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Impacto do Treinamento Intervalado de Alta Intensidade (HIIT) e Treinamento Contínuo de Moderada Intensidade na Capacidade Funcional, Modulação do Perfil Inflamatório e Marcadores de Estresse Oxidativo em Ratos Submetidos ao Infarto Agudo do Miocárdio

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“In life, we should never give up, surrendering to mediocrity, but rather we must get out of the ‘gray zone’ where everything is habit and passive resignation. We must cultivate the courage to rebel.”

“Above all, don’t fear difficult moments. The best comes from them.”

Rita Levi-Montalcini

RESUMO

Introdução: o infarto agudo do miocárdio (IAM) gera, através da interrupção do fluxo sanguíneo para o coração, a morte de cardiomiócitos, desencadeando a ativação de citocinas pró-inflamatórias e a produção de espécies reativas de oxigênio (ERO) no tecido cardíaco. O exercício físico é considerado um tratamento não-farmacológico adjuvante para pacientes com doenças cardiovasculares, atuando como agente antioxidante e anti-inflamatório, além de aumentar a capacidade funcional desses indivíduos. O treinamento contínuo de moderada intensidade (TCMI) é o principal programa de treinamento para a reabilitação de pacientes diagnosticados com doenças cardiovasculares, no entanto, o treinamento intervalado de alta intensidade (HIIT) tem surgido como alternativa mais eficaz no tratamento dessas doenças, gerando benefícios adicionais para esses pacientes. Contudo, o efeito do HIIT em biomarcadores de danos e reparos na fisiopatologia das doenças cardiovasculares ainda é pouco estudado, sendo de fundamental importância para elucidar mecanismos adaptativos do exercício físico associado ao tratamento dessas patologias. **Objetivos:** avaliar o efeito do HIIT e do TCMI na capacidade funcional, perfil inflamatório e marcadores de estresse oxidativo em ratos submetidos ao IAM. **Metodologia:** dezoito ratos Wistar machos (280g a 350g, ≈ 90 dias) foram submetidos ao IAM através da ligadura da artéria coronária descendente esquerda. Os animais realizaram o teste de esforço máximo (TEM) em esteira rolante em três momentos (cinco semanas após o IAM, na quarta e na oitava semana após o início dos protocolos de exercício) para a avaliação da capacidade funcional. Seis semanas após a indução do IAM, os animais foram alocados em três grupos experimentais: sedentário (SED; n=6), treinamento contínuo de moderada intensidade (TCMI; n=6) e treinamento intervalado de alta intensidade (HIIT; n=6). O protocolo de TCMI consistiu em 5 minutos de aquecimento (10 m/min) e 45 minutos de corrida (50-60% da velocidade máxima atingida no TEM), totalizando 50 min de exercício. O protocolo de HIIT consistiu em 8 minutos de aquecimento (10 m/min) e 10 tiros de 1 minuto (85-90% da velocidade máxima atingida no TEM) intercalados com 2 minutos (50-60% da velocidade máxima atingida no TEM) de corrida, totalizando 38 minutos de exercício. Ambos os protocolos foram realizados 5 vezes por semana, durante 8 semanas. Após o término dos protocolos de exercício, os animais foram eutanasiados e os tecidos armazenados para análises bioquímicas subsequentes. **Resultados:** todos os grupos iniciaram os protocolos de treinamento com valores similares de distância (SED; 222,4 ± 104,3 vs TCMI; 181,8 ± 66,6 e HIIT; 196,1 ± 70,4 m; P>0,05), tempo para a exaustão (SED; 730,3 ± 214,9 vs TCMI; 642,3 ± 159,3 e HIIT; 632,8 ± 159,3 s; P>0,05) e velocidade máxima (SED; 27,5 ± 6,9 vs TCMI; 25,0 ± 4,5 e HIIT; 27,5 ± 2,7 m/min; P>0,05) atingidos no TEM. Na quarta semana, tanto o grupo HIIT quanto o grupo TCMI foram capazes de aumentar a distância (SED; 190,7 ± 52,0 vs TCMI; 438,3 ± 100,1 e HIIT; 365,9 ± 63,7 m; P<0,01), tempo para exaustão (SED; 668,0 ± 129,1 vs TCMI; 1122,6 ± 154,5 e HIIT; 1006,5 ± 109,2 s; P<0,001) e velocidade máxima (SED; 25,8 ± 3,7 vs TCMI; 38,3 ± 4,0 e HIIT; 35,8 ± 3,7 m/min; P<0,001) quando comparado ao grupo SED. Na oitava semana, ambos os grupos treinados aumentaram os valores de distância (SED; 176,8 ± 38,4 vs TCMI; 522,2 ± 146,3 e HIIT; 367,58 ± 85,4 m; P<0,001), tempo para exaustão (SED; 639,3 ± 97,3 vs TCMI; 1236,6 ± 188,6 e HIIT; 1013,8 ± 149,1 s; P<0,001) e velocidade máxima (SED; 25,0 ± 3,1 vs TCMI; 41,6 ± 5,1 e HIIT; 35,8 ± 3,7 m/min; P<0,0001) quando comparado ao grupo SED. O grupo TCMI mostrou aumento da distância (P<0,01), tempo até a exaustão (P<0,05) e velocidade máxima ($P<0,05$) quando comparado ao grupo HIIT. Os níveis de IL-10 e TNF-α cardíacos não tiveram diferença entre os grupos, no entanto, a relação IL-10/TNF-α foi maior no grupo TCMI quando comparado ao grupo SED (SED; 1,9 ± 0,3 vs TCMI; 2,8 ± 0,2 e HIIT; 2,4 ± 0,6 pg/mg de proteína; $P<0,01$). No músculo gastrocnêmio, o nível de IL-10 foi maior (SED; 7,0 ± 1,0 vs TCMI; 12,9 ± 1,5 e HIIT; 12,7 ± 4,5 pg/mg de proteína; $P<0,01$), assim como a relação IL-10/TNF-α (SED; 2,1 ± 0,5 vs TCMI; 6,6 ± 1,3 e HIIT; 5,6 ± 1,4 pg/mg de proteína; $P<0.001$) em ambos os grupos treinados quando comparado ao grupo SED. O nível de TNF-α foi menor nos grupos treinados quando comparado ao grupo SED (SED; 3,4 ± 0,9 vs TCMI; 2,0 ± 0,5 e HIIT; 2,0 ± 0,5 pg/mg de proteína; $P<0,05$). O valor de 2',7'-diclorofluoresceína (DCF) foi menor no tecido cardíaco no grupo HIIT quando comparado ao grupo SED (SED; 929,2 ± 31,2 vs TCMI; 890,4 ± 45,8 e HIIT; 853,7 ± 48,1 unidades de fluorescência/mg de proteína; $P<0,05$). No músculo gastrocnêmio, o grupo HIIT demonstrou menor nível de DCF comparado ao grupo TCMI (SED; 795,1 ± 48,4 vs TCMI; 839,4 ± 90,8 e HIIT; 705,5 ± 99,2 unidades de fluorescência/mg de proteína; $P<0,05$). O nível de malondialdeído (MDA) no tecido cardíaco foi menor em ambos os grupos treinados quando comparado ao grupo SED (SED; 1054,0 ± 305,7 vs TCMI; 760,5 ± 130,4 e HIIT; 500,2 ± 163,3 nmol/mg de proteína; $P<0,05$). A concentração de glutationa total foi maior no tecido cardíaco (SED; 97,5 ± 11,2 vs TCMI; 137,8 ± 24,8 e HIIT; 125,7 ± 21,2 µmol/mg de proteína; $P<0,05$) e no músculo gastrocnêmio (SED; 49,1 ± 9,3 vs TCMI; 68,4 ± 10,8 e HIIT; 76,3 ± 9,6 µmol/mg de proteína; $P<0,05$) em ambos os grupos treinados quando comparado ao grupo SED. O valor da superóxido dismutase (SOD) no tecido cardíaco e no músculo gastrocnêmio não tiveram diferença entre os grupos ($P>0,05$). **Conclusão:** o presente estudo demonstrou que 8 semanas de treinamento físico foi capaz de aumentar a capacidade funcional através do aumento da distância, tempo para exaustão e velocidade máxima encontrados no TEM. No entanto, o treinamento contínuo de moderada intensidade foi capaz de melhorar as variáveis de capacidade funcional de forma mais proeminente do que o treinamento intervalado de alta intensidade. Ambos os protocolos de treinamento foram capazes de modular positivamente as citocinas pró-inflamatórias, bem como aumentar as concentrações de citocinas anti-inflamatórias no músculo esquelético, embora essa melhora não tenha ocorrido no músculo cardíaco. Os treinamentos contínuo e intervalado demonstraram concentrações maiores de antioxidante no músculo cardíaco e esquelético, entretanto, o treinamento intervalado de alta intensidade demonstrou valores de biomarcadores de dano oxidativo mais baixos tanto no músculo esquelético quanto no músculo cardíaco comparado ao treinamento contínuo de moderada intensidade.

