark cycle, controlled temperature ($21 \pm 2^\circ\text{C}$), 70% relative humidity, with water *ad libitum*.

Experimental Design

The animals were randomly distributed in two main groups and fed for 12 weeks with a standard diet Nuvilab (Quimtia®) (SD: 4.48% lipids; 64.98% carbohydrates and 11.75% proteins) or a modified high-fat diet (HFD: 31.07% lipids, 49.09% carbohydrates and 8.18% proteins) to induce obesity. At the end of this period, lean animals ($n = 40$) weighed 502.7 ± 45.1 grams, while the obese animals ($n = 40$) weighed 643.0 ± 86.0 grams. During the last week of treatment, all animals were acclimatized to the treadmill, which consisted of five daily 15 minutes sessions at very low speed and 0° inclination grade. A washout period of 5 days, between the last day of acclimatization and the acute exercise session, was included to avoid any adaptations or subacute effects of the last acclimation session [23]. At the end of the experimental treatment, healthy and obese animals were randomly allocated in four experimental groups: fed and rested (FER), fed and exercised (FEE), fasted and rested (FAR) and fasted and exercised (FAE), totalizing eight experimental groups with 10 animals in each group. Prior to the exercise session, animals from the FAR and FAE groups were subjected to a fasting period of 8h.

Exercise Protocol

The exercise protocol was already described in Vogt et al. (2021) and consisted of a 30min session on a treadmill, with speed and inclination corresponding to an intensity of 60% of $\text{VO}_{2\text{max}}$ (10m/min, 0° treadmill grade). The rested animals were also maintained in treadmill for 30 min. To test the acute effects of our intervention, the animals were euthanized by decapitation immediately after the exercise session. Samples of blood, liver, heart, soleus, and gastrocnemius were collected. Blood samples were centrifuged (10min, 1510g), and the serum was used to evaluate glucose, total proteins, total

cholesterol, and triglycerides levels. Glycogen and triglycerides concentrations were determined in the tissue samples. *HSP70*, *HSF1*, *PGC1a*, *SIRT1* and *AMPK* gene expression were determined in skeletal muscle samples.

Biochemical Analyses

Glucose, lactate, total proteins, total cholesterol, and triglycerides in the serum were measured using enzymatic assay kits (*Labtest Diagnóstica SA, Lagoa Santa, Minas Gerais, Brazil*) for spectrophotometer analysis. The concentrations of glucose, triglycerides and cholesterol were expressed as $\text{mg}\cdot\text{dL}^{-1}$ of plasma; lactate as $\text{mmol}\cdot\text{L}^{-1}$, and total proteins as $\text{g}\cdot\text{dL}^{-1}$. The concentration of glycogen in the tissue samples was determined as previously described [27]. Briefly, the tissue samples were first homogenized with 30% KOH, then washed with ethanol, hydrolyzed with 4N HCl, neutralized with 2M Na_2CO_3 and finally diluted with water. The levels of glycogen were determined as glucose equivalents using a glucose kit and expressed as $\text{mg glycogen}\cdot\text{g tissue}^{-1}$ (wet weight). To extract triglycerides, tissue samples were homogenized with 0.9% saline in a ratio of 10:1 (1mg of tissue to 10 μL of saline), and the concentration of triglycerides was determined with the same kit as used for the plasma assay [28]. The results were expressed as $\text{mg}\cdot\text{g}^{-1}$ (wet weight).

Substrate oxidation (^{14}C -glucose, ^{14}C -alanine, and ^{14}C -palmitate)

Tissue slices ($300 \pm 10\text{mg}$) were incubated for 60min in sealed flasks in 1mL of Krebs Henseleit buffer pH 7.4 plus 0,1% BSA, 0.1 μCi $^{\text{U-}}^{14}\text{C}$ -glucose (55mCi/mmol GE Healthcare) plus 5mM of unlabeled glucose, equilibrated with $\text{O}_2:\text{CO}_2$ (95:5, v/v) [29]. ^{14}C -alanine and ^{14}C -palmitate were incubated under the same conditions, plus 10mM or 0.05mM of unlabeled substrate, respectively. The flasks contained small glass wells inside that were attached to rubber caps and contained small strips of Whatman 3MM paper. Incubation was stopped by adding 0.25mL of 50% TCA through the rubber cap and 0.25mL of 2M NaOH was placed inside the glass wells to trap the $^{14}\text{CO}_2$ produced. Subsequently, the strips of Whatman 3MM paper were transferred to vials containing scintillation liquid [28]. Values of $^{14}\text{CO}_2$ production were expressed as μmol of ^{14}C -substrate incorporated into $^{14}\text{CO}_2\cdot\text{g of tissue}^{-1}\cdot\text{h}^{-1}$. The radioactivity was estimated by a Beckman liquid scintillation counter.

RNA isolation, cDNA synthesis, and RT-qPCR

Frozen gastrocnemius and soleus muscle samples were homogenized in Tri Reagent® (Sigma) and RNA was extracted using the chloroform-isopropanol method. RNA samples (1µg) were submitted to reverse transcription using the GoScript Reverse Transcription System (Promega) in a total volume of 20µl, according to the manufacturer's protocol. For mRNA analysis, gene-specific primers (Table 1) were mixed with GoTaq® qPCR and RT-qPCR Systems (Promega) to a final concentration of 0.2µM cDNA aliquots and run-in triplicate according to the manufacturer's protocol. Thermal cycling conditions were 2min at 95°C, 10s at 95°C, 1min at 60°C, 10s at 95°C, 1min at 60°C, 15s at 95°C (35 cycles) followed by melting curve analysis on Stratagene Mx3000P (Agilent Technologies). The $\Delta\Delta C_t$ method was used to calculate relative changes in mRNA abundance.

Table 1: Molecular sequences of the primers.

Primer sequences:

<i>HSP70 forward primer 5'-3'</i>	GTGCGGCCTTAGTAGAGGTG
<i>HSP70 reverse primer 5'-3'</i>	GGTCCACCTGCATCTTCTCT
<i>HSF1 forward primer 5'-3'</i>	TAGCCTGCCTAGACAAGAACG
<i>HSF1 reverse primer 5'-3'</i>	TCACCGAGGGGCTGAACA
<i>PGC1α forward primer 5'-3'</i>	TTGACTGGCGTCATTCAGGA
<i>PGC1α reverse primer 5'-3'</i>	CAGGGCAGCACACTCTATGT
<i>SIRT1 forward primer 5'-3'</i>	PrimePCR™ Assays BIORAD
<i>AMPK forward primer 5'-3'</i>	TCGGCAAAGTGAAGATTGGAG
<i>AMPK reverse primer 5'-3'</i>	CCAACAACATCTAAACTGCGAA
<i>β-Actin forward primer 5'-3'</i>	ATTGCTGACAGGATGCAGAA
<i>β-Actin reverse primer 5'-3'</i>	TAGAGCCACCAATCCACACAG

Statistical Analysis

The results were first analyzed with the Kolmogorov-Smirnov normality test to check the distribution of the data and, subsequently, by the Levene's homogeneity test. Normally distributed data were analyzed using two-way ANOVA to compare the effects of fasting (fed x fasting) and exercise (rest x exercise), as well as the interaction between these factors, with Bonferroni's post hoc for homogeneous data or Games-Howell for non-homogeneous data. Nonparametric data were examined with the Kruskal-Wallis non-parametric test, complemented by Dunn's post-test. Results were considered statistically different when $p < 0.05$. Analyses were performed with the Statistical Package for Social Sciences (SPSS version 25.0, IBM, Armonk, NY, USA).

Results

Since the animals did not have a regular routine of exercise, all groups can be considered sedentary. The exercise increased serum glucose levels of sedentary lean [2way ANOVA, $F(1,18)=18.269$, $p<0.001$] and obese animals [2way ANOVA, $F(1,21)=16.412$, $p=0.001$], but fasting [2way ANOVA, $F(1,21)=16.412$, $p=0.001$] and the interaction between fasting and exercise [2way ANOVA, $F(1,18)=6.442$, $p=0.021$] decreased it in lean animals. Lactate levels were 16% higher in obese than in lean animals [3way ANOVA, $F(1,38)=38.204$, $p<0.001$] (Figure 1C, D). Serum lactate levels of lean animals were different between the groups fed and rested (FER) and fed and exercise (FEE) [$\chi^2(3)=10.248$; $p=0.017$], while in obese animals both exercise [2way ANOVA, $F(1,18)=36.787$, $p<0.001$] and fasting [2way ANOVA, $F(1,18)=18.396$, $p<0.001$] increased it. Serum protein levels were 16% higher in obese than lean animals [3way ANOVA, $F(1,42)=72.053$, $p<0.001$] (Figure 1E, FD). In lean animals, serum protein levels did not alter, while in obese groups, the interaction between exercise and fasting was significant [2way ANOVA, $F(1,21)=5.179$, $p=0.033$]. The levels of triglycerides were similar between lean and obese animals and were not affected by fasting or exercise (Figure 1G, H). In lean animals' total cholesterol levels, the interaction between fasting and exercise was significant [2way ANOVA, $F(1,20)=4.680$, $p=0.043$] and cholesterol was higher in lean animals that exercised. In obese animals, total cholesterol levels decreased during fasting [2way ANOVA, $F(1,17)=9.052$, $p=0.008$] in both groups.

Liver glycogen concentration was 70% higher in the obese than lean group [$\chi^2(3)=2.725$; $p=0.006$] (Figure 2 A, B). In lean animals, liver glycogen concentration decreased during fasting in relation to fed values [$\chi^2(3)=17.646$; $p=0.001$] and a similar pattern was developed by obese animals [2way ANOVA, $F(1,18)=19.962$, $p<0.001$]. Glycogen concentration in all types of muscles of lean animals were not affected by exercise, fasting or their interaction (Figure 2C, E, G). When compared to lean animals, gastrocnemius glycogen concentration decreased by 60% in obese animals [3way ANOVA, $F(1,37)=32.626$, $p<0.001$] (Figure 2C, D). In obese animals, the concentration of glycogen in gastrocnemius muscle decreased after the exercise [2way ANOVA, $F(1,18)=7.328$, $p=0.014$]. In soleus muscle of obese animals, the interaction between fasting and exercise was significant [2way ANOVA, $F(1,16)=9.475$, $p=0.007$] (Figure 2F). In the heart of obese animals, glycogen concentration was not modified (Figure 2H).

Triglycerides' concentration in the liver and muscles of lean animals were not affected by any of the interventions (Figure 3A, C, E, G). The same happened in the liver,

soleus, and heart muscles of obese animals (Figure 3B, F, H). In gastrocnemius of obese animals, triglycerides' concentration decreased in fasting groups [2way ANOVA, $F(1,20)=7.105$, $p=0.015$] (Figure 3D). Triglycerides' concentration in the gastrocnemius [$\chi^2(3)=4.039$; $p<0.001$] and soleus [3way ANOVA, $F(1,36)=17.859$, $p<0.001$] of obese animals were 31% higher and 46% lower than lean animals, respectively.

Glucose, palmitate, and alanine oxidation in gastrocnemius muscle were not affected by fasting and exercise in lean animals (Figure 4A, C, E). When compared with lean animals, glucose [3way ANOVA, $F(1,41)=4.502$, $p=0.040$] and alanine [3way ANOVA, $F(1,42)=5.442$, $p=0.025$] oxidation decreased by 26% and 38% respectively in obese animals. In gastrocnemius of obese animals (Figure 4B, D, F), the exercise increased glucose oxidation [2way ANOVA, $F(1,20)=10.646$, $p=0.004$], while fasting decreased glucose [2way ANOVA, $F(1,20)=7.640$, $p=0.012$] and palmitate oxidation [2way ANOVA, $F(1,21)=5.408$, $p=0.030$].

In soleus muscle, glucose oxidation decreased 37% in obese animals in relation to lean animals [$\chi^2(3)=-2.793$; $p=0.005$] (Figure 5A, B). In lean animals, glucose oxidation was not affected by fasting or exercise, while in obese animals, fasting increased it [2way ANOVA, $F(1,19)=4.721$, $p=0.043$]. Palmitate oxidation in soleus muscle (Figures 5C, D), increased after fasting in lean animals [2way ANOVA, $F(1,23)=6.545$, $p=0.018$], while in the obese animals, palmitate oxidation decreased in the group of animals that exercised in the fed state in relation to the group that was only submitted to fasting [$\chi^2(3)=9.333$; $p=0.025$]. In this muscle, alanine oxidation was not altered by any condition (Figures 5E, F).

The interaction between fasting and exercise affected *AMPK* gene expression in the gastrocnemius of lean animals (Figure 6A) [2way ANOVA, $F(1,16)=6.319$, $p=0.023$], while fasting decreased *AMPK* expression in obese animals (Figure 6B) [2way ANOVA, $F(1,16)=5.026$, $p=0.039$]. When compared with lean animals, *AMPK* expression in gastrocnemius decreased 15% in obese animals [3way ANOVA, $F(1,32)=5.504$, $p=0.025$] and the opposite occurred with *SIRT1* expression (Figure 6G, H), which increased 28% [3way ANOVA, $F(1,32)=5.150$, $p=0.030$]. The expression of *HSP70*, *HSF1* and *PGC1 α* genes were not influenced by any of the interventions.

The expression of *AMPK* was also affected by the interaction between fasting and exercise in the soleus muscle of sedentary lean animals [2way ANOVA, $F(1,16)=8.972$, $p=0.009$] (Figure 7A). In obese animals, *AMPK* expression decreased 29% in relation to lean group [3way ANOVA, $F(1,32)=7.544$, $p=0.010$]. The expression of *HSP70*, *HSF1*, *SIRT1* and *PGC1 α* genes were not influenced by any of the interventions.

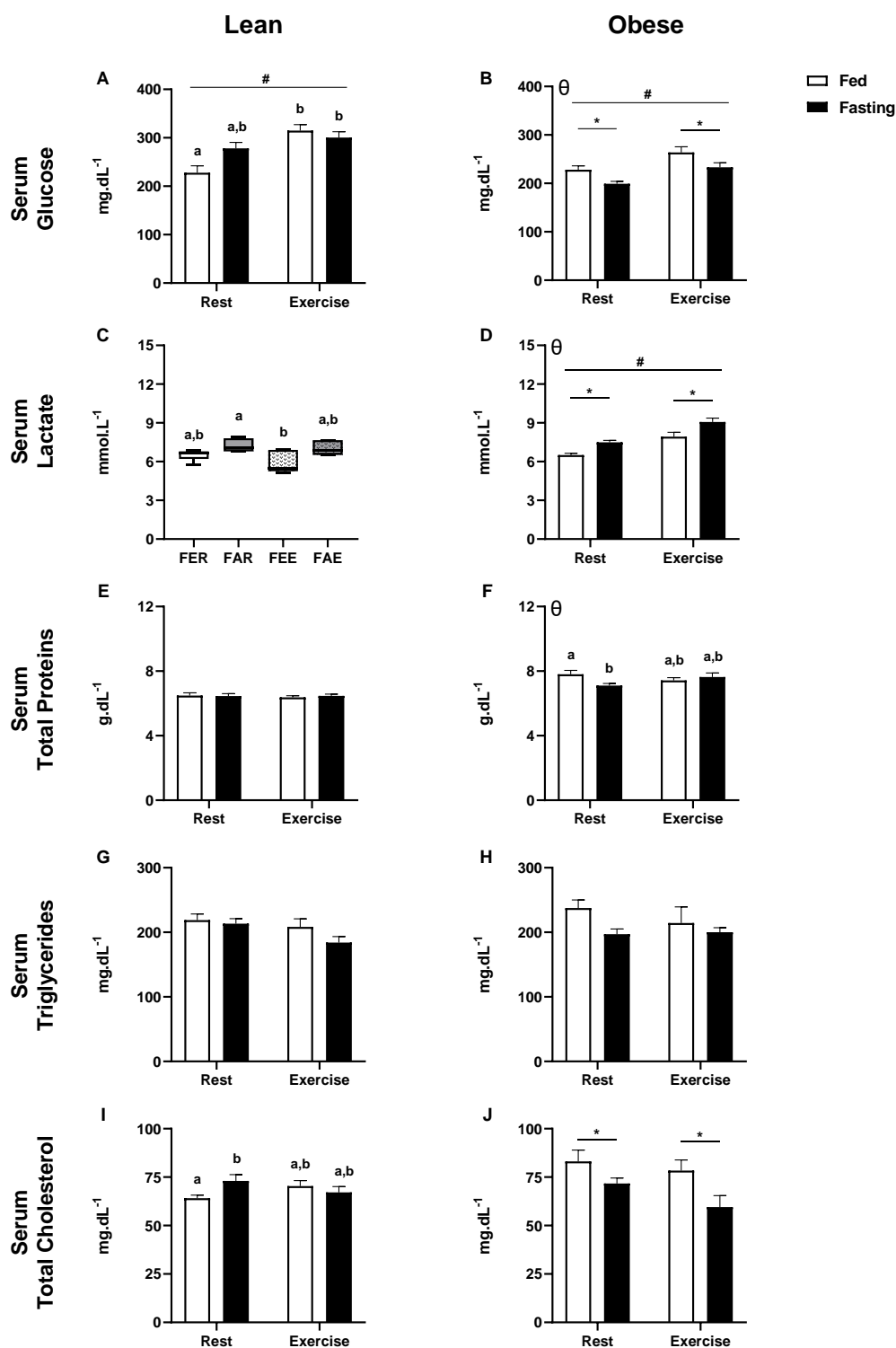


Figure 1. Serum concentration of metabolites in sedentary lean (left) and obese (right) animals at the end of the exercise protocol. (A, B) glucose; (C, D) lactate; (E, F) total proteins; (G, H) triglycerides and (I, J) total cholesterol. Animal groups: fed rest (FER), fasted rest (FAR), fed exercise (FEE) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc), Kruskal - Wallis' test or multifactorial ANOVA. Results were considered different when $p < 0.05$. #: significant differences between rest and exercise. *: significant differences between fed and fasting. θ : significant differences between lean and obese. Letters represent significant differences between each experimental group.

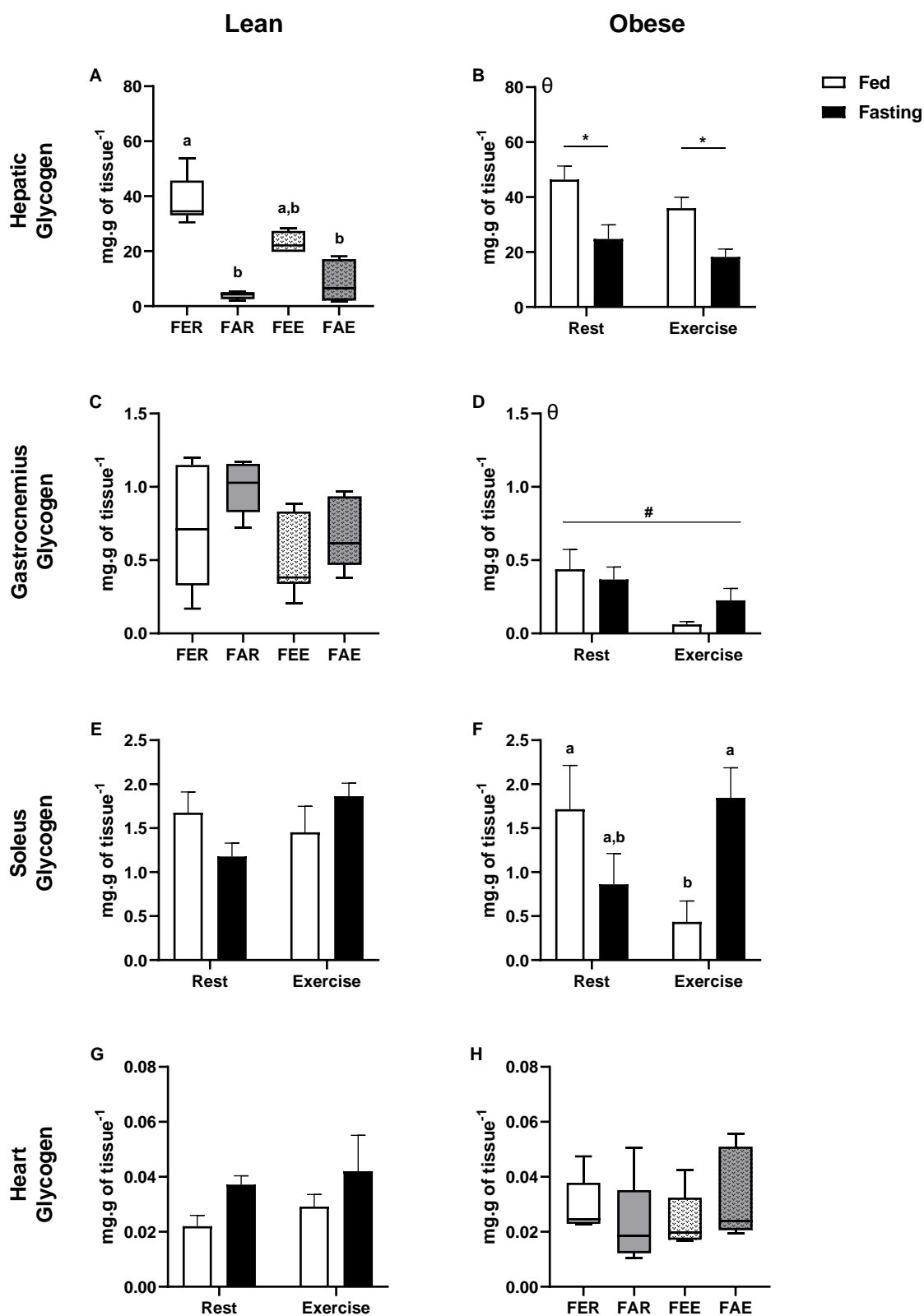


Figure 2. Glycogen concentration in sedentary lean (left) and obese (right) animals at the end of the exercise protocol. (A, B) liver; (C, D) gastrocnemius; (E, F) soleus and (G, H) heart. Animal groups: fed rest (FER), fasted rest (FAR), fed exercise (FEE) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc), Kruskal-Wallis's test or multifactorial ANOVA. Results were considered different when $p < 0.05$. #: significant differences between rest and exercise. *: significant differences between fed and fasting. θ : significant differences between sedentary lean and obese. Letters represent significant differences between each experimental group.

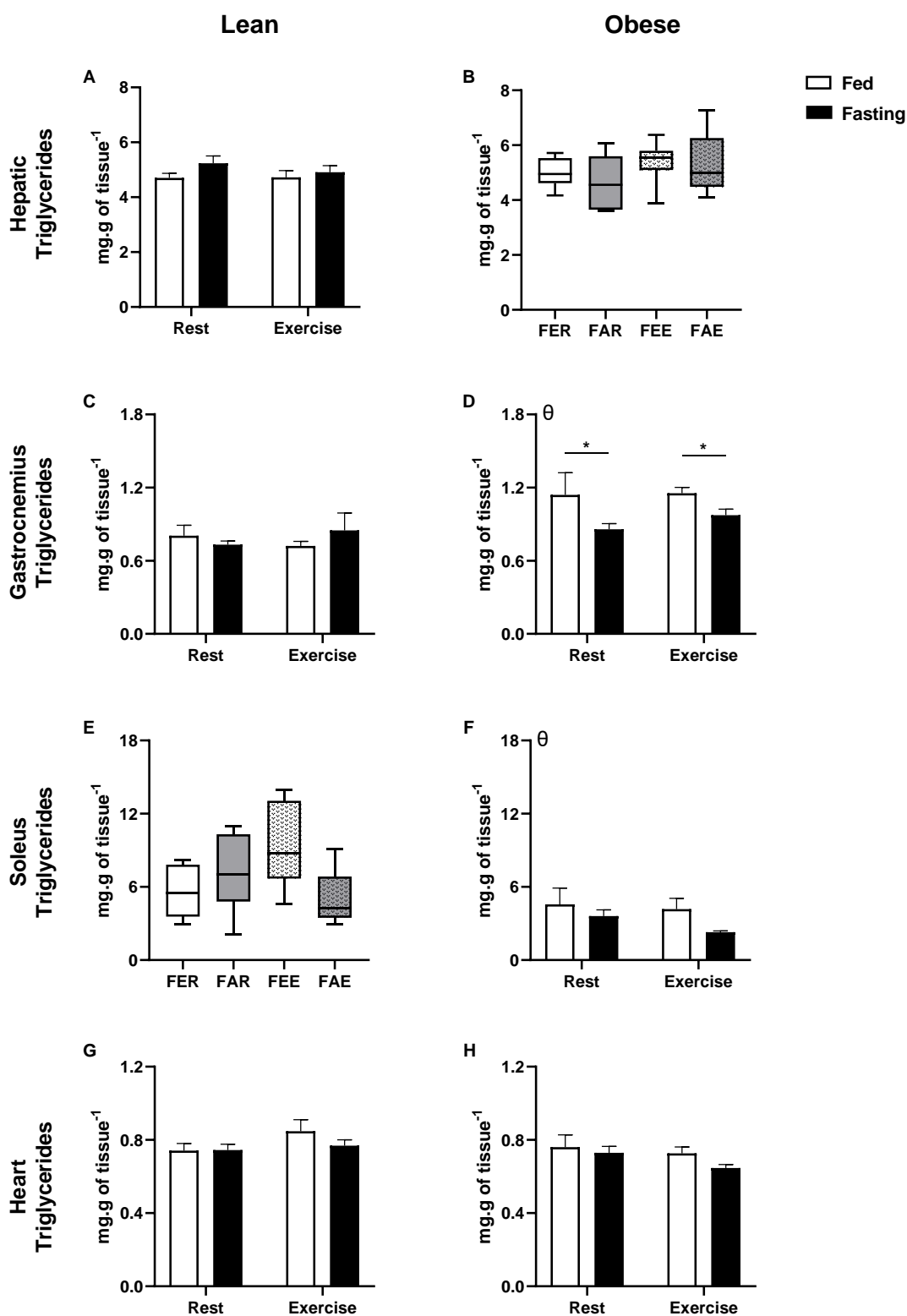


Figure 3. Triglycerides concentration in sedentary lean (left) and obese (right) animals at the end of the exercise protocol. (A, B) liver; (C, D) gastrocnemius; (E, F) soleus and (G, H) heart. Animal groups: fed rest (FER), fasted rest (FAR), fed exercise (FEE) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc), Kruskal-Wallis test or multifactorial ANOVA. Results were considered different when $p < 0.05$. *: significant differences between fed and fasting. θ : significant differences between sedentary lean and obese.

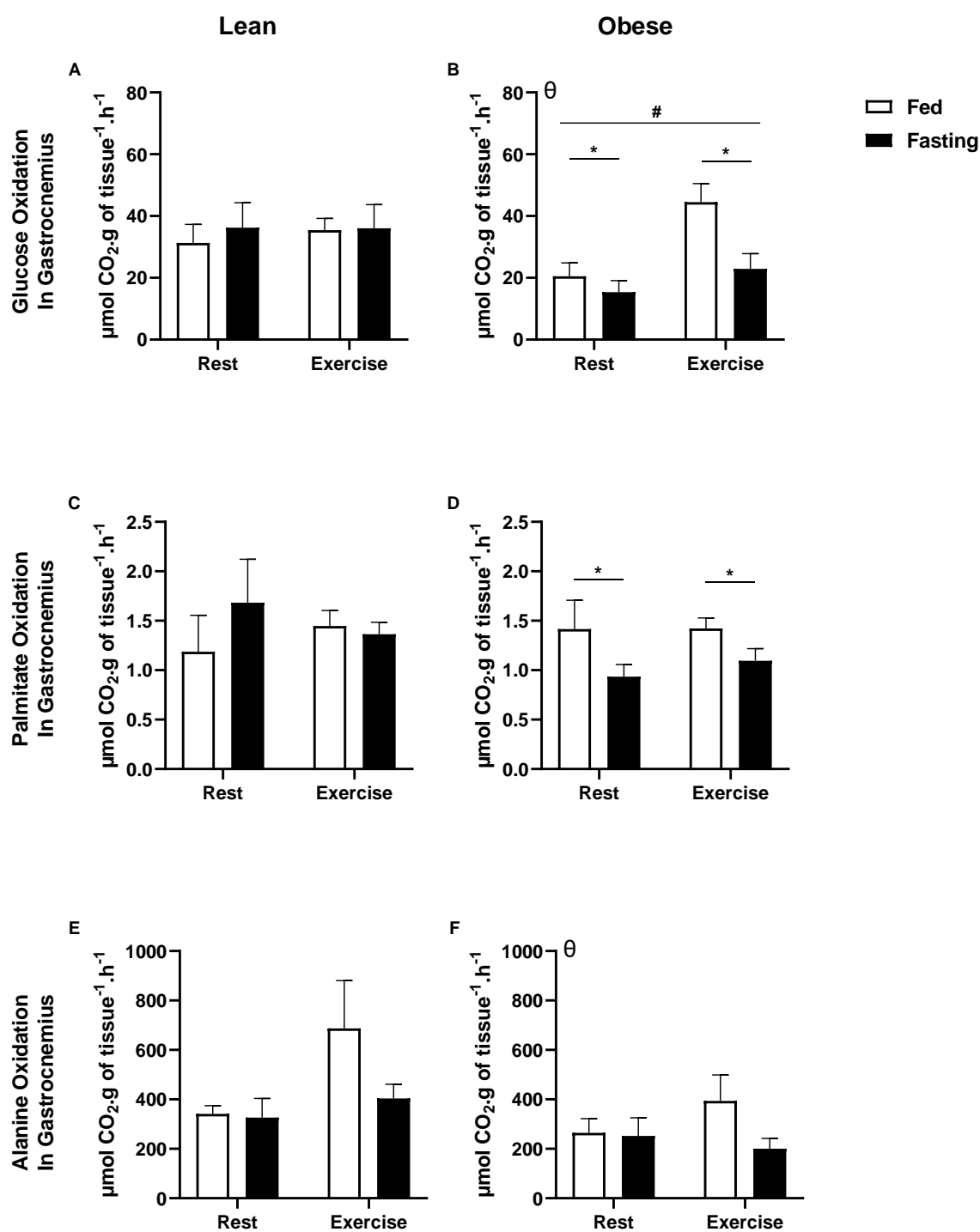


Figure 4. Oxidation of metabolites in gastrocnemius of sedentary lean (left) and obese (right) animals at the end of the exercise protocol. (A, B) glucose; (C, D), palmitate; (E, F) alanine. Data were analyzed by two-way ANOVA (Bonferroni post hoc) or multifactorial ANOVA. Results were considered different when $p < 0.05$. *: significant differences between fed and fasting. #: significant differences between rest and exercise. θ : significant differences between sedentary lean and obese.

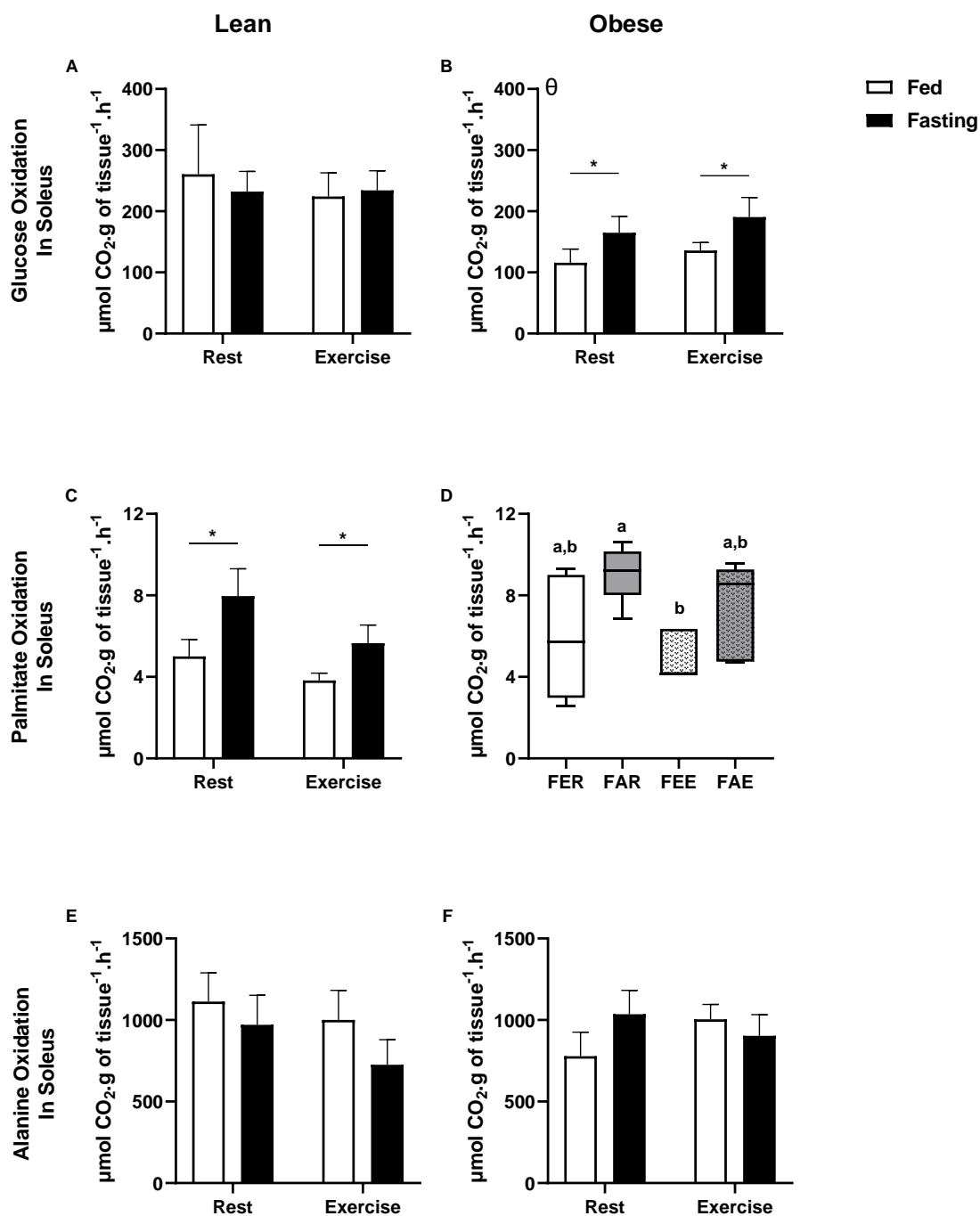


Figure 5. Oxidation of metabolites in soleus of sedentary lean (left) and obese (right) animals at the end of the exercise protocol. (A, B) glucose; (C, D), palmitate; (E, F) alanine. Data were analyzed by two-way ANOVA (Bonferroni post hoc), Kruskal-Wallis test or multifactorial ANOVA. Results were considered different when $p < 0.05$. *: significant differences between fed and fasting. θ : significant differences between sedentary lean and obese. Letters represent significant differences between each experimental group.