Palavras Chaves: Infarto Agudo do Miocárdio. Exercício Físico. HIIT. Inflamação. Estresse Oxidativo.

ABSTRACT

Introduction: acute myocardial infarction (AMI) generates, through the heart blood flow interruption, the cardiomyocytes death, triggering the proinflammatory cytokines activation and reactive oxygen species (ROS) production in the cardiac tissue. Physical exercise is considered an adjuvant non-pharmacological treatment for patients with cardiovascular disease, acting as an antioxidant and anti-inflammatory agent, also to increase the functional capacity of these individuals. The moderate intensity continuous training (MICT) is the main training program for the rehabilitation of patients diagnosed with cardiovascular disease, however, the high intensity interval training (HIIT) has emerged as more effective alternative in these diseases treatment, generating additional benefits for these patients. Nevertheless, the effect of HIIT on damage and repair biomarkers in the cardiovascular disease pathophysiology is still poorly studied, and it has a fundamental importance to elucidate adaptive physical exercise mechanisms associated with the treatment of these diseases. **Aims:** to evaluate the effect of HIIT and MICT on the functional capacity, inflammatory profile and oxidative stress markers of myocardial-infarcted rats. **Methods:** eighteen male Wistar rats (280g to 350g, ≈ 90 days) were submitted to AMI through ligation of the left descending coronary artery. The animals perform the maximal treadmill exercise test (MET) at three moments (five weeks after MI, on the fourth and eighth weeks after the beginning of exercise protocols) to assess functional capacity. Six weeks after AMI induction, the animals were allocated into three experimental groups: sedentary (SED; n = 6), moderate intensity continuous training (MICT; n = 6) and high intensity interval training (HIIT; n = 6). The MICT protocol was consisted in 5 min of warming-up (10m/min) and 45 min of running (50-60% of maximal velocity presented in MET), totalizing 50 min of exercise. The HIIT protocol was consisted in 8 min of warming-up (10m/min) and ten 1 min sprints (85-90% of the maximal velocity presented in MET) interleaved with 2 min (50-60% of the maximal velocity presented in the test) of running, totalizing 38 min of exercise. Both protocols were performed 5 times a week for 8 weeks. After the end of the exercise protocols, the animals were euthanized and the tissues stored for subsequent biochemical analyzes. **Results:** all groups started training protocols with similar distance (SED; 222.4 ± 104.3 vs MICT; 181.8 ± 66.6 and HIIT; 196.1 ± 70.4 m; $P>0.05$), time to exhaust (SED; 730.3 ± 214.9 vs MICT; 642.3 ± 159.3 and HIIT; 632.8 ± 159.3 s; $P>0.05$) and maximal velocity (SED; 27.5 ± 6.9 vs MICT; 25.0 ± 4.5 and HIIT; 27.5 ± 2.7 m/min; $P>0.05$) reached in MET. In the fourth week, both HIIT and MICT were able to increase the distance (SED; 190.7 ± 52.0 vs MICT; 438.3 ± 100.1 and HIIT; 365.9 ± 63.7 m; $P<0.01$), time to exhaustion (SED; 668.0 ± 129.1 vs MICT; 1122.6 ± 154.5 and HIIT; 1006.5 ± 109.2 s; $P<0.001$) and maximal velocity (SED; 25.8 ± 3.7 vs MICT; 38.3 ± 4.0 and HIIT; 35.8 ± 3.7 m/min; $P<0.001$) when compared to the SED group. In the eighth week, both trained groups increased the distance values (SED; 176.8 ± 38.4 vs MICT; 522.2 ± 146.3 and HIIT; 367.58 ± 85.4 m; $P<0.001$), time to exhaustion (SED; 639.3 ± 97.3 vs MICT; 1236.6 ± 188.6 and HIIT; 1013.8 ± 149.1 s; $P<0.0001$) and maximal velocity (SED; 25.0 ± 3.1 vs MICT; 41.6 ± 5.1 and HIIT; 35.8 ± 3.7 m/min; $P<0.0001$) when compared to the SED group. The MICT group showed an increase in distance ($P<0.01$), time to exhaustion ($P<0.05$) and maximal velocity ($P<0.05$) when compared with the HIIT group. The heart IL-10 and TNF-α levels did not differ between groups, however, the IL-10/TNF-α ratio was higher in the MICT group when compared to the SED group (SED; 1.9 ± 0.3 vs

MICT; 2.8 ± 0.2 and HIIT; 2.4 ± 0.6 pg/mg of protein; *P*<0.01). In the gastrocnemius muscle, IL-10 level was higher (SED; 7.0 ± 1.0 vs MICT; 12.9 ± 1.5 and HIIT; 12.7 ± 4.5 pg/mg of protein; *P*<0.01), as well the IL-10/TNF-α ratio (SED; 2.1 ± 0.5 vs MICT; 6.6 ± 1.3 and HIIT; 5.6 ± 1.4 pg/mg of protein; *P*<0.001) in both trained groups when compared to the SED group. The TNF-α level was lower in the exercise groups when compared to the SED group (SED; 3.4 ± 0.9 vs MICT; 2.0 ± 0.5 and HIIT; 2.0 ± 0.5 pg/mg of protein; *P*<0.05). The value of 2',7'-dichlorofluorescein (DCF) was lower in cardiac tissue in the HIIT group when compared to the SED group (SED; 929.2 ± 31.2 vs MICT; 890.4 ± 45.8 and HIIT; 853.7 ± 48.1 fluorescence units/mg of protein; *P*<0.05). In the gastrocnemius muscle, the HIIT group showed lower DCF concentration compared to the MICT (SED; 795.1 ± 48.4 vs MICT; 839.4 ± 90.8 and HIIT; 705.5 ± 99.2 fluorescence units/mg of protein; *P*<0.05). The malondialdehyde (MDA) level in cardiac tissue was lower in both trained groups compared to the SED group (SED; 1054.0 ± 305.7 vs MICT; 760.5 ± 130.4 and HIIT; 500.2 ± 163.3 nmol/mg of protein; *P*<0.05). The total glutathione concentration was higher in cardiac tissue (SED; 97.5 ± 11.2 vs MICT; 137.8 ± 24.8 and HIIT; 125.7 ± 21.2 µmol/mg of protein; *P*<0.05) and in gastrocnemius muscle (SED; 49.1 ± 9.3 vs MICT; 68.4 ± 10.8 and HIIT; 76.3 ± 9.6 µmol/mg of protein; *P*<0.05) in both trained groups when compared to the SED group. The superoxide dismutase (SOD) value in cardiac tissue and gastrocnemius muscle did not differ between groups (*P*>0.05). **Conclusion:** the present study demonstrated that 8 weeks of physical training was able to increase functional capacity by increasing the distance, time to exhaustion and maximal velocity found in MET. However, moderate-intensity continuous training was able to enhance functional capacity variables more prominently than high intensity interval training. Both training protocols were able to modulate positively pro-inflammatory cytokines, as well as increasing anti-inflammatory cytokines concentrations in skeletal muscle, although this improvement did not occurred in cardiac muscle. Continuous and interval training demonstrated higher antioxidant concentrations in cardiac and skeletal muscle, however, high intensity interval training demonstrated lower oxidative damage biomarkers values in both skeletal muscle and cardiac muscle compared to moderate-intensity continuous training.

Keywords: Acute Myocardial Infarction. Physical Exercise. HIIT. Inflamation. Oxidative Stress.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

AMPK	5' proteína cinase ativada por AMP
Ca^{2+}	Íon cálcio
CTE	Cadeia de transporte de elétrons
DAC	Doença arterial coronariana
DAMP	Padrões moleculares associados a danos
DC	Débito cardíaco
EF	Exercício físico
ERO	Espécies reativas de oxigênio
FCR	Frequência cardíaca de reserva
FCmáx	Frequência cardíaca máxima
FE	Fração de ejeção
H_2O_2	Peróxido de hidrogênio
HIIT	Treinamento intervalado de alta intensidade (high intensity interval training)
HSP	Proteínas de choque térmico
IAM	Infarto agudo do miocárdio
IFN-γ	Interferon γ
IGF-1	Fator de crescimento semelhante à insulina tipo 1
IL-1	Interleucina 1
IL-1β	Interleucina 1β
IL-ra	Antagonista do receptor de interleucina 1
IL-6	Interleucina 6
IL-10	Interleucina 10
IL-18	Interleucina 18
iNOS	Óxido nítrico síntase indutível
LDL	Lipoproteína de baixa densidade
MMP	Metaloproteinase de matriz
NADH	Nicotinamida adenina dinucleotídeo
NF-κβ	Fator nuclear κ β
NOS	Óxido nítrico sintase
Nox	NADPH oxidase
NK	Célula *natural killer*

Nrf2	Fator nuclaer eritróide 2 relacionado ao fator 2
O_2	Molécula de oxigênio
O_2-$^{\bullet}$	Ânion radical superóxido
$HO^{\bullet}$	Radical hidroxila
TGF-β	Fator de crescimento transformador β
TNF-α	Fator de necrose tumoral α
p38MAPK	p38 proteína cinase ativada por mitógeno
PGC1-α	Coativador-1α do receptor ativado por proliferador de peroxissimo
SERCA	Ca^{2+}-ATPase do retículo sarcoplasmático
SOD	Superóxido dismutase
SOD-Cu	Cobre-superóxido dismutase
SOD-Mn	Manganês-superóxido dismutase
VE	Ventrículo esquerdo
$\dot{V}O_{2máx}$	Consumo máximo de oxigênio

SUMÁRIO

1 INTRODUÇÃO

As doenças cardiovasculares são a maior causa de mortes no mundo, levando mais de 17 milhões de pessoas a óbito somente em 2016 (WHO, 2016). No Brasil, somente em 2018, mais de 380 mil pessoas morreram devido às doenças cardiovasculares. O Infarto Agudo do Miocárdio (IAM) e outras doenças isquêmicas do coração são a principal causa de mortes por doenças cardiovasculares no Brasil (SBC, 2019).

O evento do IAM decorre da interrupção do fluxo sanguíneo através das artérias coronárias para o leito miocárdico, causando isquemia e morte celular (VAFAIE, 2016). A partir da necrose tecidual, há a liberação de padrões moleculares associados a danos (DAMP) pelas células, aumentando assim a produção de citocinas pelas células cardíacas e do sistema imune (ONG et al., 2018). Ademais, o aumento da produção de espécies reativas de oxigênio (ERO) pelas células lesadas gera interações entre biomoléculas celulares, causando danos ao DNA, peroxidação lipídica e de proteínas (SALLAM; LAHER, 2016). Esse cenário de aumento de agentes inflamatórios e de produção de ERO no músculo cardíaco, associado às alterações estruturais e hemodinâmicas causadas pela perda de cardiomiócitos, trará consequências à função cardíaca, comprometendo a capacidade funcional do indivíduo (WEBB et al., 2006).