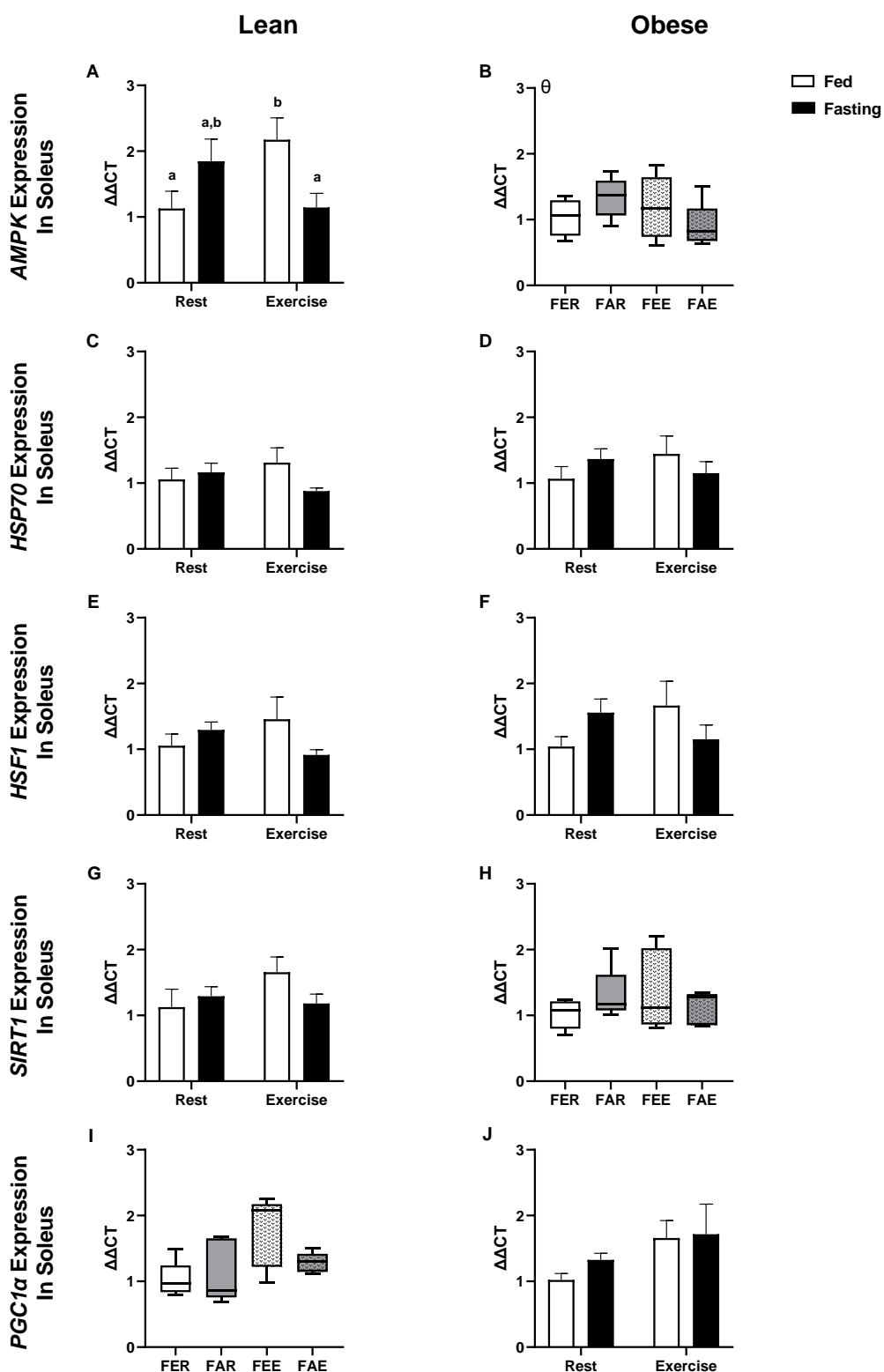


Figure 7. Gene expression in soleus of sedentary lean (left) and obese (right) animals at the end of the exercise protocol. (A, B) *AMPK*; (C, D) *HSP70*; (E, F) *HSF1*; (G, H) *SIRT1* and (I, J) *PGC1 α* . All quantifications were obtained at the end of the exercise protocol. Animal groups: fed rest (FER), fasted rest (FAR), fed exercise (FEE) and fasted exercise (FAE). Data were analyzed by two-way ANOVA (Bonferroni post hoc), Kruskal-Wallis test or multifactorial ANOVA. Results were considered different when $p < 0.05$. θ : significant differences between sedentary lean and obese. Letters represent significant differences between each experimental group.

Discussion

The metabolic effects of aerobic exercise during different periods of fasting has been investigated by many research groups [23,30–36]. Most of these studies focused on lean subjects, and, to our best knowledge, the immediate metabolic effects of fasting aerobic exercise were not yet investigated in obese rats. In the present study, both lean and obese animals did not have a regular routine of exercise and therefore, can be considered sedentary. Both groups presented high basal glucose concentration (Figure 1). These results may be attributed to the IR that can be induced by both the sedentary behavior and the HFD [23]. Despite of this, serum glucose of lean animals increased after exercise in both fed and fasted groups. This elevation of glycemia during the exercise could result from a combination of factors: higher sympathetic tonus causing liver glycogenolysis; IR state caused by sedentary behavior; increased availability of free fatty acids (FA); and the glucose-sparing effect. This higher glucose levels in lean fasted groups were accompanied by decreased liver glycogen. In the obese animals, glucose levels were higher in fasted than in fed animals and in exercised than rested animals. In these groups, liver glycogen concentration was 70% higher than in lean animals, however, as in lean animals, fasting reduced liver glycogen. This reduction was expected, given that liver glycogen is an important source of glucose during periods of food deprivation [37,38]. These findings differ from our previous data, in which obese animals glycemia were equal in all groups 12h after the exercise bout [23], suggesting that, at least in obese animals, these alterations in glucose metabolism have a short duration.

In lean animals, glycogen concentration in the muscles was not affected by fasting, exercise or the association of both interventions. Gastrocnemius and soleus were chosen as examples of glycolytic and oxidative muscles, respectively [39]. In soleus and gastrocnemius of obese animals, glycogen decreased immediately after the exercise in fed state. Also in obese animals, gastrocnemius glycogen concentration decreased when compared to lean animals. In our previous study [23], glycogen decreased in both muscles of fasted animals 12h after the exercise. However, in this study, muscle glycogen concentration of obese animals that exercised while fasting was not different from rested animals, suggesting that the association of exercise and fasting had a glycogen-sparing effect. Similar findings were already described in previous studies using healthy rats [17,40].

Although lactate is traditionally considered a good biomarker of exercise intensity, it is also a predictor of metabolic diseases [41,42]. Higher lactate levels in obese subjects can be associated with loss of oxidative capacity. This reduced mitochondrial activity

increases the glycolytic flux increasing lactate synthesis and therefore, its serum concentration [41]. Lactate can also bind to hydroxycarboxylic acid receptor 1 (HCAR-1) in white adipose tissue and consequently, inhibits lipolysis [43]. This inhibition of lipolysis will culminate in reduction of the expected increased FAO during exercise. In lean animals, lactate levels reduced in the exercised and fed group but was restored to control levels in the group that exercise while fasting. In obese animals, exercise and fasting increased serum lactate in relation to fed and rest. In obese animals exposed to the same protocol, lactate levels of the fasted and exercised groups were similar to rested and fed group 12h after the exercise [23], suggesting that these are short term effects.

Obese animals developed higher concentration of triglycerides than lean animals only in gastrocnemius. This finding can be related to the fact that both groups were sedentary. The balance between lipid synthesis and degradation is not always modified in obese subjects, instead they develop higher FA uptake and increase the content of FA transporters in the cellular membrane, which may lead to increased lipid accumulation [44,45]. In addition, the decreased FAO may also contribute to the higher concentration of lipids [46]. In lean animals, the concentration of triglycerides in all the analyzed tissues were not affected by fasting and exercise, while the cholesterol levels increased in fasted and rested animals. In obese animals, fasting reduced serum total cholesterol and triglycerides concentration in gastrocnemius while exercise had no effect on circulating lipids.

The capacity to oxidate glucose, alanine and palmitate was investigated in gastrocnemius and soleus to identify which would be the preferred substrate in each intervention of this study. Glucose oxidation decreased in both muscles of obese animals in relation to lean ones. In gastrocnemius of obese animals, fasting decreased glucose oxidation in relation to fed state, however the exercise was able to restore it. The effects of fasting on soleus were the opposite than gastrocnemius: glucose oxidation increased during fasting and was not affected by the exercise. These results are consistent with the metabolic characteristics of both muscles [47].

Palmitate oxidation was not altered by the HFD. These findings contradict previous studies that showed decreased palmitate oxidation and activity of oxidative metabolism enzymes such as carnitine palmitoyltransferase I (CPT1) [6,8,25,26,45]. During low-intensity exercise, FAO increase in both types of muscles in healthy and obese subjects [48,49]. In gastrocnemius of obese animals, fasting decreased palmitate oxidation and the exercise did not alter it. As expected, fasting increased palmitate oxidation in soleus of lean animals. In obese animals, palmitate oxidation decreased in fed-exercised animals and were similar to rested animals in the group that exercise during fasting,

suggesting that the combination of exercise and fast can restore palmitate oxidation in this muscle. Therefore, the results indicate that the preferential energy substrate during fasting and exercise is different in each muscle.

When the obese animals fasted, serum protein levels reduced, while the exercise had no effect on protein levels. In our previous data with obese rats [23], total proteins decreased 12h after the exercise during fasting, and it is important to point that in the first study the animals were refed during the recovery after the exercise. However, even after refeeding, protein levels reduced in the group that exercised during fasting. Taken together, these results indicate a short-term effect of the association between fasting and exercise on protein metabolism. To investigate a possible impact of fasting and exercise on muscle protein metabolism, alanine oxidation was also investigated in soleus and gastrocnemius. Alanine oxidation decreased in gastrocnemius of obese animals in relation to lean animals, but in soleus of obese and lean animals, it did not modify. These findings are in agreement with previous descriptions that amino acids are not an important energetic source during short time aerobic exercise [50–52].

When compared to lean animals, *AMPK* expression decreased in both muscles of obese animals and *SIRT1* expression increased only in gastrocnemius of obese. In lean animals, *AMPK* expression increased with the association of fasting and exercise in gastrocnemius while in soleus, it increased only in fed-exercised animals. The lack of alteration in *SIRT1* and *PGC1 α* expression in lean animals can be related to the time of sampling, because the activation of *AMPK* after exercise is an early event (minutes) while *SIRT1* and *PGC1 α* activation usually occur more than one hour after the exercise [39,53]. In obese animals, fasting decreased *AMPK* expression in gastrocnemius and had no effect in soleus, while exercise had no effect on both muscles. In gastrocnemius of obese animals, the decreased *AMPK* expression was combined with decreased glycogen concentration and glucose oxidation and increased triglycerides concentration. These were expected findings, because *AMPK* increase FAO by decreasing malonyl-CoA concentration, inhibiting lipid synthesis. *AMPK* also has important effects on carbohydrate metabolism, promoting glucose uptake through *GLUT1* and *GLUT4* translocation to plasmatic membrane [10,11,54]. Taken together, these findings suggest that *AMPK* is more sensitive in lean than obese animals, and that *AMPK* response is different in oxidative and glycolytic muscles.

SIRT1 activation is one of transcriptional adjustments induced by *AMPK* and can switch muscular metabolism from glycogenolysis to ketogenesis and FAO [55]. These effects are triggered by *PGC1 α* deacetylation and *PPAR α* activation [55,56]. In the present study both *SIRT1* and *PGC1 α* expression in both muscles were not affected by

exercise or fasting. It is possible that a longer period of time after the exercise was necessary to see alterations in the expression of these factors [39,53].

The HSR is another important molecular pathway triggered by exercise and many types of environmental-related stresses [57]. This pathway starts with increased expression of HSF1, which in turn activates the transcription of many genes, in particular the HSPs [58–62]. Besides their actions as molecular chaperones, the HSPs also have anti-inflammatory actions and many metabolic effects that can prevent IR and the development of DM2 [57,63]. Exercise is considered a potent inducer of *HSP70* expression on muscle, and this is considered one of the mechanisms involved in the beneficial effects of exercise [57,62–64]. Depletion of energetic substrates such as glycogen associated with nutritional stresses potentiate this exercise induced *HSP70* expression. Surprisingly, neither HSF1 or *HSP70* was modified by the HFD, fasting or exercise. This could be caused by the time the samples were collected, the intensity and duration of the exercise and the HFD regimen.

Conclusion

In summary, the HFD induced obesity, increased serum lactate and liver glycogen and decreased *AMPK* expression and glucose oxidation in soleus and gastrocnemius. The association of fasting and exercise had different effects in gastrocnemius, a glycolytic muscle, in relation to soleus, an oxidative muscle in obese animals. In soleus, the combination of fasting and exercise had a glycogen-sparing effect associated with increased palmitate oxidation, while in gastrocnemius, it increased glucose oxidation. In addition, *AMPK* was more sensitive to the interventions in lean than obese animals and its response was different between oxidative and glycolytic muscles. In conclusion, these findings contribute to the knowledge about the effects of conducting exercise while fasting, specifically in obese subjects. However, more studies are still necessary before this approach of exercise can be recommended to obese people.

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Conflicts of Interest: The authors declare no conflict of interest.