Visto a importância de se encontrar terapias adjuvantes para o tratamento do quadro clínico e funcional desses pacientes, o exercício físico (EF) regular se torna uma ferramenta muito importante para esse manejo. O EF faz parte do programa de reabilitação de pacientes diagnosticados com doenças cardíacas, melhorando o seu desempenho funcional e qualidade de vida (LAVIE; ARENA; FRANKLIN, 2016). Ademais, o EF tem sido utilizado no tratamento de inúmeras doenças ligadas a desordens inflamatórias. Seu mecanismo de ação compreende a indução um padrão anti-inflamatório, uma vez que seu efeito pode ser imunorregulador, alterando a síntese de diversas citocinas e outros marcadores inflamatórios (PEDERSEN, 2017). Além do seu potencial anti-inflamatório, o EF tem papel antioxidante através das respostas adaptativas celulares, aumentando a resistência à danos oxidativos gerados pela exposição às ERO (MATTSON, 2008; PEAKE et al., 2015).

Atualmente, o treinamento contínuo de moderada intensidade é a modalidade de EF mais utilizado para a reabilitação cardiovascular (PIEPOLI et al., 2014). No entanto, novas evidências têm demonstrado a eficácia do treinamento intervalado de alta intensidade (da sigla em inglês HIIT – high intensity interval training) no tratamento de pacientes com doenças cardiovasculares, podendo ser realizado com segurança e gerando melhorias na fisiopatologia da doença, capacidade funcional e consumo máximo de oxigênio ($\dot{V}O_{2máx}$) (QUINDRY et al., 2019).

A intensidade, o volume e a frequência de treinamento físico aeróbico para pacientes com doenças cardiovasculares já está bem estabelecido na literatura. No entanto, ainda hoje, não se tem respostas concretas acerca da dose-resposta de exercício físico sobre parâmetros bioquímicos e cardiometabólicos e quanto o treinamento intenso pode afetar mecanismos celulares de lesão e/ou proteção, não somente no tecido cardíaco, mas em outros tecidos nos quais esses agentes poderiam atuar.

O presente trabalho se insere na área de doenças crônicas e investigou o potencial do EF como regulador de processo inflamatório crônico, estresse oxidativo e melhora da capacidade funcional em ratos Wistar submetidos à cirurgia de infarto do miocárdio. Portanto, a realização deste estudo justifica-se pela necessidade de se ampliar o conhecimento acerca da modulação do estresse oxidativo e perfil inflamatório causado por ambos os treinamentos, para que, posteriormente, os resultados possam ajudar na prática clínica de reabilitação de pacientes pós-IAM.

2 REVISÃO DA LITERATURA

2.1 INFARTO AGUDO DO MIOCÁRDIO

2.1.1 Epidemiologia

As doenças cardiovasculares são a principal causa de mortes no mundo. De acordo com a Organização Mundial da Saúde, 17,9 milhões de pessoas morreram em 2016 por doenças do sistema cardiovascular, o que caracteriza 31% das mortes registradas no ano em todo o mundo. O IAM e o acidente vascular encefálico foram responsáveis por 85% de todas as mortes ocorridas por doenças cardiovasculares (WHO, 2016).

Nos Estados Unidos, entre 2013 e 2016, a prevalência de doenças cardiovasculares em adultos acima de 20 anos foi de 48%, cerca de 121 milhões de pessoas. A prevalência de doença arterial coronariana (DAC) no mesmo período foi de 6,7%, cerca de 18,2 milhões de americanos. Em 2016, 830 mil pessoas foram a óbito decorrente de algum evento cardiovascular e 110 mil pessoas por IAM (BENJAMIN et al., 2019). Na Europa, o número de mortes por doenças cardiovasculares chega a 4 milhões, 45% do total de mortes no continente, sendo 1,8 milhões por DAC (TOWNSEND et al., 2015).

No Brasil, assim como em outros países, as doenças cardiovasculares são a principal causa de mortes. De acordo com a Sociedade Brasileira de Cardiologia, mais de 4,5 milhões de pessoas morreram por doenças cardiovasculares de 2004 a 2017, sendo 383.961 somente em 2017. Quando se faz um comparativo do número de mortes de 2004 a 2017, se encontra um aumento gradativo, chegando em 35%. As doenças isquêmicas do coração, como o IAM, são responsáveis por cerca de 30% de todas as mortes por doenças cardiovasculares, totalizando mais de um milhão de mortes de 2004 a 2017 (SBC, 2019).

2.1.2 Etiologia

O IAM é definido como um evento isquêmico que ocorre no miocárdio devido à interrupção do fluxo sanguíneo para o local, o que ocasiona a morte de cardiomiócitos e necrose miocárdica. A isquemia ocorre principalmente pela ruptura, fissura ou erosão da placa aterosclerótica, resultando em um trombo que bloqueará o lúmen do vaso, interrompendo a irrigação do coração (ANDERSON; MORROW, 2017; THYGESEN et al., 2019; VAFAIE, 2016).

A DAC é uma patologia crônica com efeito progressivo ao longo dos anos, sendo o IAM o resultante da piora desse processo. Os fatores de risco para o desenvolvimento de um evento isquêmico de IAM podem ser divididos em não-modificáveis: gênero, idade (homens acima de 45 anos e mulheres acima dos 55 anos) e histórico familiar; fatores de risco modificáveis: dieta rica em gordura, tabagismo, ingestão excessiva de álcool, obesidade, hipertensão arterial, hiperglicemia, dislipidemia e inatividade física; fatores de risco emergentes: níveis sanguíneos aumentados de proteína C-reativa, fibrinogênio, homocisteína e lipoproteína (a), além de calcificações nas artérias coronárias (BOATENG; SANBORN, 2013; MACK; GOPAL, 2016).

Os sintomas físicos relacionados com o IAM envolvem angina, dispneia, fadiga e diaforese, podendo ser associados com náuseas, tontura e taquicardia (ANDERSON; MORROW, 2017). Para o diagnóstico clínico de IAM são necessários alguns critérios, tais como valores sanguíneos de troponina (C e I) aumentados, mudança característica no segmento ST e onda Q no eletrocardiograma, exame de imagem mostrando hipo ou acinesia na parede ventricular e trombo intracoronário evidenciado por angioplastia (ANDERSON; MORROW, 2017; VAFAIE, 2016).