References

- [1] R. Stienstra, C. Duval, M. Müller, S. Kersten, PPARs, obesity, and inflammation, *PPAR Res.* 2007 (2007). <https://doi.org/10.1155/2007/95974>.
- [2] A. Wirth, M. Wabitsch, H. Hauner, The prevention and treatment of obesity, *Dtsch. Arztebl. Int.* 111 (2014) 705–713. <https://doi.org/10.3238/arztebl.2014.0705>.
- [3] S. Carbone, M.G. Del Buono, C. Ozemek, C.J. Lavie, Obesity, risk of diabetes and role of physical activity, exercise training and cardiorespiratory fitness, *Prog. Cardiovasc. Dis.* 62 (2019) 327–333. <https://doi.org/10.1016/j.pcad.2019.08.004>.
- [4] A. Chobot, K. Górowska-Kowolik, M. Sokołowska, P. Jarosz-Chobot, Obesity and diabetes—Not only a simple link between two epidemics, *Diabetes. Metab. Res. Rev.* 34 (2018) 1–9. <https://doi.org/10.1002/dmrr.3042>.
- [5] W. Herzog, Reflections on obesity, exercise, and musculoskeletal health, *J. Sport Heal. Sci.* 9 (2020) 108–109. <https://doi.org/10.1016/j.jshs.2019.11.004>.
- [6] J.-Y. Kim, R.C. Hickner, R.L. Cortright, G.L. Dohm, J.A. Houmard, Lipid oxidation is reduced in obese human skeletal muscle, *Am. J. Physiol. Metab.* 279 (2000) E1039–E1044. <https://doi.org/10.1152/ajpendo.2000.279.5.E1039>.
- [7] B.H. Goodpaster, L.M. Sparks, Metabolic Flexibility in Health and Disease, *Cell Metab.* 25 (2017) 1027–1036. <https://doi.org/10.1016/j.cmet.2017.04.015>.
- [8] E. Zacharewicz, M.K.C. Hesselink, P. Schrauwen, Exercise counteracts lipotoxicity by improving lipid turnover and lipid droplet quality, *J. Intern. Med.* 284 (2018) 505–518. <https://doi.org/10.1111/joim.12729>.
- [9] A.K. Saha, K.M. Allen, A.K. Saha, AMP-Activated Protein Kinase: Its Regulation by Different Sites, *Curr. Res. Diabetes Obes. J.* 2 (2017) 1–3. <https://doi.org/10.19080/crdoj.2017.02.555597>.
- [10] D. Samovski, J. Sun, T. Pietka, R.W. Gross, R.H. Eckel, X. Su, P.D. Stahl, N.A. Abumrad, Regulation of AMPK activation by CD36 links fatty acid uptake to β -oxidation, *Diabetes.* 64 (2015) 353–359. <https://doi.org/10.2337/db14-0582>.
- [11] M. Foretz, P.C. Even, B. Viollet, AMPK activation reduces hepatic lipid content by increasing fat oxidation in vivo, *Int. J. Mol. Sci.* 19 (2018). <https://doi.org/10.3390/ijms19092826>.
- [12] C. Cantó, J. Auwerx, PGC-1 α , SIRT1 and AMPK, an energy sensing network that controls energy expenditure, *Curr. Opin. Lipidol.* 20 (2009) 98–105. <https://doi.org/10.1097/MOL.0b013e328328d0a4>.
- [13] M. Krause, T.G. Heck, A. Bittencourt, S.P. Scomazzon, P. Newsholme, R. Curi, P.I. Homem de Bittencourt, The Chaperone Balance Hypothesis: The Importance of the Extracellular to Intracellular HSP70 Ratio to Inflammation-Driven Type 2

- Diabetes, the Effect of Exercise, and the Implications for Clinical Management, *Mediators Inflamm.* 2015 (2015) 1–12. <https://doi.org/10.1155/2015/249205>.
- [14] E.G. Noble, K.J. Milne, C.W.J. Melling, Heat shock proteins and exercise: a primer, *Appl Physiol Nutr Metab.* 33 (2008) 1050–1065. <https://doi.org/10.1139/H08-069>.
- [15] M.S. Krause, L.P. Oliveira Jr, E.M.S. Silveira, D.R. Vianna, J.S. Rossato, B.S. Almeida, M.F. Rodrigues, A.J.M. Fernandes, J.A.B. Costa, R. Curi, P.I.H. de Bittencourt Jr, MRP1/GS-X pump ATPase expression: is this the explanation for the cytoprotection of the heart against oxidative stress-induced redox imbalance in comparison to skeletal muscle cells?, *Cell Biochem. Funct.* 25 (2007) 23–32.
- [16] K.D. Sarge, S.P. Murphy, R.I. Morimoto, Activation of Heat Shock Gene Transcription by Heat Shock Factor 1 Involves Oligomerization, Acquisition of DNA-Binding Activity, and Nuclear Localization and Can Occur in the Absence of Stress, *Mol. Cell. Biol.* 13 (1993) 1392–1407.
- [17] G.L. Dohm, E.B. Tapscott, H.A. Barakat, G.J. Kasperek, Influence of fasting on glycogen depletion in rats during exercise, *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 55 (1983) 830–833. <https://doi.org/10.1152/jap.1983.55.3.830>.
- [18] T.P. Aird, R.W. Davies, B.P. Carson, Effects of fasted vs fed-state exercise on performance and post-exercise metabolism: A systematic review and meta-analysis, *Scand. J. Med. Sci. Sport.* 28 (2018) 1476–1493. <https://doi.org/10.1111/sms.13054>.
- [19] K. Van Proeyen, K. Szulc, H. Nielens, M. Ramaekers, P. Hespel, Beneficial metabolic adaptations due to endurance exercise training in the fasted state, *J. Appl. Physiol.* 110 (2011) 236–245. <https://doi.org/10.1152/jap.00907.2010>.
- [20] K. Iwayama, R. Kurihara, Y. Nabekura, R. Kawabuchi, I. Park, M. Kobayashi, H. Ogata, M. Kayaba, M. Satoh, K. Tokuyama, Exercise Increases 24-h Fat Oxidation Only When It Is Performed Before Breakfast, *EBioMedicine.* 2 (2015) 2003–2009. <https://doi.org/10.1016/j.ebiom.2015.10.029>.
- [21] N. Vicente-Salar, A.U. Otegui, E.R. Collado, Endurance training in fasting conditions: biological adaptations and body weight management, *Nutr. Hosp.* 32 (2015) 2409–2420. <https://doi.org/10.3305/nh.2015.32.6.9488>.
- [22] J. Vilaça-Alves, F. Muller, C. Rosa, R. Payan-Carreira, R. Lund, F. Matos, N. Garrido, F.J. Saavedra, V.M. Reis, Cardiorespiratory, enzymatic and hormonal responses during and after walking while fasting, *PLoS One.* 13 (2018) 1–10. <https://doi.org/10.1371/journal.pone.0193702>.
- [23] É.L. Vogt, M.C. Von Dentz, D.S. Rocha, J.F.A. Model, L.S. Kowalewski, S.K. de Souza, V. de O. Girelli, P.I.H. de Bittencourt, R. Friedman, M. Krause, A.S. Vinagre, Metabolic and molecular subacute effects of a single moderate-intensity exercise bout, performed in the fasted state, in obese male rats, *Int. J. Environ. Res. Public Health.* 18 (2021) 1–17. <https://doi.org/10.3390/ijerph18147543>.
- [24] A. Petridou, A. Siopi, V. Mougios, Exercise in the management of obesity, *Metabolism.* 92 (2019) 163–169. <https://doi.org/10.1016/j.metabol.2018.10.009>.

- [25] J.A. Houmard, Intramuscular lipid oxidation and obesity, *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 294 (2008) 1111–1116. <https://doi.org/10.1152/ajpregu.00396.2007>.
- [26] J.F. Horowitz, Regulation of Lipid Mobilization and Oxidation during Exercise in Obesity, *Exerc Sport Sci Rev.* 29 (2001) 42–46. <https://doi.org/10.1097/00003677-200101000-00009>.
- [27] J.F.A. Model, M.V. Lima, R. Ohlweiler, É.L. Vogt, D.S. Rocha, S.K. De Souza, P. Türck, A.S. da R. Araújo, A.S. Vinagre, Molecular and Cellular Endocrinology Liraglutide improves lipid and carbohydrate metabolism of ovariectomized rats, *Mol. Cell. Endocrinol.* 524 (2021). <https://doi.org/10.1016/j.mce.2021.111158>.
- [28] J.F.A. Model, M.V. Lima, R. Ohlweiler, E. Sarapio, É.L. Vogt, D.S. Rocha, S.K. de Souza, A.S. Vinagre, Liraglutide treatment counteracts alterations in adipose tissue metabolism induced by orchietomy in rats, *Life Sci.* 278 (2021). <https://doi.org/10.1016/j.lfs.2021.119586>.
- [29] A.G. Cozer, M. Trapp, T.L. Martins, L.S. De Fraga, C. Vieira Marques, J.F.A. Model, V. Schein, L.C. Kucharski, R.S.M. Da Silva, Effects of Stanniocalcin-1 on glucose flux in rat brown adipose tissue, *Biochimie.* 138 (2017) 50–55. <https://doi.org/10.1016/j.biochi.2017.04.008>.
- [30] O. Bjorkman, L.S. Eriksson, Splanchnic glucose metabolism during leg exercise in 60-hour-fasted human subjects, *Am J Physiol Endocrinol Metab.* 8 (1983) E443–E448.
- [31] M. Gleeson, P.L. Greenhaff, R.J. Maughan, Influence of a 24 h fast on high intensity cycle exercise performance in man, *Eur J Appl Physiol.* 57 (1988) 653–659.
- [32] J.J. Knapik, B.H. Jones, C. Meredith, W.J. Evans, Influence of a 3.5 day fast on physical performance, *Eur J Appl Physiol.* 56 (1987) 428–432.
- [33] H.E. Koubi, D. Desplanches, C. Gabrielle, J.-M.M. Cottet-Emard, B. Sempore, R.J. Favier, Exercise endurance and fuel utilization: a reevaluation of the effects of fasting, *J Appl Physiol.* 70 (1991) 1337–1343. <https://doi.org/10.1152/jappl.1991.70.3.1337>.
- [34] R.J. Maughan, M. Gleeson, Influence of a 36 h fast followed by refeeding with glucose, glycerol or placebo on metabolism and performance during prolonged exercise in man, *Eur J Appl Physiol.* 57 (1988) 570–576.
- [35] S.F. Loy, R.K. Conlee, W.W. Winder, A.G. Nelson, D.A. Arnall, G. Fisher, Effects of 24-hour fast on cycling endurance time at two different intensities, *J Appl Physiol.* 61 (1986) 654–659.
- [36] B.A. Zinker, K. Britz, G.A. Brooks, Effects of a 36-hour fast on human endurance and substrate utilization, *J Appl Physiol.* 69 (1990) 1849–1855.
- [37] E.A. Newsholme, The control of fuel utilization by muscle during exercise and starvation, *Diabetes.* 28 (1979) 1–7. <https://doi.org/10.2337/diab.28.1.s1>.
- [38] M. Alatrach, C. Agyin, J. Adams, R.A. DeFronzo, M.A. Abdul-Ghani, Decreased basal hepatic glucose uptake in impaired fasting glucose, *Diabetologia.* 60 (2017) 1325–1332. <https://doi.org/10.1007/s00125-0174252-0>.

- [39] C. Cantó, Z. Gerhart-hines, J.N. Feige, M. Lagouge, J.C. Milne, P.J. Elliott, P. Puigserver, J. Auwerx, AMPK regulates energy expenditure by modulating NAD + metabolism and SIRT1 activity, *Nature*. 458 (2009) 1056–1060. <https://doi.org/10.1038/nature07813.AMPK>.
- [40] L.F. Aragón-Vargas, Effects of Fasting on Endurance Exercise, *Sport. Med.* 16 (1993) 255–265.
- [41] N.T. Broskey, K. Zou, G.L. Dohm, J.A. Houmard, Plasma Lactate as a Marker for Metabolic Health, *Exerc. Sport Sci. Rev.* 48 (2020) 119–124. <https://doi.org/10.1249/jes.0000000000000220>.
- [42] H. Westerblad, J.D. Bruton, A. Katz, Skeletal muscle: Energy metabolism, fiber types, fatigue and adaptability, *Exp. Cell Res.* 316 (2010) 3093– 3099. <https://doi.org/10.1016/j.yexcr.2010.05.019>.
- [43] G.A. Brooks, Lactate as a fulcrum of metabolism, *Redox Biol.* 35 (2020) 101454. <https://doi.org/10.1016/j.redox.2020.101454>.
- [44] B.C. Bergman, L. Perreault, A. Strauss, S. Bacon, A. Kerege, K. Harrison, J.T. Brozinick, D.M. Hunerdosse, M.C. Playdon, W. Holmes, H.H. Bui, P. Sanders, P. Siddall, T. Wei, M.K. Thomas, M.S. Kuo, R.H. Eckel, Intramuscular triglyceride synthesis: Importance in muscle lipid partitioning in humans, *Am. J. Physiol. - Endocrinol. Metab.* 314 (2018) E152–E164. <https://doi.org/10.1152/ajpendo.00142.2017>.
- [45] D.E. Kelley, B. Goodpaster, R.R. Wing, J.-A.A. Simoneau, Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss, *Am. J. Physiol. - Endocrinol. Metab.* 277 (1999) E1130–E1141. <https://doi.org/10.1152/ajpendo.1999.277.6.e1130>.
- [46] G. Perseghin, P. Scifo, F. De Cobelli, E. Pagliato, A. Battezzati, C. Arcelloni, A. Vanzulli, G. Testolin, G. Pozza, A. Del Maschio, L. Luzi, Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: A 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents, *Diabetes*. 48 (1999) 1600–1606. <https://doi.org/10.2337/diabetes.48.8.1600>.
- [47] Y.J. Kim, H.J. Kim, W.J. Lee, J.K. Seong, A comparison of the metabolic effects of treadmill and wheel running exercise in mouse model, *Lab. Anim. Res.* 36 (2020) 1–8. <https://doi.org/10.1186/s42826-019-0035-8>.
- [48] S. Klein, E.F. Coyle, R.R. Wolfe, Fat metabolism during low-intensity exercise in endurance-trained and untrained men, *Am J Physiol.* 267 (Endoc (1994) E934–E940.
- [49] D.P.C. Van Aggel-Leijssen, W.H.M. Saris, A.J.M. Wagenmakers, J.M. Senden, M.A. Van Baak, Effect of exercise training at different intensities on fat metabolism of obese men, *J Appl Physiol.* 92 (2002) 1300–1309.
- [50] W.M. Sherman, Metabolism of sugars and physical performance, *Am. J. Clin. Nutr.* 62 (1995). <https://doi.org/10.1093/ajcn/62.1.228S>.
- [51] M. Hargreaves, R. Snow, Amino Acids and Endurance Exercise, *Int. J. Sport Nutr. Exerc. Metab.* 11 (2001) 133–145.

- [52] M.J. Gibala, Regulation of Skeletal Muscle Amino Acid Metabolism During Exercise, *Int. J. Sport Nutr. Exerc. Metab.* 11 (2001) 87–108.
- [53] N.B. Ruderman, X.J. Xu, L. Nelson, J.M. Cacicedo, A.K. Saha, F. Lan, Y. Ido, AMPK and SIRT1: a long-standing partnership?, *Am. J. Physiol. - Endocrinol. Metab.* 298 (2010) E751–E760. <https://doi.org/10.1152/ajpendo.00745.2009>.
- [54] S. Galic, K. Loh, L. Murray-Segal, G.R. Steinberg, Z.B. Andrews, B.E. Kemp, AMPK signaling to acetyl-CoA carboxylase is required for fasting and cold-induced appetite but not thermogenesis, *Elife.* 7 (2018) 1–22. <https://doi.org/10.7554/eLife.32656>.
- [55] C. Cantó, L.Q. Jiang, A.S. Deshmukh, C. Matak, A. Coste, M. Lagouge, J.R. Zierath, J. Auwerx, Interdependence of AMPK and SIRT1 for Metabolic Adaptation to Fasting and Exercise in Skeletal Muscle, *Cell Metab.* 11 (2010) 213–219. <https://doi.org/10.1016/j.cmet.2010.02.006>.
- [56] A. Purushotham, T.T. Schug, Q. Xu, S. Surapureddi, X. Guo, X. Li, Hepatocyte-Specific Deletion of SIRT1 Alters Fatty Acid Metabolism and Results in Hepatic Steatosis and Inflammation, *Cell Metab.* 9 (2009) 327–338. <https://doi.org/10.1016/j.cmet.2009.02.006>.
- [57] A.E. Archer, A.T. Von Schulze, P.C. Geiger, Exercise, heat shock proteins and insulin resistance, *Philos. Trans. R. Soc. B Biol. Sci.* 373 (2017). <https://doi.org/10.1098/rstb.2016.0529>.
- [58] I.S. Singh, J.-R. He, S. Calderwood, J.D. Hasday, A High Affinity HSF-1 Binding Site in the 5'-Untranslated Region of the Murine Tumor Necrosis Factor- α Gene Is a Transcriptional Repressor, *J. Biol. Chem.* 277 (2002) 4981–4988. <https://doi.org/10.1074/jbc.M108154200>.
- [59] L. Wu, C. Hu, M. Huang, M. Jiang, L. Lu, J. Tang, Heat shock transcription factor 1 attenuates TNF α -induced cardiomyocyte death through suppression of NF κ B pathway, *Gene.* 527 (2013) 89–94. <https://doi.org/10.1016/j.gene.2013.05.024>.
- [60] P.L. Hooper, G. Balogh, E. Rivas, K. Kavanagh, L. Vigh, The importance of the cellular stress response in the pathogenesis and treatment of type 2 diabetes, *Cell Stress Chaperones.* 19 (2014) 447–464. <https://doi.org/10.1007/s12192-014-0493-8>.
- [61] J. Chung, A.-K.A.K. Nguyen, D.C. Henstridge, A.G. Holmes, M.H.S.S. Chan, J.L. Mesa, G.I. Lancaster, R.J. Southgate, C.R. Bruce, S.J. Duffy, I. Horvath, R. Mestri, M.J. Watt, P.L. Hooper, B.A. Kingwell, L. Vigh, A. Hevener, M.A. Febbraio, HSP72 protects against obesity-induced insulin resistance, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 1739–1744. <https://doi.org/10.1073/pnas.0705799105>.
- [62] K.A. Huey, B.M. Meador, Contribution of IL-6 to the Hsp72, Hsp25, and α β -crystallin responses to inflammation and exercise training in mouse skeletal and cardiac muscle, *J. Appl. Physiol.* 105 (2008) 1830–1836. <https://doi.org/10.1152/jappphysiol.90955.2008>.
- [63] M. Krause, M.S. Ludwig, T.G. Heck, H.K. Takahashi, Heat shock proteins and heat therapy for type 2 diabetes: Pros and cons, *Curr. Opin. Clin. Nutr. Metab. Care.* 18 (2015) 374–380. <https://doi.org/10.1097/MCO.000000000000183>.

- [64] L. Figueiredo, R.B. Nunes, B. Marmett, L.B.P.C. de Sá, A.K. Arbex, Antinflammatory Effects of Physical Exercise on Obesity, *Open J. Endocr. Metab. Dis.* 07 (2017) 44–51. <https://doi.org/10.4236/ojemd.2017.71005>.

Credit Author Statement

Title: Acute Effects of a Single Moderate-Intensity Exercise Bout Performed in Fast or Fed States on Cell Metabolism and Signaling: Comparison between Lean and Obese Rats

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Highlights

Title: Acute Effects of a Single Moderate-Intensity Exercise Bout Performed in Fast or Fed States on Cell Metabolism and Signaling: Comparison between Lean and Obese Rats

Highlights:

- Exercise and fasting induced different effects in glycolytic and oxidative muscles of obese rats
- In oxidative muscles, fasting and exercise spared glycogen and increased palmitate oxidation
- Fasting and exercise increased glucose oxidation in glycolytic muscles of obese animals
- AMPK response is more sensitive to exercise and fasting in lean than obese rats
- AMPK response is different between oxidative and glycolytic muscles

6. DISCUSSÃO GERAL

Apesar dos efeitos metabólicos do exercício aeróbio durante diferentes períodos de jejum vir sendo investigados por muitos grupos de pesquisa, não se tem conhecimento ainda do volume e intensidade necessários para que se consolidem possíveis efeitos benéficos desta prática (BJORKMAN; ERIKSSON, 1983; GLEESON; GREENHAFF; MAUGHAN, 1988; KNAPIK *et al.*, 1987; KOUBI *et al.*, 1991; LOY *et al.*, 1986; MAUGHAN; GLEESON, 1988; VOGT *et al.*, 2021; ZINKER; BRITZ; BROOKS, 1990).

Imediatamente após o exercício, a glicemia dos animais obesos em jejum foi maior que nos alimentados e nos exercitados do que em repouso. Após 12h, a glicemia dos animais obesos foi igual em todos os grupos avaliados, apesar de ter sido observada uma diminuição quando comparada à glicemia dos ratos obesos logo após o exercício [3way ANOVA, $F(1,46)=1035,742$, $p<0,000$]. Isso sugere que, pelo menos em animais obesos, essas alterações no metabolismo da glicose têm curta duração (Figura 11A, B).

Por muito tempo o lactato foi considerado apenas um subproduto da via glicolítica, mas atualmente vem sendo considerado um bom preditor de doenças metabólicas (BROSKEY *et al.*, 2020). Embora seja um bom indicador de intensidade do exercício, o propósito da formação de lactato é a regeneração de NAD^+ consumido na via glicolítica, para que seja mantido o fluxo glicolítico para produção de energia (WESTERBLAD; BRUTON; KATZ, 2010). Por natureza, indivíduos obesos apresentam maiores concentrações de lactato sanguíneo quando comparados a indivíduos saudáveis, devido a uma perda de função mitocondrial e capacidade oxidativa. Dessa maneira, um menor fluxo

mitocondrial leva a um aumento no fluxo glicolítico e culmina com maior produção de lactato (BROSKEY *et al.*, 2020). Esse aumento na produção de lactato leva a um aumento na concentração sanguínea de lactato, o que foi observado nos ratos obesos quando comparados aos magros imediatamente após o exercício. Nos animais obesos, tanto o exercício como o jejum foram capazes de aumentar as concentrações circulantes de lactato imediatamente após o exercício, apesar destas modificações não serem capazes de perdurar pelas 12 horas seguidas ao exercício, onde a lactatemia retornou a valores normais em todos os grupos obesos (Figura 11C, D).

Broskey e colaboradores (2020) ainda afirmam que indivíduos acometidos por síndrome metabólica apresentam concentrações plasmáticas de lactato aproximadas a de ciclistas treinados se exercitando a uma carga de 300W. Esse lactato, por sua vez, pode inibir a lipólise no tecido adiposo branco ligando-se ao receptor *Hydroxycarboxylic Acid Receptor 1* (HCAR-1) (BROOKS, 2020), o que pode acarretar em uma inibição do aumento da oxidação de ácidos graxos em indivíduos obesos observadas no presente estudo.

Embora não tenha sido observada diferença entre as concentrações séricas de triglicerídeos de ratos magros e obesos logo após o protocolo de exercício, 12 horas após, os ratos obesos apresentavam menores concentrações do substrato [3way ANOVA, $F(1,46)=5,321$, $p=0,026$] (Figura 11G, H) em comparação aos triglicerídeos imediatamente após o exercício. Entretanto, não foram observadas diferenças na concentração de colesterol total ao longo do tempo de recuperação (Figura 11I, J).

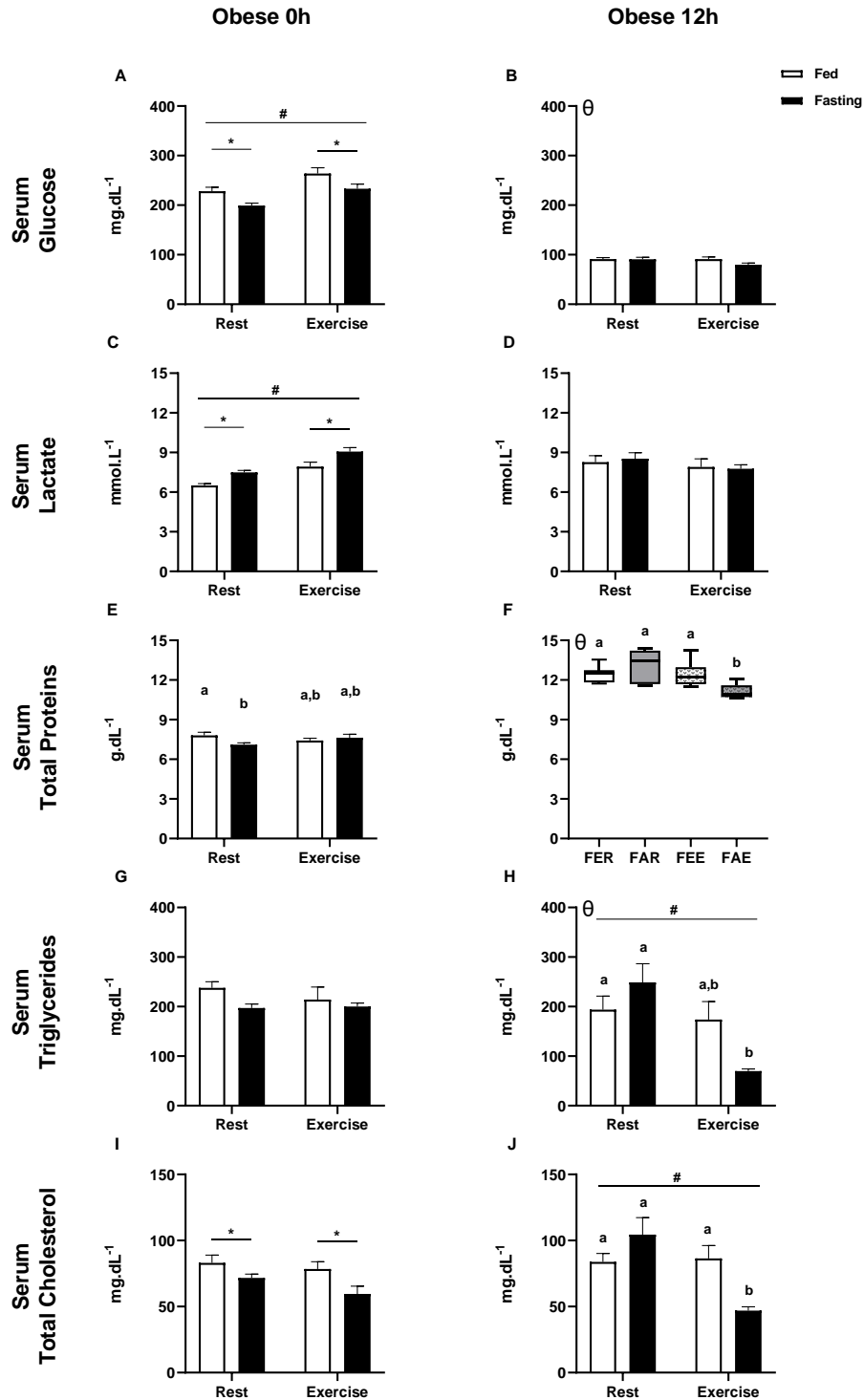


Figura 11. Concentração sérica de substratos dos animais obesos 0h (esquerda) e 12h (direita) após o protocolo de exercício. (A, B) glicose; (C, D) lactato; (E, F) proteínas totais; (G, H) triglicerídeos e (I, J) colesterol total. Grupos: controle alimentado (FER), controle jejum (FAR), exercício alimentado (FEE) e exercício em jejum (FAE). Os dados foram submetidos a 2way ANOVA (*post hoc* de Bonferroni), Kruskal-Wallis ou ANOVA multifatorial. Os resultados foram considerados significativamente diferentes quando $p < 0,05$. #: diferenças significativas entre repouso e exercício. *: diferenças significativas entre alimentados e jejum. θ : diferenças significativas entre 0h e 12h. Letras representam diferenças significativas entre cada grupo experimental.

Imediatamente após o exercício, ratos obesos apresentaram menor diminuição de glicogênio hepático do que seus controles magros, o que pode ser resultado de uma menor produção hepática de glicose, culminando em uma menor glicemia, comparados aos magros. Os efeitos no conteúdo de glicogênio hepático 12 horas após o exercício mostram que o grupo obeso que realizou o protocolo de exercício alimentado apresentou a menor concentração do substrato, fenômeno esse que já foi demonstrado na literatura (ARAGÓN-VARGAS, 1993; DOHM *et al.*, 1983). Além disso, ao longo das primeiras 12 horas de recuperação do exercício houve um aumento no conteúdo de glicogênio hepático dos animais obesos [3way ANOVA, $F(1,44)=22,227$, $p<0,000$] (Figura 12A, B).

O conteúdo de glicogênio no gastrocnêmio foi menor nos indivíduos obesos do que nos magros, diminuindo imediatamente após o exercício nos obesos (Figura 2, artigo 2). Doze horas após (Figura 12C, D), os animais obesos não apresentaram quaisquer diferenças entre os grupos avaliados, apesar de apresentarem um maior conteúdo de glicogênio neste músculo quando comparados aos obesos imediatamente após o exercício [3way ANOVA, $F(1,44)=5,577$, $p=0,023$]. Imediatamente após o exercício, não foram observadas diferenças entre magros e obesos no conteúdo de glicogênio no sóleo, bem como não houve diferença entre 0h e 12h.

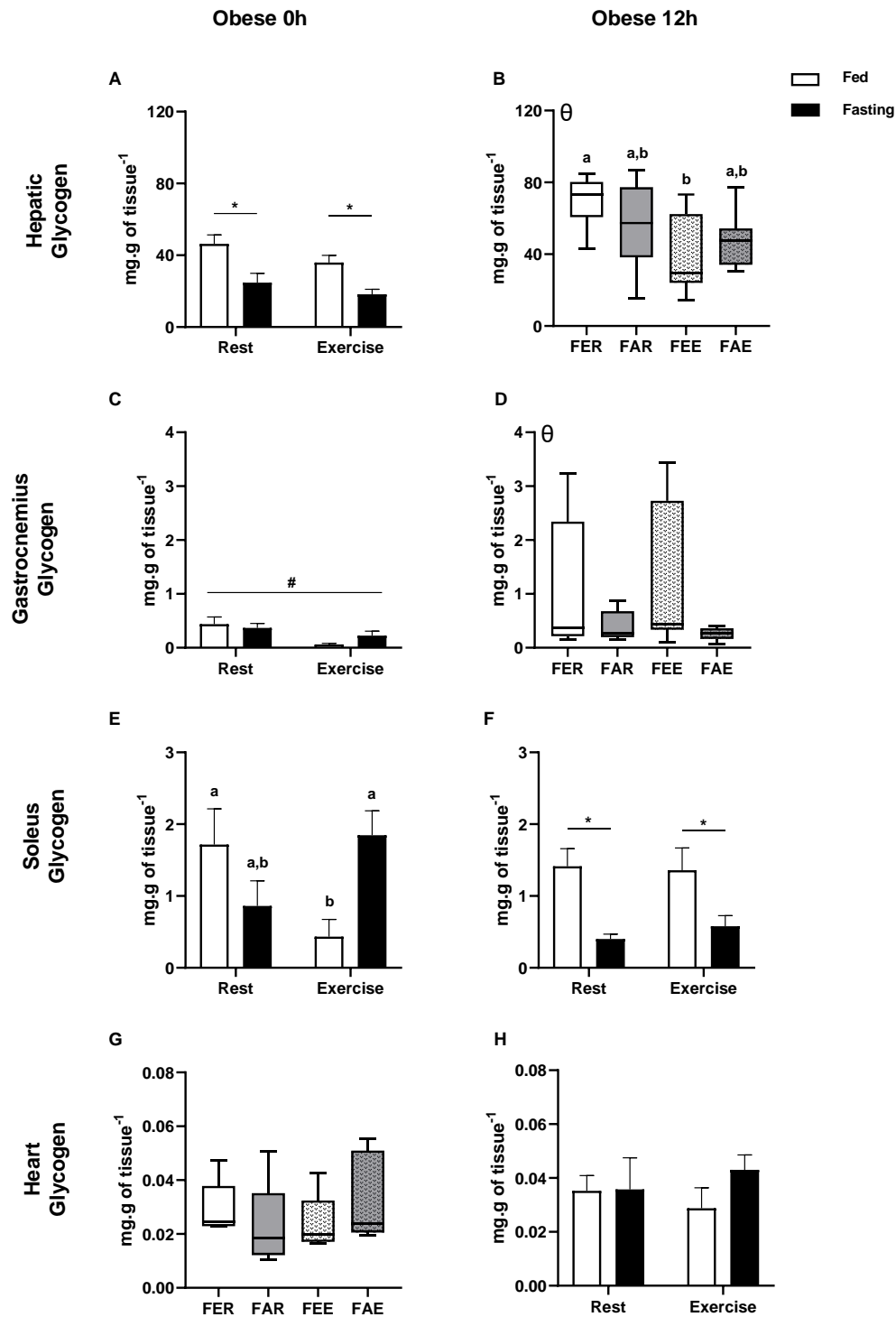


Figura 12. Conteúdo de glicogênio dos animais obesos 0h (esquerda) e 12h (direita) após o protocolo de exercício. (A, B) hepático; (C, D) no gastrocnêmio; (E, F) no sóleo e; (G, H) no coração. Grupos: controle alimentado (FER), controle jejum (FAR), exercício alimentado (FEE) e exercício em jejum (FAE). Os dados foram submetidos a 2way ANOVA (*post hoc* de Bonferroni), Kruskal-Wallis ou ANOVA multifatorial. Os resultados foram considerados significativamente diferentes quando $p < 0,05$. #: diferenças significativas entre repouso e exercício. *: diferenças significativas entre alimentados e jejum. θ: diferenças significativas entre 0h e 12h. Letras representam diferenças significativas entre cada grupo experimental.

O conteúdo de triglicerídeos hepáticos não apresentou diferenças entre ratos magros e obesos, tampouco entre os grupos avaliados. Entretanto, 12 horas depois, ratos obesos apresentaram maior conteúdo quando comparados aos ratos sem recuperação [3way ANOVA, $F(1,45)=121,314$, $p<0,000$] (Figura 13A, B).

Os músculos avaliados mostraram diferentes padrões de estoque lipídico imediatamente após o exercício. No músculo gastrocnêmio, os obesos apresentaram maiores concentrações de triglicerídeos imediatamente após o exercício e o jejum reduziu essas concentrações. Doze horas após, as modificações nas concentrações de TGIM do gastrocnêmio não se sustentaram, apesar dos obesos apresentarem concentrações significativamente maiores do que imediatamente após o exercício [3way ANOVA, $F(1,40)=105,475$, $p<0,000$] (Figura 13C, D). De maneira oposta ao gastrocnêmio, no sóleo os ratos obesos apresentaram menores concentrações de TGIM 12 horas após o exercício [3way ANOVA, $F(1,41)=7,725$, $p=0,008$] (Figura 13E, F).

No que diz respeito ao coração, o conteúdo de triglicerídeos não apresentou diferença entre ratos magros e obesos imediatamente após o exercício. Apesar disso, quando comparados, ratos obesos 12 horas após o exercício apresentaram um conteúdo 7 vezes maior quando comparado aos ratos avaliados imediatamente após o exercício [3way ANOVA, $F(1,45)=1686,235$, $p<0,000$] (Figura 13G, H).