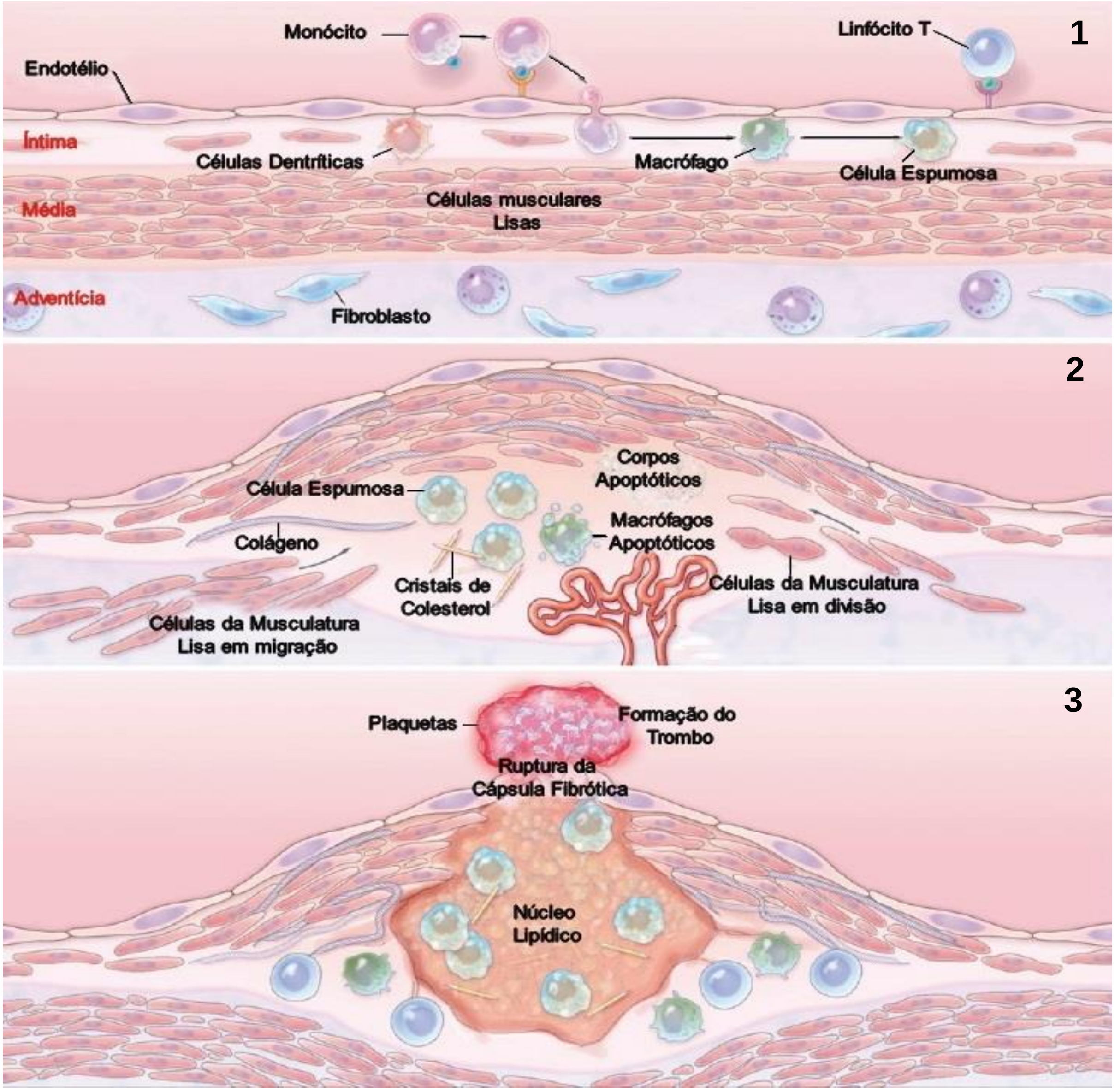
O mecanismo primário para o desenvolvimento da DAC é a exposição progressiva a fatores de risco e consequente processo de aterogênese (GIMBRONE; GARCÍA-CARDEÑA, 2013). Esse processo é desencadeado por alterações no tecido endotelial, onde há modificações nos padrões de síntese e secreção de mediadores pelo endotélio vascular. O endotélio, uma camada de células localizada na parte mais interna do vaso sanguíneo, entre o lúmen do vaso e a musculatura lisa, tem função de secretar substâncias que agem de forma parácrina, além de manter a homeostase e a função vasomotora. A síntese de mediadores pelas células do endotélio é responsável por modular a resposta das células da musculatura lisa adjacente, o que interfere diretamente na complacência vascular. O endotélio é responsável pela produção tanto de fatores vasodilatadores (óxido nítrico e prostaciclina) como vasoconstritores (endotelina, prostaglandinas, leucotrienos, angiotensina II), o que em situações não-patológicas geram um estado homeostático. No entanto, em situações de disfunção ou doença, como no caso da aterosclerose, esse mecanismo de ineficiência vasodilatadora compromete o tônus vascular (HEUSCH et al., 2014).

Fatores de risco como fumo, dislipidemia e hipertensão arterial sistêmica desencadeiam variações em processos hemodinâmicos, oxidativos e/ou bioquímicos no sistema cardiovascular, o que, associado à inflamação característica desses processos, é capaz de aumentar a permeabilidade do endotélio, promovendo a infiltração de monócitos e partículas de lipoproteínas de baixa densidade (LDL). Ademias, ocorre modificações no tônus vasomotor, disfunções trombóticas, migração e proliferação de células da musculatura lisa (MATSUZAWA et al., 2015; NABEL; BRAUNWALD, 2012; SANTOS-GALLEGO; PICATOSTE; BADIMÓN, 2014).

O vaso sanguíneo, através do fluxo laminar, está suscetível a diversas forças hemodinâmicas, tais como estresse de cisalhamento e pressão hidrostática. Em condições normais, esse fluxo desencadeia a produção de óxido nítrico pela óxido nítrico sintase endotelial, aumentando a dilatação do vaso (GIMBRONE; GARCÍA-CARDEÑA, 2013). Já em condições patológicas, o fluxo laminar irregular e baixo estresse de cisalhamento, associado ao aumento e deposição de moléculas de apolipoproteína B na camada íntima do vaso, faz com que haja a síntese de moléculas de adesão intercelular, moléculas de adesão vascular e E-Selectina nas células endoteliais, gerando uma atração de monócitos para a região subendotelial. Na túnica vascular, os macrófagos fagocitam as lipoproteínas infiltradas no vaso formando células espumosas (*foam cells*), além de promover a síntese de mediadores inflamatórios que aumentarão e sustentarão o processo de inflamação local. O endotélio lesado gera um aumento de ERO, o que, juntamente com o processo de inflamação instalado, gera uma migração de células musculares lisa e deposição de colágeno no local, resultando na formação da placa aterosclerótica (Figura 1) (EELEN et al., 2018; HEUSCH et al., 2014; SWIRSKI; NAHRENDORF, 2013)

Ademais, os macrófagos infiltrados na túnica do vaso são capazes de produzir enzimas proteolíticas chamadas de metaloproteinases de matriz (MMP) que poderão degradar a camada fibrosa da placa, tornando-a mais fina e suscetível ao rompimento. Quando essa camada se rompe, todo o material da placa é extravasado para o sangue e a cascata pró-trombótica é desencadeada. Os fatores de coagulação sanguínea entram em contato com os mediadores pró-coagulantes secretados pelos macrófagos contidos na placa, formando o trombo que obstruirá a luz do vaso, caracterizando o IAM (HEUSCH et al., 2014).

Figura 1 – Processo fisiopatológico de formação da placa aterosclerótica.



Fonte: Adaptado de Nabel e Braunwald, 2012.

2.1.3 Fisiopatologia

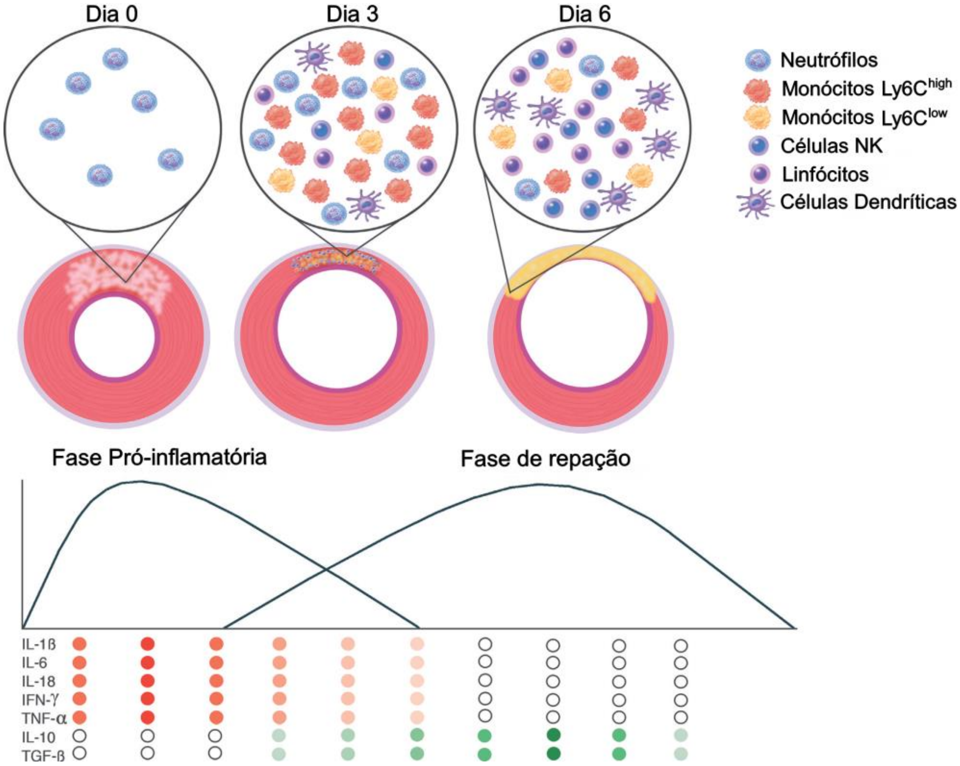
O mecanismo inicial para o evento de IAM é o desequilíbrio entre a oferta e a demanda de oxigênio para a musculatura cardíaca. As primeiras mudanças estruturais encontradas nos cardiomiócitos acontecem em torno de 10 a 15 minutos após o início do IAM e compreendem a diminuição do glicogênio celular, relaxamento das miofibrilas, rompimento do sarcolema celular, hemorragia e infiltração de leucócitos. Nesse ponto, observa-se alterações mitocondriais progressivas a partir dos 10 primeiros minutos após a isquemia (HEUSCH; GERSH, 2017; THYGESEN et al., 2019).

A cascata de isquemia se inicia na oclusão coronária e o leito miocárdico irrigado pela artéria, torna-se isquêmico. As células do tecido miocárdico, a partir da interrupção do aporte de oxigênio, mudam seu metabolismo para anaeróbico, o que as torna menos eficientes na manutenção da produção de ATP, acumulando assim, a partir do metabolismo celular, ácido lático. A diminuição de ATP atrelada à acidose intracelular desregula a homeostase iônica, levando ao aumento descontrolado de Ca^{2+}, digestão do citoesqueleto e sarcolema e produção de ERO, ocasionando morte celular (HEUSCH; GERSH, 2017; MCALINDON et al., 2014).

2.1.3.1 Inflamação

O processo de inflamação no tecido cardíaco decorrente do IAM possui duas fases distintas. A primeira, que começa logo após o evento e se estende por até três dias, tem como principal objetivo a retirada de restos celulares decorrentes da morte dos cardiomiócitos. A segunda, que começa no terceiro dia e se estende até o sexto, tem como característica a contenção e supressão da resposta pró-inflamatória anterior, gerando uma fase de reparação tecidual (Figura 2) (WESTMAN et al., 2016).