De maneira geral, o quadro observado 12 horas após a realização do protocolo de exercício apresentou resultados promissores e metabolicamente relevantes. A diminuição da glicemia concomitantemente ao aumento do

conteúdo de glicogênio tanto no gastrocnêmio quanto no fígado demonstra um aumento na captação de glicose e manutenção na capacidade de síntese do substrato e consequente manutenção na sensibilidade insulínica.

De maneira semelhante, a diminuição do conteúdo de triglicerídeos séricos paralelamente ao aumento no conteúdo de triglicerídeos no gastrocnêmio, fígado e coração, parece apresentar um aumento na captação do substrato, culminando com maior síntese de triglicerídeos teciduais. Todavia, este aumento na concentração de triglicerídeos dentro dos tecidos periféricos deve ser avaliado com cautela, já que TAG não são sinalizadores intracelulares, mas sim seus metabólitos como DAG e ceramidas (JOCKEN *et al.*, 2010). Assim, um aumento na concentração de TAG intracelular não deve ser associada a uma piora na sensibilidade insulínica à primeira vista, visto que há outros metabólitos com papel mais importante no desenvolvimento ou manutenção deste quadro (BERGMAN *et al.*, 2018; HANNUN *et al.*, 1986).

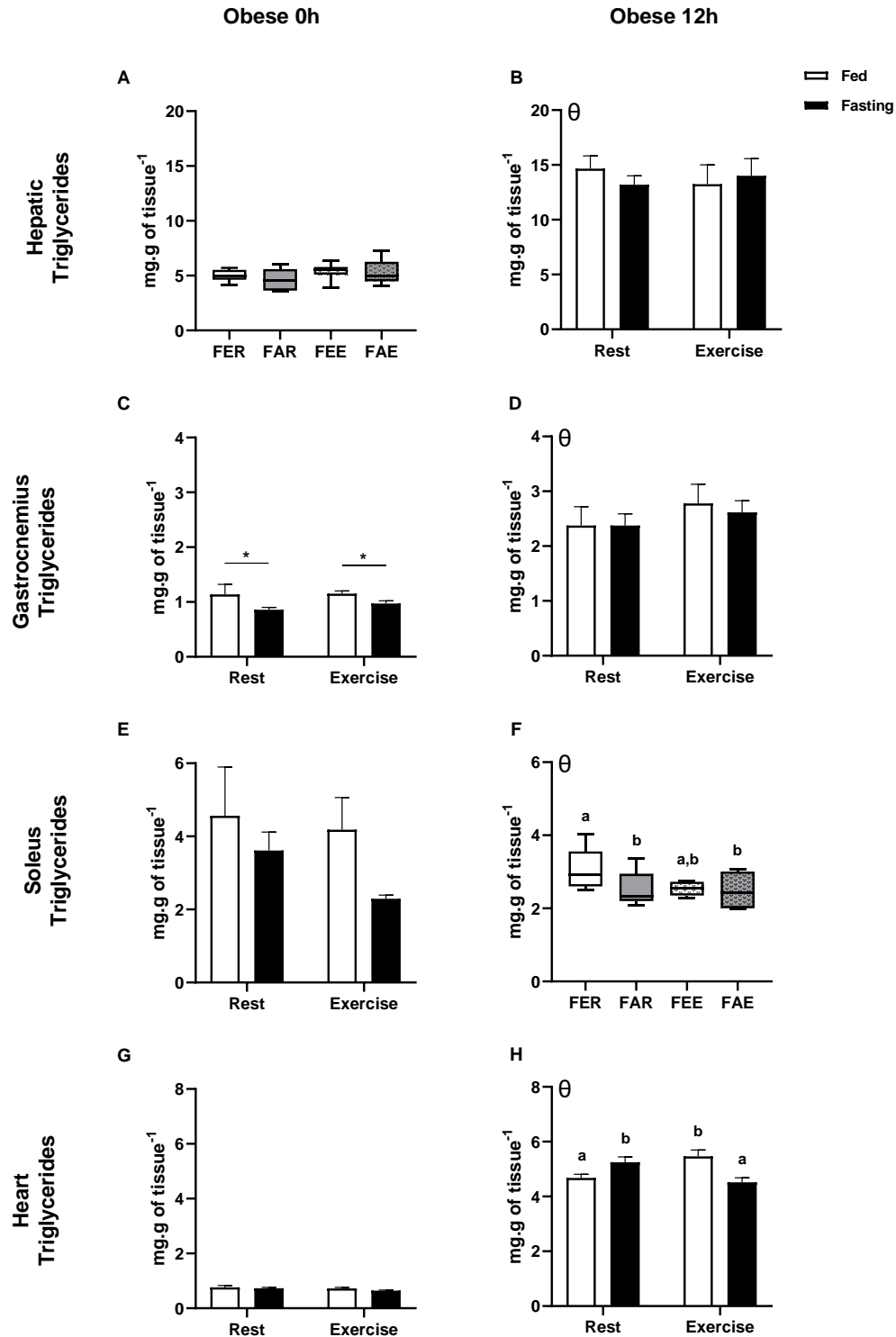


Figura 13. Conteúdo de triglicerídeos dos animais obesos 0h (esquerda) e 12h (direita) após o protocolo de exercício. (A, B) hepático; (C, D) no gastrocnêmio; (E, F) no sóleo e; (G, H) no coração. Grupos: controle alimentado (FER), controle jejum (FAR), exercício alimentado (FEE) e exercício em jejum (FAE). Os dados foram submetidos a 2way ANOVA (*post hoc* de Bonferroni), Kruskal-Wallis ou ANOVA multifatorial. Os resultados foram considerados significativamente diferentes quando $p < 0,05$. #: diferenças significativas entre repouso e exercício. *: diferenças significativas entre alimentados e jejum. θ: diferenças significativas entre 0h e 12h. Letras representam diferenças significativas entre cada grupo experimental.

Devido a seus perfis metabólicos diferentes, onde o músculo gastrocnêmio apresenta marcada capacidade glicolítica e o sóleo, oxidativa (CANTÓ *et al.*, 2009), ambos apresentaram diferentes padrões de oxidação de glicose. Ratos obesos obtiveram menor oxidação de glicose tanto no gastrocnêmio quanto no sóleo, enquanto, no sóleo, o jejum foi capaz de aumentar a oxidação de glicose (Figura 5A, B, artigo 2). No gastrocnêmio o efeito foi oposto. O aumento na oxidação de glicose observado no gastrocnêmio dos obesos em resposta ao exercício pode ser atribuído a uma maior captação de glicose imposta pelo exercício, culminando com diminuição da glicemia nestes indivíduos.

Imediatamente após o exercício, não foram observadas diferenças entre ratos magros e obesos na oxidação de palmitato nos músculos gastrocnêmio e sóleo. O gastrocnêmio dos ratos magros não foi responsivo a nenhuma das intervenções, enquanto o jejum foi capaz de diminuir a oxidação do ácido graxo nos animais obesos. Já no sóleo dos ratos magros, o jejum aumentou a oxidação de palmitato, enquanto nos obesos, os animais do grupo exercitado alimentado (FEE) apresentaram menor oxidação de palmitato quando comparados ao grupo jejuado em repouso (FAR).

Embora aminoácidos não representem uma importante fonte energética durante o exercício (SHERMAN, 1995), ratos magros apresentaram uma maior oxidação de alanina imediatamente após a sessão de exercício, no músculo gastrocnêmio. Talvez essa menor oxidação de alanina por parte dos obesos possa ser explicada por uma menor atividade do ciclo de Krebs, via esta que se encontra diminuída por uma menor capacidade oxidativa.

É de extrema importância que o controle energético celular realizado pela via AMPK-SIRT1-PGC1 α seja restabelecido, em indivíduos obesos, em prol de uma flexibilidade metabólica funcional que seja capaz de diminuir a inflamação crônica de baixo grau e resistência insulínica. Ratos obesos apresentaram maior expressão de *SIRT1* no gastrocnêmio logo após o exercício, quando comparados aos magros, e menor expressão de *AMPK*, tanto no gastrocnêmio quanto no sóleo (Figura 6A, B; Figura 7A, B, artigo 2, respectivamente).

A via de choque térmico HSF1-HSP70 apresenta benefícios anti-inflamatórios importantes para indivíduos acometidos por obesidade e/ou resistência insulínica. Um conteúdo diminuído de HSP70 intracelular está relacionado a um prejuízo na resposta contra doenças crônicas pela oxidação e glicação de proteínas, formação de espécies reativas de oxigênio e nitrogênio, agregação proteica e inflamação (CHUNG *et al.*, 2008; HOOPER *et al.*, 2014; HUEY; MEADOR, 2008). Apesar de não terem sido observadas diferenças na expressão dos genes codificadores da *HSP70*, *HSF1*, *SIRT1* e *PGC1 α* em resposta a nenhuma intervenção, nos ratos obesos exercitados (em jejum ou alimentados) logo após a intervenção, o imunoc conteúdo de HSP70 e de SIRT1 aumentou em resposta ao exercício 12 horas após sua realização. Esse aumento na HSP70 e na SIRT1 é importante não apenas para o controle metabólico, mas também por sua participação na proteostase celular e resolução da inflamação, principalmente em um modelo de inflamação crônica de baixo grau como o do presente trabalho.

Em resumo, as principais alterações metabólicas no soro, fígado e coração podem ser observadas na figura 14, enquanto os principais resultados

obtidos no gastrocnêmio e no sóleo estão resumidos nas figuras 15 e 16, respectivamente.

	Magros 0h		Magros vs.	Obesos 0h		Obesos 12h		Obesos 0h
	Jejum	Exercício	Obesos 0h	Jejum	Exercício	Jejum	Exercício	vs. 12h
Glicose Sérica	—	● ↑	Obesos ↓	↓	↑	—	—	12h ↓
Lactato Sérico	—	●	Obesos ↑	↑	↑	—	—	—
Proteínas Totais Séricas	—	—	Obesos ↑	—	●	—	●	12h ↑
Triglicerídeos Séricos	—	—	—	—	—	—	● ↓	12h ↓
Colesterol Total Sérico	—	●	Obesos ↑	↓	—	—	● ↓	—
Glicogênio Hepático	—	●	Obesos ↑	↓	—	—	●	12h ↑
Glicogênio Coração	—	—	—	—	—	—	—	—
Triglicerídeos Hepáticos	—	—	—	—	—	—	—	12h ↑
Triglicerídeos Coração	—	—	—	—	—	—	●	12h ↑

Figura 14. Resumo das alterações metabólicas no soro, fígado e coração. Setas para cima: aumento significativo; seta para baixo: diminuição significativa; linha horizontal: ausência de alteração; círculo verde: interação entre exercício e jejum.

	Magros 0h		Magros vs.	Obesos 0h		Obesos 12h		Obesos 0h
	Jejum	Exercício	Obesos 0h	Jejum	Exercício	Jejum	Exercício	vs. 12h
Glicogênio	—	—	Obesos ↓	—	↓	—	—	12h ↑
Triglicerídeos	—	—	Obesos ↑	↓	—	—	—	12h ↑
Oxidação de Glicose	—	—	Obesos ↓	↓	↑	—	—	—
Oxidação de Palmitato	—	—	—	↓	—	—	—	—
Oxidação de Alanina	—	—	Obesos ↓	—	—	—	—	—
Expressão de AMPK	—	●	Obesos ↓	↓	—	—	—	—
Expressão de HSP70	—	—	—	—	—	—	—	—
Expressão de HSF1	—	—	—	—	—	—	—	—
Expressão de SIRT1	—	—	Obesos ↑	—	—	—	—	—
Expressão de PGC1 α	—	—	—	—	—	—	—	—
Imunoconteúdo de HSP70	—	—	—	—	—	—	↑	—
Imunoconteúdo de SIRT1	—	—	—	—	—	—	↑	—

Figura 15. Resumo das alterações metabólicas e moleculares no músculo gastrocnêmio. Setas para cima: aumento significativo; seta para baixo: diminuição significativa; linha horizontal: ausência de alteração; círculo verde: interação entre exercício e jejum.

	Magros 0h		Magros vs.	Obesos 0h		Obesos 12h		Obesos 0h
	Jejum	Exercício	Obesos 0h	Jejum	Exercício	Jejum	Exercício	vs. 12h
Glicogênio	—	—	—	—	●	↓	—	—
Triglicerídeos	—	—	Obesos ↓	—	—	—	●	12h ↓
Oxidação de Glicose	—	—	Obesos ↓	↑	—	—	—	—
Oxidação de Palmitato	↑	—	—	—	●	—	—	—
Oxidação de Alanina	—	—	—	—	—	—	—	—
Expressão de AMPK	—	●	Obesos ↓	—	—	—	—	—
Expressão de HSP70	—	—	—	—	—	—	—	—
Expressão de HSF1	—	—	—	—	—	—	—	—
Expressão de SIRT1	—	—	—	—	—	—	—	—
Expressão de PGC1 α	—	—	—	—	—	—	—	—
Imunoconteúdo de HSP70	—	—	—	—	—	—	↑	—
Imunoconteúdo de SIRT1	—	—	—	—	—	—	↑	—

Figura 16. Resumo das alterações metabólicas e moleculares no músculo sóleo. Seta para cima: aumento significativo; seta para baixo: diminuição significativa; linha horizontal: ausência de alteração; círculo verde: interação entre exercício e jejum.

7. CONCLUSÕES GERAIS

Contrariamente à hipótese principal do presente trabalho, o exercício aeróbio realizado em jejum não foi capaz de potencializar as respostas do jejum ou exercício quando realizadas separadamente. A associação de jejum e exercício teve efeitos imediatos diferentes no gastrocnêmio (músculo glicolítico) em relação ao sóleo (músculo oxidativo) em animais obesos. No sóleo, a combinação de jejum e exercício teve um efeito poupador de glicogênio associado ao aumento da oxidação do palmitato, enquanto no gastrocnêmio, aumentou a oxidação da glicose. Além disso, a AMPK foi mais sensível às intervenções em animais magros do que em obesos e sua resposta foi diferente entre os músculos oxidativos e glicolíticos.

Nos ratos obesos, o exercício em jejum provocou modificações metabólicas positivas adicionais que perduraram por até 12 horas após sua realização. A redução de triglicerídeos e colesterol total sanguíneos (ambos marcadores de risco cardiovascular) pode ser particularmente importante para indivíduos obesos e diabéticos. Embora não tenha sido observada interação entre exercício e jejum, 12 horas após sua realização, no imunoconteúdo de HSP70 e SIRT1, foram encontradas diferenças significativas no metabolismo glicídico e lipídico dos animais obesos.

Embora seja difícil extrapolar estes achados em ratos para a população humana obesa, eles contribuem para o conhecimento sobre os efeitos da realização de exercícios em jejum, especificamente em indivíduos obesos. No entanto, as alterações metabólicas induzidas (principalmente no que diz respeito à diminuição dos triglicerídeos e do colesterol total) e o fato de poderem durar

até 12 horas após a sessão de exercício são promissores achados que precisam ser investigados em humanos.

8. PERSPECTIVAS

Conforme mostrado no presente trabalho, existem respostas imediatas e tardias à realização do exercício aeróbio de moderada intensidade, tanto em ratos magros como obesos. Embora muitas respostas não sejam resultado da interação entre exercício e jejum, mas do exercício *per se*, essa modalidade pode representar uma importante ferramenta no combate a doenças metabólicas.

Com base no exposto, uma das perspectivas do estudo é a utilização de protocolos de treinamento ao invés da realização de uma única sessão de exercício, bem como a avaliação do imunoconteúdo de outras proteínas de interesse, em diferentes tempos pós exercício.

9. REFERÊNCIAS

AIRD, T. P.; DAVIES, R. W.; CARSON, B. P. Effects of fasted vs fed-state exercise on performance and post-exercise metabolism: A systematic review and meta-analysis. **Scandinavian Journal of Medicine and Science in Sports**, [s. l.], v. 28, n. 5, p. 1476–1493, 2018.

ALLEN, Katherine M; SAHA, Asish K. AMP-Activated Protein Kinase: Its Regulation by Different Sites. **Current Research in Diabetes & Obesity Journal**, [s. l.], v. 2, n. 5, p. 1–3, 2017.

ANTON, Stephen D. *et al.* Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. **Obesity**, [s. l.], v. 26, n. 2, p. 254–268, 2018.

ARAGÓN-VARGAS, Luis F. Effects of Fasting on Endurance Exercise. **Sports Medicine**, [s. l.], v. 16, n. 4, p. 255–265, 1993.

ARCHER, Ashley E.; VON SCHULZE, Alex T.; GEIGER, Paige C. Exercise, heat shock proteins and insulin resistance. **Philosophical Transactions of the Royal Society B: Biological Sciences**, [s. l.], v. 373, n. 1738, 2017.