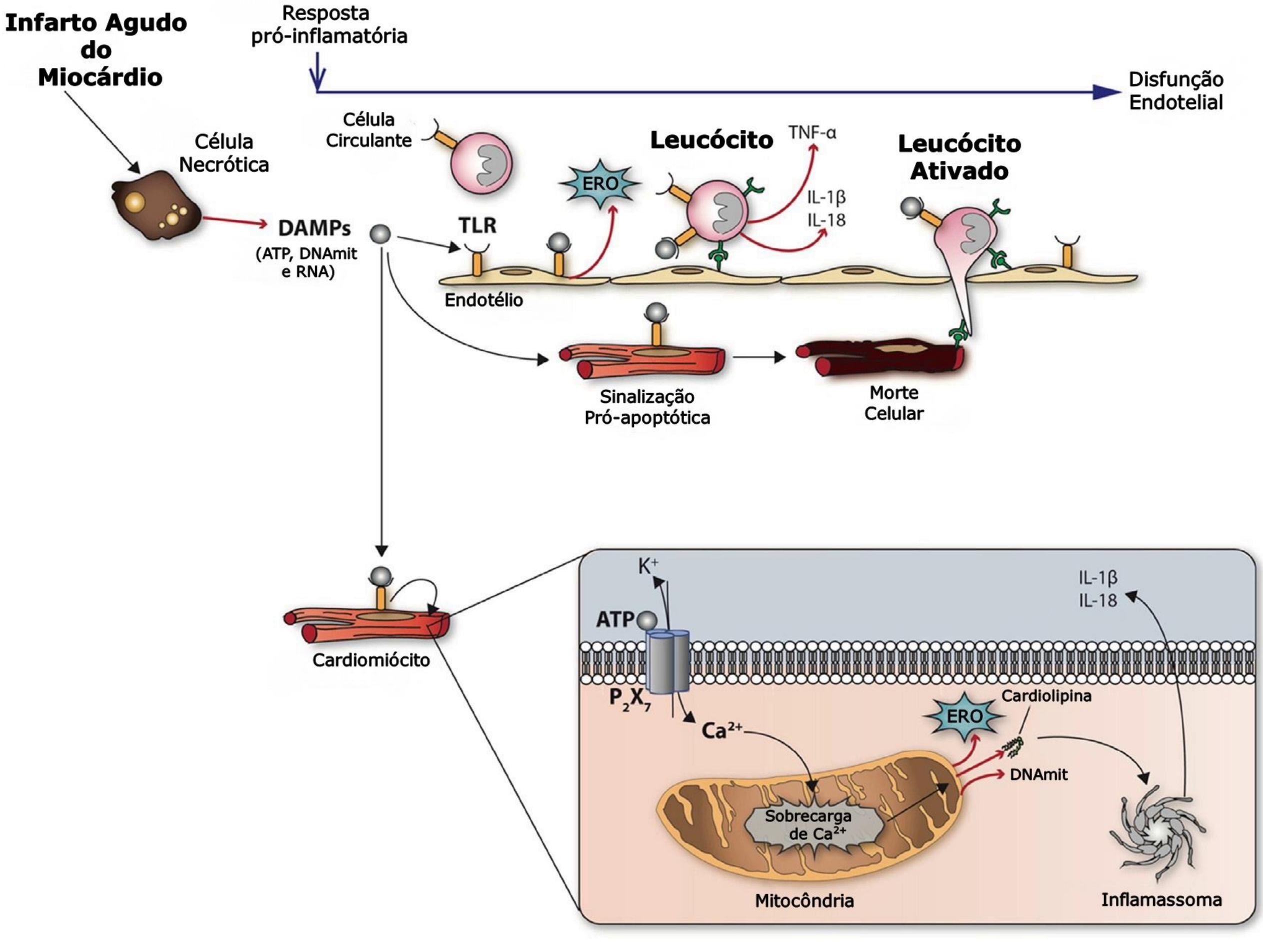
Figura 2 – Representação das respostas de citocinas de acordo com as fases de inflamação após o IAM no músculo cardíaco.



Fonte: Adaptado de Westman et al., 2016.

A morte dos cardiomiócitos é a principal via de sinalização para o processo inflamatório inicial, visto que há liberação de DAMP pelas células necrosadas. Os DAMP são produtos liberados por células mortas ou tecidos lesados que podem incluir ATP, DNA mitocondrial, RNA de cadeia dupla, fibronectina, ácido úrico, além de outros componentes celulares. A zona adjacente ao dano também pode ser capaz de desencadear uma reação inflamatória, sendo capaz de produzir e secretar citocinas pró-inflamatórias. As moléculas de DAMP, ao se ligarem aos chamados receptores do tipo toll (uma família de receptores de reconhecimento de padrões) existentes nas membranas de células imunes, anunciam que o tecido foi lesado, o que gera uma cascata intracelular de ativação através do fator nuclear κ β (NF-κβ), atuando na liberação de citocinas pró-inflamatórias (Figura 3) (ONG et al., 2018; WESTMAN et al., 2016).

Figura 3 – Processo de produção de citocinas pró-inflamatórias decorrente da ativação de leucócitos e cardiomiócitos após o evento do IAM.



Fonte: Adaptado de Ong et al., 2018.

Outro fator que desencadeia a formação de citocinas pró-inflamatórias é a ativação dos inflamassomas (plataformas proteicas intracelulares com receptores similares ao domínio de oligomerização ligante de nucleotídeo que estão associados à uma molécula de caspase-1), estimuladas pelos DAMP. A ativação da caspase-1 faz com que a célula secrete interleucina 1 β (IL-1β) e interleucina 18 (IL-18) que estavam em suas formas inativas, gerando mais inflamação e recrutamento de células para o local da lesão (Figura 3) (ONG et al., 2018; WESTMAN et al., 2016).

Os neutrófilos são o primeiro tipo de célula de defesa a chegar no local da lesão, visto que tem a expressão de receptores de membrana para o reconhecimento de DAMP aumentada (LATET et al., 2015). Eles agem fagocitando os detritos celulares resultantes da morte miocitária, degradando a matriz celular através da secreção de MMP, além de secretar fatores quimioatrativos de monócitos. As células *natural killer* (NK) e linfócitos T também são atraídos para o local da lesão, secretando fator de necrose tumoral α (TNF-α), IL-1β e interferon γ (IFN-γ) (WESTMAN et al., 2016).

Os monócitos possuem fenótipos diferentes e atuam, dependendo da fase de inflamação decorrente do IAM, de maneiras antagônicas. No modelo animal, o fenótipo inicial (Ly6C^{high}) possui a função de síntese de mediadores pró-inflamatórios, sendo atraídos para o local e secretando citocinas pró-inflamatórias como interleucina 1 (IL-1), Interleucina 6 (IL-6), TNF-α e MMP, além de fagocitar cardiomiócitos em processo de apoptose/necrose, neutrófilos e outros detritos envolvidos na morte celular (LIU et al., 2017; NAHRENDORF; PITTET; SWIRSKI, 2010; PRABHU; FRANGOGIANNIS, 2016). Os monócitos também são capazes de agir na zona adjacente ao infarto, ativando fibroblastos, secretando MMP e fatores pró-apoptóticos (SWIRSKI; NAHRENDORF, 2013).

A IL-1 tem o papel de estimular e aumentar a expressão de moléculas de adesão nas células endoteliais, promover a síntese de quimiocinas pelas células mononucleares de defesa, modular a expressão gênica de fibroblastos e das MMP produzidas por eles, o que terá um papel muito importante no processo de cicatrização e remodelamento cardíaco (KAIN; PRABHU; HALADE, 2014). Já o TNF-α tem um papel inotrópico negativo gerado a partir do desacoplamento de receptores beta-adrenérgicos da adenilciclase através da inibição da proteína G. Ademais, o aumento de TNF-α está associado com hipertrofia de cardiomiócitos e com a ativação de MMP, o que gera remodelamento de matriz extracelular (FELDMAN et al., 2000).