ARMSTRONG, R. B.; PHELPS, R. O. Muscle fiber type composition of the rat hindlimb. **The American Journal of Anatomy**, [s. l.], v. 171, p. 259–272, 1984.

ARNER, Peter *et al.* Adrenergic regulation of lipolysis in situ at rest and during exercise. **Journal of Clinical Investigation**, [s. l.], v. 85, n. 3, p. 893–898, 1990.

ASAOKA, Yoshinori *et al.* Protein kinase C, calcium and phospholipid degradation. **Trends in Biochemical Sciences**, [s. l.], v. 17, n. 10, p. 414–417, 1992.

BANDET, Cécile L. *et al.* Sphingolipid metabolism: New insight into ceramide-induced lipotoxicity in muscle cells. **International Journal of Molecular Sciences**, [s. l.], v. 20, n. 3, p. 1–26, 2019.

BECKER, Jörg; CRAIG, Elizabeth A. Heat-shock proteins as molecular chaperones. **European Journal of Biochemistry**, [s. l.], v. 219, p. 11–23, 1994.

BERGMAN, Bryan C. *et al.* Intramuscular triglyceride synthesis: Importance in muscle lipid partitioning in humans. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 314, n. 2, p. E152–E164, 2018.

BERTHOLDT, Lærke *et al.* Impact of training state on fasting-induced regulation of adipose tissue metabolism in humans. **Journal of Applied Physiology**, [s. l.], v. 124, n. 3, p. 729–740, 2018.

BHUTTA, Maimoona Shahid; GALLO, Elisa S.; BORENSTEIN, Ronen. Multifaceted role of AMPK in viral infections. **Cells**, [s. l.], v. 10, n. 5, p. 1–26, 2021.

BJORKMAN, Ola; ERIKSSON, Ljusk Siw. Splanchnic glucose metabolism during leg exercise in 60-hour-fasted human subjects. **Am J Physiol Endocrinol Metab**, [s. l.], v. 8, p. E443–E448, 1983.

BONI, Lawrence T; RANDO, Robert R. The nature of protein kinase C activation by physically defined phospholipid vesicles and diacylglycerols. **Journal of Biological Chemistry**, [s. l.], v. 260, n. 19, p. 10819–10825, 1985.

BOSMA, Madeleen *et al.* Perilipin 2 improves insulin sensitivity in skeletal muscle despite elevated intramuscular lipid levels. **Diabetes**, [s. l.], v. 61, n. 11, p. 2679–2690, 2012.

BROOKS, George A. Lactate as a fulcrum of metabolism. **Redox Biology**, [s. l.], v. 35, n. January, p. 101454, 2020. Disponível em: <https://doi.org/10.1016/j.redox.2020.101454>.

BROSKEY, Nicholas T. *et al.* Plasma Lactate as a Marker for Metabolic Health. **Exercise and Sport Sciences Reviews**, [s. l.], v. 48, n. 3, p. 119–124, 2020.

BUJAK, Adam L. *et al.* AMPK activation of muscle autophagy prevents fasting-induced hypoglycemia and myopathy during aging. **Cell Metabolism**, [s. l.], v. 21, n. 6, p. 883–890, 2015. Disponível em: <http://dx.doi.org/10.1016/j.cmet.2015.05.016>.

CANTÓ, Carles *et al.* AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity. **Nature**, [s. l.], v. 458, n. 7241, p. 1056–1060, 2009.

CANTÓ, Carles *et al.* Interdependence of AMPK and SIRT1 for Metabolic Adaptation to Fasting and Exercise in Skeletal Muscle. **Cell Metabolism**, [s. l.], v. 11, n. 3, p. 213–219, 2010. Disponível em: <http://dx.doi.org/10.1016/j.cmet.2010.02.006>.

CARBONE, Salvatore *et al.* Obesity, risk of diabetes and role of physical activity, exercise training and cardiorespiratory fitness. **Progress in Cardiovascular Diseases**, [s. l.], v. 62, n. 4, p. 327–333, 2019. Disponível em: <https://doi.org/10.1016/j.pcad.2019.08.004>.

CARLING, David. AMPK hierarchy: a matter of space and time. **Cell Research**, [s. l.], v. 29, n. 6, p. 425–426, 2019. Disponível em: <http://dx.doi.org/10.1038/s41422-019-0171-6>.

CHEN, Li *et al.* Mechanisms Linking Inflammation to Insulin Resistance. **International Journal of Endocrinology**, [s. l.], v. 2015, 2015.

CHOBOT, Agata *et al.* Obesity and diabetes—Not only a simple link between two epidemics. **Diabetes/Metabolism Research and Reviews**, [s. l.], v. 34, n. 7, p. 1–9, 2018.

CHUNG, Jason *et al.* HSP72 protects against obesity-induced insulin resistance. **Proceedings of the National Academy of Sciences of the United States of America**, [s. l.], v. 105, n. 5, p. 1739–1744, 2008.

CIVITARESE, Anthony E. *et al.* Glucose ingestion during exercise blunts exercise-induced gene expression of skeletal muscle fat oxidative genes. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 289, n. 6, p. 1023–1029, 2005.

COZER, Aline G. *et al.* Effects of Stanniocalcin-1 on glucose flux in rat brown adipose tissue. **Biochimie**, [s. l.], v. 138, p. 50–55, 2017.

DA NOBREGA, Antonio Claudio Lucas. The Subacute Effects of Exercise: Concept, Characteristics, and Clinical Implications. **Exercise and Sport Sciences Reviews**, [s. l.], v. 33, n. 2, p. 84–87, 2005.

DAEMEN, Sabine; VAN POLANEN, Nynke; HESSELINK, Matthijs K.C. The effect of diet and exercise on lipid droplet dynamics in human muscle tissue. **Journal of Experimental Biology**, [s. l.], v. 121, 2018.

DE BOCK, K. *et al.* Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. **Journal of Applied Physiology**, [s. l.], v. 104, n. 4, p. 1045–1055, 2008.

DE TODA, I. Martínez; DE LA FUENTE, M. The role of Hsp70 in oxi-inflamm-aging and its use as a potential biomarker of lifespan. **Biogerontology**, [s. l.], v. 16, n. 6, p. 709–721, 2015.

DOBROWSKY, R. T. *et al.* Ceramide activates heterotrimeric protein phosphatase 2A. **Journal of Biological Chemistry**, [s. l.], v. 268, n. 21, p. 15523–15530, 1993.

DOHM, G. L. *et al.* Influence of fasting on glycogen depletion in rats during exercise. **Journal of Applied Physiology Respiratory Environmental and Exercise Physiology**, [s. l.], v. 55, n. 3, p. 830–833, 1983.

DOHM, G. L. *et al.* Metabolic responses to exercise after fasting. **Journal of Applied Physiology**, [s. l.], v. 61, n. 4, p. 1363–1368, 1986.

DRAZNIN, B. *et al.* Effect of dietary macronutrient composition on AMPK and SIRT1 expression and activity in human skeletal muscle. **Hormone and Metabolic Research**, [s. l.], v. 44, n. 9, p. 650–655, 2012.

FEDER, Martin E.; HOFMANN, Gretchen E. Heat-Shock Proteins, Molecular Chaperones, and the Stress Response: Evolutionary and Ecological Physiology. **Annual Review of Physiology**, [s. l.], v. 61, p. 243–282, 1999.

FISHER, Ffolliott F. *et al.* FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. **Genes and Development**, [s. l.], v. 26, n. 3, p. 271–281, 2012.

FORETZ, Marc; EVEN, Patrick C.; VIOLLET, Benoit. AMPK activation reduces hepatic lipid content by increasing fat oxidation in vivo. **International Journal of Molecular Sciences**, [s. l.], v. 19, n. 9, 2018.

FUCHO, Raquel *et al.* Ceramides and mitochondrial fatty acid oxidation in obesity. **FASEB Journal**, [s. l.], v. 31, n. 4, p. 1263–1272, 2017.

FUJITA, Naoto *et al.* Changes in lipid metabolism and capillary density of the skeletal muscle following low-intensity exercise training in a rat model of obesity with hyperinsulinemia. **PLoS ONE**, [s. l.], v. 13, n. 5, p. 1–20, 2018.

GALBO, Thomas *et al.* Free fatty acid-induced pp2a hyperactivity selectively impairs hepatic insulin action on glucose metabolism. **PLoS ONE**, [s. l.], v. 6, n. 11, p. 1–9, 2011.

GALIC, Sandra *et al.* AMPK signaling to acetyl-CoA carboxylase is required for fasting-and cold-induced appetite but not thermogenesis. **eLife**, [s. l.], v. 7, p. 1–22, 2018.

GEMMINK, Anne *et al.* Dissociation of intramyocellular lipid storage and insulin resistance in trained athletes and type 2 diabetes patients; involvement of perilipin 5?. **Journal of Physiology**, [s. l.], v. 596, n. 5, p. 857–868, 2018.

GLEESON, M; GREENHAFF, P L; MAUGHAN, R J. Influence of a 24 h fast on high intensity cycle exercise performance in man. **Eur J Appl Physiol**, [s. l.], v. 57, p. 653–659, 1988.

GOMEZ-MUÑOZ, Antonio *et al.* Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. **Progress in Lipid Research**, [s. l.], v. 61, p. 51–62, 2016. Disponível em: <http://dx.doi.org/10.1016/j.plipres.2015.09.002>.

GOODPASTER, Bret H *et al.* Skeletal muscle lipid content and insulin resistance: Evidence for a paradox in endurance-trained athletes. **Journal of Clinical Endocrinology and Metabolism**, [s. l.], v. 86, n. 12, p. 5755–5761, 2001.

GOODPASTER, Bret H.; SPARKS, Lauren M. Metabolic Flexibility in Health and Disease. **Cell Metabolism**, [s. l.], v. 25, n. 5, p. 1027–1036, 2017. Disponível em: <http://dx.doi.org/10.1016/j.cmet.2017.04.015>.

GRYGIEL-GÓRNIAK, Bogna. Peroxisome proliferator-activated receptors and their ligands: Nutritional and clinical implications - A review. **Nutrition Journal**, [s. l.], v. 13, n. 1, p. 1–10, 2014.

HANNUN, Yusuf A. *et al.* Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. **Journal of Biological Chemistry**, [s. l.], v. 261, n. 27, p. 12604–12609, 1986.

HERZOG, Walter. Reflections on obesity, exercise, and musculoskeletal health. **Journal of Sport and Health Science**, [s. l.], v. 9, n. 2, p. 108–109, 2020.

HESHKA, S.; ALLISON, D. B. Is obesity a disease?. **International Journal of Obesity**, [s. l.], v. 25, p. 1401–1406, 2001.

HILDEBRANDT, Audrey L.; NEUFER, P. Darrell. Exercise attenuates the fasting-induced transcriptional activation of metabolic genes in skeletal muscle. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 278, n. 6 41-6, p. 1078–1086, 2000.

HOOPER, Philip L. *et al.* The importance of the cellular stress response in the pathogenesis and treatment of type 2 diabetes. **Cell Stress and Chaperones**, [s. l.], v. 19, n. 4, p. 447–464, 2014.

HOROWITZ, Jeffrey F. Regulation of Lipid Mobilization and Oxidation during Exercise in Obesity. **Exerc Sport Sci Rev**, [s. l.], v. 29, n. 1, p. 42–46, 2001.

HOTTA, Yuhei *et al.* Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver. **Endocrinology**, [s. l.], v. 150, n. 10, p. 4625–4633, 2009.

HOUMARD, Joseph A. Intramuscular lipid oxidation and obesity. **American Journal of Physiology - Regulatory Integrative and Comparative Physiology**, [s. l.], v. 294, n. 4, p. 1111–1116, 2008.

HUEY, Kimberly A.; MEADOR, Benjamin M. Contribution of IL-6 to the Hsp72, Hsp25, and $\alpha\beta$ -crystallin responses to inflammation and exercise training in mouse skeletal and cardiac muscle. **Journal of Applied Physiology**, [s. l.], v. 105, n. 6, p. 1830–1836, 2008.

ITANI, S. I. *et al.* Involvement of protein kinase C in human skeletal muscle insulin resistance and obesity. **Diabetes**, [s. l.], v. 49, n. 8, p. 1353–1358, 2000.

IWAYAMA, Kaito *et al.* Exercise Increases 24-h Fat Oxidation Only When It Is Performed Before Breakfast. **EBioMedicine**, [s. l.], v. 2, n. 12, p. 2003–2009, 2015. Disponível em: <http://dx.doi.org/10.1016/j.ebiom.2015.10.029>.

JEON, Sang Min. Regulation and function of AMPK in physiology and diseases. **Experimental & molecular medicine**, [s. l.], v. 48, n. 7, p. e245, 2016. Disponível em: <http://dx.doi.org/10.1038/emm.2016.81>.

JOCKEN, Johan W.E. *et al.* Skeletal muscle lipase content and activity in obesity and type 2 diabetes. **Journal of Clinical Endocrinology and Metabolism**, [s. l.], v. 95, n. 12, p. 5449–5453, 2010.

KEIPERT, Susanne *et al.* Skeletal muscle mitochondrial uncoupling drives endocrine cross-talk through the induction of FGF21 as a myokine. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 306, n. 5, p. 469–482, 2014.

KELLEY, David E. *et al.* Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 277, n. 6 40-6, p. E1130–E1141, 1999.

KIM, Jong Yeon *et al.* Evidence of a malonyl-CoA-insensitive carnitine palmitoyltransferase I activity in red skeletal muscle. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 282, n. 5 45-5, p. 1014–1022, 2002.

KIM, Jong-Yeon *et al.* Lipid oxidation is reduced in obese human skeletal muscle. **American Journal of Physiology-Endocrinology and Metabolism**, [s. l.], v. 279, p. E1039–E1044, 2000. Disponível em: <https://www.physiology.org/doi/10.1152/ajpendo.2000.279.5.E1039>.

KLEIN-WIERINGA, Inge R. *et al.* Adipocytes Modulate the Phenotype of Human Macrophages through Secreted Lipids. **The Journal of Immunology**, [s. l.], v. 191, n. 3, p. 1356–1363, 2013.

KLEINERT, Maximilian *et al.* mTORC2 and AMPK differentially regulate muscle triglyceride content via Perilipin 3. **Molecular Metabolism**, [s. l.], v. 5, n. 8, p. 646–655, 2016. Disponível em: <http://dx.doi.org/10.1016/j.molmet.2016.06.007>.

KNAPIK, Joseph J *et al.* Influence of a 3.5 day fast on physical performance. **Eur J Appl Physiol**, [s. l.], v. 56, p. 428–432, 1987.

KOUBI, H. E. *et al.* Exercise endurance and fuel utilization: a reevaluation of the effects of fasting. **J Appl Physiol**, [s. l.], v. 70, n. 3, p. 1337–1343, 1991.

KRAUSE, Maurício S *et al.* MRP1/GS-X pump ATPase expression: is this the explanation for the cytoprotection of the heart against oxidative stress-induced redox imbalance in comparison to skeletal muscle cells?. **Cell Biochemistry and Function**, [s. l.], v. 25, p. 23–32, 2007.

KRAUSE, Mauricio *et al.* The Chaperone Balance Hypothesis: The Importance of the Extracellular to Intracellular HSP70 Ratio to Inflammation-Driven Type 2 Diabetes, the Effect of Exercise, and the Implications for Clinical Management. **Mediators of Inflammation**, [s. l.], v. 2015, p. 1–12, 2015.

KRAUSE, Mauricio *et al.* The regulatory roles of NADPH oxidase, intra- and extra-cellular HSP70 in pancreatic islet function, dysfunction and diabetes. **Clinical Science**, [s. l.], v. 128, n. 11, p. 789–803, 2015.

KURZ, Jennifer *et al.* Ceramides as Novel Disease Biomarkers. **Trends in Molecular Medicine**, [s. l.], v. 25, n. 1, p. 20–32, 2019. Disponível em: <http://dx.doi.org/10.1016/j.molmed.2018.10.009>.

LARSEN, Philip J.; TENNAGELS, Norbert. On ceramides, other sphingolipids and impaired glucose homeostasis. **Molecular Metabolism**, [s. l.], v. 3, n. 3, p. 252–260, 2014. Disponível em: <http://dx.doi.org/10.1016/j.molmet.2014.01.011>.

LEE, Paul *et al.* Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. **Cell Metabolism**, [s. l.], v. 19, n. 2, p. 302–309, 2014. Disponível em: <http://dx.doi.org/10.1016/j.cmet.2013.12.017>.

LOY, Steven F *et al.* Effects of 24-hour fast on cycling endurance time at two different intensities. **J Appl Physiol**, [s. l.], v. 61, n. 2, p. 654–659, 1986.

LUMENG, Carey N *et al.* Obesity induces a phenotypic switch in adipose tissue macrophage polarization Find the latest version : Obesity induces a phenotypic switch in adipose tissue macrophage polarization. **J Clin Invest**, [s. l.], v. 117, n. 1, p. 175–184, 2007. Disponível em: http://fride.org/download/PB_81_From_Lisbon_to_NY.pdf.

MA, Xinran *et al.* Deciphering the roles of PPAR γ in adipocytes via dynamic change of transcription complex. **Frontiers in Endocrinology**, [s. l.], v. 9, n. AUG, p. 1–10, 2018.

MAHFOUZ, Rana *et al.* Characterising the inhibitory actions of ceramide upon insulin signaling in different skeletal muscle cell models: A mechanistic insight. **PLoS ONE**, [s. l.], v. 9, n. 7, p. 1–9, 2014.

MAUGHAN, R J; GLEESON, M. Influence of a 36 h fast followed by refeeding with glucose , glycerol or placebo on metabolism and performance during prolonged exercise in man. **Eur J Appl Physiol**, [s. l.], v. 57, p. 570–576, 1988.

MCGARRY, J. Denis; BROWN, Nicholas F. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. **European Journal of Biochemistry**, [s. l.], v. 244, n. 1, p. 1–14, 1997.

MENGESTE, Abel M; RUSTAN, Arild C; LUND, Jenny. Skeletal muscle energy metabolism in obesity. **Obesity (Silver Spring)**, [s. l.], v. 29, p. 1582–1595, 2021.

MODEL, Jorge Felipe Argenta *et al.* Liraglutide improves lipid and carbohydrate metabolism of ovariectomized rats. **Molecular and Cellular Endocrinology**, [s. l.], v. 524, p. 1–11, 2021.

MODEL, Jorge Felipe Argenta *et al.* Liraglutide treatment counteracts alterations in adipose tissue metabolism induced by orchietomy in rats. **Life Sciences**, [s. l.], v. 278, n. May, 2021.

MONTGOMERY, Magdalene K.; DE NARDO, William; WATT, Matthew J. Impact of Lipotoxicity on Tissue “Cross Talk” and Metabolic Regulation. **Physiology**, [s. l.], v. 34, n. 2, p. 134–149, 2019.

MORALES, Pablo Esteban; BUCAREY, Jose Luis; ESPINOSA, Alejandra. Muscle lipid metabolism: Role of lipid droplets and perilipins. **Journal of Diabetes Research**, [s. l.], v. 2017, 2017.

MORIGNY, Pauline *et al.* Adipocyte lipolysis and insulin resistance. **Biochimie**, [s. l.], v. 125, p. 259–266, 2016. Disponível em: <http://dx.doi.org/10.1016/j.biochi.2015.10.024>.

MORTON, James P. *et al.* Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. **Journal of Applied Physiology**, [s. l.], v. 101, p. 176–182, 2006.

NEWSHOLME, E. A. The control of fuel utilization by muscle during exercise and starvation. **Diabetes**, [s. l.], v. 28, n. SUPPL. 1, p. 1–7, 1979.

NEWSHOLME, Philip; DE BITTENCOURT JR, Paulo I. Homem. The fat cell senescence hypothesis: A mechanism responsible for abrogating the resolution of inflammation in chronic disease. **Current Opinion in Clinical Nutrition and Metabolic Care**, [s. l.], v. 17, n. 4, p. 295–305, 2014.

NIMPTSCH, Katharina; KONIGORSKI, Stefan; PISCHON, Tobias. Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. **Metabolism: Clinical and Experimental**, [s. l.], v. 92, p. 61–70, 2019. Disponível em: <https://doi.org/10.1016/j.metabol.2018.12.006>.

NOBLE, Earl G; MILNE, Kevin J; MELLING, C W James. Heat shock proteins and exercise: a primer. **Appl Physiol Nutr Metab**, [s. l.], v. 33, p. 1050–1065, 2008.

PARDO, Patricia S; BORIEK, Aladin M. The physiological roles of Sirt1 in skeletal muscle. **AGING**, [s. l.], v. 3, n. 4, p. 430–437, 2011.

PAWLAK, Michal; LEFEBVRE, Philippe; STAELS, Bart. Molecular mechanism of PPAR α action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. **Journal of Hepatology**, [s. l.], v. 62, n. 3, p. 720–733, 2015. Disponível em: <http://dx.doi.org/10.1016/j.jhep.2014.10.039>.

PERALDI, Pascal *et al.* Tumor necrosis factor (TNF)- β inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. **Journal of Biological Chemistry**, [s. l.], v. 271, n. 22, p. 13018–13022, 1996.

PERSEGHIN, Gianluca *et al.* Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: A ¹H-¹³C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. **Diabetes**, [s. l.], v. 48, n. 8, p. 1600–1606, 1999.

PETERSEN, Max C.; SHULMAN, Gerald I. Mechanisms of insulin action and insulin resistance. **Physiological Reviews**, [s. l.], v. 98, n. 4, p. 2133–2223, 2018.

PETERSEN, Max C.; SHULMAN, Gerald I. Roles of Diacylglycerols and Ceramides in Hepatic Insulin Resistance. **Trends in Pharmacological Sciences**, [s. l.], v. 38, n. 7, p. 649–665, 2017. Disponível em: <http://dx.doi.org/10.1016/j.tips.2017.04.004>.

PRIEUR, Xavier *et al.* Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. **Diabetes**, [s. l.], v. 60, n. 3, p. 797–809, 2011.

PURUSHOTHAM, Aparna *et al.* Hepatocyte-Specific Deletion of SIRT1 Alters Fatty Acid Metabolism and Results in Hepatic Steatosis and Inflammation. **Cell Metabolism**, [s. l.], v. 9, n. 4, p. 327–338, 2009. Disponível em: <http://dx.doi.org/10.1016/j.cmet.2009.02.006>.

RODGERS, Joseph T *et al.* Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1. **Letters to Nature**, [s. l.], v. 434, n. March, p. 3–8, 2005.

RODGERS, Joseph T. *et al.* Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1. **Nature**, [s. l.], v. 434, n. 7029, p. 113–118, 2005.

RODRIGUES, Bruno *et al.* Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. **Cardiovascular Diabetology**, [s. l.], v. 6, p. 1–7, 2007.

RODRÍGUEZ-HERNÁNDEZ, Heriberto *et al.* Obesity and inflammation: Epidemiology, risk factors, and markers of inflammation. **International Journal of Endocrinology**, [s. l.], v. 2013,

2013.

SALO, David C.; DONOVAN, Casey M.; DAVIES, Kelvin J.A. HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. **Free Radical Biology and Medicine**, [s. l.], v. 11, n. 3, p. 239–246, 1991.

SAMOVSKI, Dmitri *et al.* Regulation of AMPK activation by CD36 links fatty acid uptake to β -oxidation. **Diabetes**, [s. l.], v. 64, n. 2, p. 353–359, 2015.

SAMUEL, Varman T.; SHULMAN, Gerald I. The pathogenesis of insulin resistance: Integrating signaling pathways and substrate flux. **Journal of Clinical Investigation**, [s. l.], v. 126, n. 1, p. 12–22, 2016.

SARGE, Kevin D; MURPHY, Shawn P; MORIMOTO, Richard I. Activation of Heat Shock Gene Transcription by Heat Shock Factor 1 Involves Oligomerization, Acquisition of DNA-Binding Activity, and Nuclear Localization and Can Occur in the Absence of Stress. **Molecular and Cellular Biology**, [s. l.], v. 13, n. 3, p. 1392–1407, 1993.

SAWAI, Hirofumi; HANNUN, Yusuf A. Ceramide and sphingomyelinases in the regulation of stress responses. **Chemistry and Physics of Lipids**, [s. l.], v. 102, n. 1–2, p. 141–147, 1999.

SCHAFFER, Jean E. Lipotoxicity: when tissues overeat. **Current Opinion in Lipidology**, [s. l.], v. 14, p. 281–287, 2003.

SEVERINSEN, Mai Charlotte Krogh; PEDERSEN, Bente Klarlund. Muscle-Organ Crosstalk: The Emerging Roles of Myokines. **Endocrine reviews**, [s. l.], v. 41, n. 4, p. 1–16, 2020.

SHERMAN, William M. Metabolism of sugars and physical performance. **American Journal of Clinical Nutrition**, [s. l.], v. 62, n. 1 SUPPL., 1995.

SHULMAN, Gerald I. Cellular mechanisms of insulin resistance. **The Journal of Clinical Investigation**, [s. l.], v. 106, n. 2, p. 171–176, 2000.

SIMONEAU, Jean-Aimé; BOUCHARD, Claude. Genetic determinism of fiber type proportion in human skeletal muscle. **The FASEB Journal**, [s. l.], v. 9, p. 1091–1095, 1995.

SIMONEAU, J. A.; BOUCHARD, C. Human variation in skeletal muscle fiber-type proportion and enzyme activities. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], v. 20, p. 567–572, 1989.

SINGH, Ishwar S *et al.* A High Affinity HSF-1 Binding Site in the 5'-Untranslated Region of the Murine Tumor Necrosis Factor- α Gene Is a Transcriptional Repressor. **The Journal of Biological Chemistry**, [s. l.], v. 277, n. 7, p. 4981–4988, 2002.

STEINHAUSER, Matthew L. *et al.* The circulating metabolome of human starvation. **JCI insight**, [s. l.], v. 3, n. 16, p. 1–16, 2018.

STIENSTRA, Rinke *et al.* PPARs, obesity, and inflammation. **PPAR Research**, [s. l.], v. 2007, 2007.

SZTALRYD, Carole; KIMMEL, Alan R. Perilipins: Lipid droplet coat proteins adapted for tissue-specific energy storage and utilization, and lipid cytoprotection. **Biochimie**, [s. l.], v. 96, n. 1, p. 96–101, 2014. Disponível em: <http://dx.doi.org/10.1016/j.biochi.2013.08.026>.

TAMARGO-GÓMEZ, Isaac; MARIÑO, Guillermo. AMPK: Regulation of metabolic dynamics in the context of autophagy. **International Journal of Molecular Sciences**, [s. l.], v. 19, n. 12, 2018.

THIERION, Elodie; ODOM, Duncan T. Charting the transcriptional regulatory changes in mouse liver during fasting. **Hepatology**, [s. l.], v. 67, n. 1, p. 436–438, 2018.

UNGER, Roger H. Lipid overload and overflow: Metabolic trauma and the metabolic syndrome. **Trends in Endocrinology and Metabolism**, [s. l.], v. 14, n. 9, p. 398–403, 2003.

VAN PRAAG, H. *et al.* Exercise, Energy Intake, Glucose Homeostasis, and the Brain. **Journal of Neuroscience**, [s. l.], v. 34, n. 46, p. 15139–15149, 2014.

VAN PROEYEN, Karen *et al.* Beneficial metabolic adaptations due to endurance exercise training in the fasted state. **Journal of Applied Physiology**, [s. l.], v. 110, n. 1, p. 236–245, 2011.

VERBOVEN, K. *et al.* Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. **Scientific Reports**, [s. l.], v. 8, n. 4677, p. 1–8, 2018.

VICENTE-SALAR, Néstor; OTEGUI, Aritz Urdampilleta; COLLADO, Enrique Roche. Endurance training in fasting conditions: biological adaptations and body weight management. **Nutricion Hospitalaria**, [s. l.], v. 32, n. 6, p. 2409–2420, 2015.

VILAÇA-ALVES, José *et al.* Cardiorespiratory, enzymatic and hormonal responses during and after walking while fasting. **PLoS ONE**, [s. l.], v. 13, n. 3, p. 1–10, 2018.

VILLARROYA, Joan *et al.* Lactate induces expression and secretion of fibroblast growth factor-21 by muscle cells. **Endocrine**, [s. l.], v. 61, n. 1, p. 165–168, 2018. Disponível em: <http://dx.doi.org/10.1007/s12020-018-1612-6>.

VOGT, Éverton Lopes *et al.* Metabolic and molecular subacute effects of a single moderate-intensity exercise bout, performed in the fasted state, in obese male rats. **International Journal of Environmental Research and Public Health**, [s. l.], v. 18, n. 14, p. 1–17, 2021.

WAHREN, John. Glucose Turnover During Exercise in Healthy Man and in Patients with Diabetes Mellitus. **Diabetes**, [s. l.], v. 28, p. 82–88, 1979.

WALKER, Amy K. *et al.* Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. **Genes and Development**, [s. l.], v. 24, n. 13, p. 1403–1417, 2010.

WANG, Yiguo *et al.* The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. **Nature**, [s. l.], v. 460, n. 7254, p. 534–537, 2009. Disponível em: <http://dx.doi.org/10.1038/nature08111>.

WENDEL, Angela A. *et al.* Glycerol-3-phosphate acyltransferase (GPAT)-1, but not GPAT4, incorporates newly synthesized fatty acids into triacylglycerol and diminishes fatty acid oxidation*. **Journal of Biological Chemistry**, [s. l.], v. 288, n. 38, p. 27299–27306, 2013.

WESTERBLAD, Håkan; BRUTON, Joseph D; KATZ, Abram. Skeletal muscle: Energy metabolism, fiber types, fatigue and adaptability. **Experimental Cell Research**, [s. l.], v. 316, p. 3093–3099, 2010. Disponível em: <http://dx.doi.org/10.1016/j.yexcr.2010.05.019>.

WIRTH, Alfred; WABITSCH, Martin; HAUNER, Hans. The prevention and treatment of obesity. **Deutsches Ärzteblatt international**, [s. l.], v. 111, n. 42, p. 705–713, 2014.

WOLINS, Nathan E.; MITTENDORFER, Bettina. The athlete's paradOXpat. **Journal of Physiology**, [s. l.], v. 596, n. 5, p. 755–756, 2018.

WU, Lianpin *et al.* Heat shock transcription factor 1 attenuates TNF α -induced cardiomyocyte death through suppression of NF κ B pathway. **Gene**, [s. l.], v. 527, p. 89–94, 2013. Disponível em: <http://dx.doi.org/10.1016/j.gene.2013.05.024>.

WU, Z. *et al.* Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1 transcription and mitochondrial biogenesis in muscle cells. **Proceedings of the National Academy of Sciences**, [s. l.], v. 103, n. 39, p. 14379–14384, 2006.

YENARI, Mldori A *et al.* The neuroprotective potential of heat shock protein 70 (HSP70). **Molecular Medicine Today**, [s. l.], v. 5, p. 525–531, 1999. Disponível em: https://ac.els-cdn.com/S1357431099015993/1-s2.0-S1357431099015993-main.pdf?_tid=db9d995e-d5dc-42cd-ab04-7039e9b71088&acdnat=1547124621_df7aff77435e14e45615a3757aee06b6.

YUSUF, Salim *et al.* Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. **Lancet**, [s. l.], v. 366, p. 1640–1649, 2005.

ZACHAREWICZ, E.; HESSELINK, M. K.C.; SCHRAUWEN, P. Exercise counteracts lipotoxicity by improving lipid turnover and lipid droplet quality. **Journal of Internal Medicine**, [s. l.], v. 284, n. 5, p. 505–518, 2018.

ZEYDA, Maximilian; STULNIG, Thomas M. Obesity, inflammation, and insulin resistance - A mini-review. **Gerontology**, [s. l.], v. 55, n. 4, p. 379–386, 2009.

ZINKER, Bradley A; BRITZ, Karen; BROOKS, George A. Effects of a 36-hour fast on human endurance and substrate utilization. **J Appl Physiol**, [s. l.], v. 69, n. 5, p. 1849–1855, 1990.

ZOUHAL, Hassane *et al.* Exercise Training and Fasting: Current Insights. **Open Access Journal of Sports Medicine**, [s. l.], v. 11, p. 1–28, 2020.

ANEXO I

Comprovante de aprovação pelo Comitê de Ética no Uso de Animais

Prezado Pesquisador ANAPAUOLA SOMMER VINAGRE,

Informamos que o projeto de pesquisa Efeitos de uma sessão aguda de exercício aeróbio, realizado em estado alimentado ou jejum, sobre marcadores metabólicos e expressão de genes relacionados ao metabolismo lipídico em ratos wistar machos, encaminhado para análise em 26/12/2017, foi aprovado no(a) Comissão de Ética no Uso de Animais com o seguinte parecer:

A Comissão De Ética No Uso De Animais aprovou o trabalho "34271 - Efeitos de uma sessão aguda de exercício aeróbio, realizado em estado alimentado ou jejum, sobre marcadores metabólicos e expressão de genes relacionados ao metabolismo lipídico em ratos wistar machos" em reunião realizada em 05/03/2018 - Sala 330 do Anexo I da Reitoria - Campus Centro - Porto Alegre - RS, em seus aspectos éticos e metodológicos, para a utilização de um total de 160 ratos Wistar, machos, com 60 dias de idade, provenientes do Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL) da UFRGS, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

CEUA/UFRGS

Atenciosamente,

Comissão de Ética no Uso de Animais