A fase de reparação e cicatrização pós-infarto ocorre devido a supressão e contenção do processo inflamatório gerado na fase aguda e depende da produção de mediadores anti-inflamatórios e de sinalização intracelular para a inibição da resposta inata inflamatória (PRABHU; FRANGOGIANNIS, 2016). Nessa fase, os monócitos de reparação ($Ly6C^{low}$) atuam em mecanismos de angiogênese e síntese de matriz extracelular a partir da secreção do fator de crescimento do endotélio vascular e do fator de crescimento transformador β (TGF-β) (PRABHU; FRANGOGIANNIS, 2016; SWIRSKI; NAHRENDORF, 2013). Ademais, além da diminuição dos monócitos do tipo $Ly6C^{high}$ e aumento dos monócitos do tipo $Ly6C^{low}$, há um aumento de IL-10 produzida principalmente por células T reguladoras e células mononucleadas M2, agindo na supressão da síntese de IL-1, IL-6 e TNF-α. O TGF-β também age inibindo a expressão de citocinas e quimiocinas, além de aumentar a produção de inibidores teciduais de MMP, para que assim o processo de cicatrização possa ocorrer de forma efetiva (LIBBY; NAHRENDORF; SWIRSKI, 2016; WESTMAN et al., 2016).

Ainda na fase de reparo cardíaco, ocorre a diferenciação da população de fibroblastos cardíacos em miofibroblastos sintéticos através do aumento da produção de TGF-β. Esse fenótipo modificado desenvolve fibras de estresse com expressão de proteínas contráteis como a α-actina do músculo liso e a isoforma embrionária de miosina. Assim, após o evento isquêmico, fibroblastos intersticiais que sobrevivem ao evento, assim como os fribroblastos recrutados da zona adjacente ao evento devido ao aumento de citocinas pró-inflamatórias na fase inicial, podem se diferenciar em miofibloblastos que irão constituir a área cicatricial pós-infarto (PRABHU; FRANGOGIANNIS, 2016; SHINDE; FRANGOGIANNIS, 2014).

2.1.3.2 Estresse Oxidativo

O termo clássico e inicial data de 1985 e descreve o estresse oxidativo (EO) como um desequilíbrio entre a produção de agentes pró-oxidantes e uma diminuição das defesas antioxidantes do organismo, sendo a ação pró-oxidante predominante (SIES, 1985). Os danos aos componentes celulares, de um modo geral, se dão pelo aumento de ERO. Esse termo é utilizado para espécies químicas derivadas do oxigênio, podendo ser radicais livres (ânion radical superóxido e radical hidroxila) ou não-radicalares (peróxido de hidrogênio) (KIYUNA et al., 2018). Os elétrons desemparelhados das ERO interagem com qualquer macromolécula celular suscetível à doação, tais como lipídeos, proteínas e DNA, causando dano celular, déficit energético e a morte da célula (BAGATINI et al., 2011; KEVIN; NOVALIJA; STOWE, 2005; TSUTSUI; KINUGAWA; MATSUSHIMA, 2011). As principais fontes de geração de ERO são cadeia de transporte de elétrons a (CTE) na mitocôndria, as enzimas NADPH oxidase (Nox), xantina oxidase e óxido nítrico sintase (NOS) (INCALZA et al., 2018; MÜNZEL et al., 2017). As espécies reativas de nitrogênio, como NO, dióxido de nitrogênio, peroxinitrito e nitritos/nitratos, também representam um importante papel, visto que essas espécies também são capazes de interagir com macromoléculas e interferir na homeostase celular (SIES; BERNDT; JONES, 2017).

O processo de liberação de energia passa pela redução tetravalente da molécula de oxigênio (O_2) na CTE na mitocôndria. Pelo fato de o mecanismo aceptor final de elétrons não ser inteiramente eficiente, cerca de 0,2 a 4,0% acaba não sendo reduzido e vaza na forma de ânion radical superóxido (O_2-$^{\bullet}$) da CTE. Esse processo se torna mais acelerado em situações de maior demanda de O_2 e quando há lesões mitocondriais. Ademais, a própria mitocôndria e seu DNA estão mais suscetíveis à ação oxidativa e consequente dano devido à proximidade com a CTE, o que afeta a transcrição de RNA_{mit} e síntese proteica, resultando em um declínio de sua função (IDE et al., 2012; KEVIN; NOVALIJA; STOWE, 2005).

Os mecanismos de defesa celular compreendem agentes antioxidantes enzimáticos, sendo essas enzimas responsáveis por balancear os efeitos deletérios das ERO. A SOD é a mais eficiente enzima antioxidante celular e a primeira linha de defesa contra a produção de ERO (IGHODARO; AKINLOYE, 2018). O O_2-$^{\bullet}$ é dismutado na matriz mitocondrial pela manganês-superóxido dismutase (SOD-Mn) em peróxido de hidrogênio (H_2O_2), sendo o restante transportado através de canais iônicos para o citoplasma, para então ser dismutado pela cobre-superóxido dismutase (SOD-Cu). Posteriormente, o H_2O_2 que se encontra no citoplasma é reduzido à H_2O e O_2 pela ação da glutationa peroxidase ou pela catalase. No entanto, parte desse H_2O_2 reage com metais de transição, geralmente o Fe^{2+}/Fe^{3+}, a partir da reação de Fenton e Haber-Weiss, gerando radical hidroxila ($HO^{\bullet}$) (BAGATINI et al., 2011; SACK et al., 2018).

Vários processos biológicos são regulados a partir do estado redox celular, ativando fatores de transcrição e desencadeando respostas fisiológicas a diversos estímulos (SACK et al., 2018; SIES; BERNDT; JONES, 2017). As respostas adaptativas ao aumento da produção de ERO também estão ligadas à ativação gênica de fatores de transcrição. Para a manutenção da homeostase, o H_2O_2 pode ativar o fator nuclear eritróide 2 relacionado ao fator 2 (Nrf2) gerando mecanismos de síntese de defesas antioxidantes; assim como a ativação do NF-κβ, induzindo mecanismos de respostas inflamatórias. O acionamento desses e de diversos outros fatores de transcrição geram mecanismos adaptativos, além de proliferação, diferenciação, apoptose e necrose celular (CHEN; MALTAGLIATI, 2018; ESPINOSA-DIEZ et al., 2015; PACKER; CADENAS, 2007; RAY; HUANG; TSUJI, 2012; SIES, 2018).

O radical hidroxila é considerado o mais perigoso das ERO. Apesar da sua meia-vida ser bastante curta, possui um grande potencial de reatividade, causando danos em biomoléculas próximas ao local de sua produção. O dano na molécula de DNA ocorre a partir da reação com as bases nitrogenadas e com o esqueleto de desoxirribose da molécula (VALKO et al., 2007).

A partir dos danos celulares causado pelo IAM, há o aumento de mitocôndrias com função diminuída, resultante da redução da atividade do citocromo C-oxidase e danos peroxidativos na membrana mitocondrial, gerando assim um maior número ERO e consequente ciclo vicioso de dano oxidativo ao DNA, peroxidação de lipídeos e proteínas, além de morte celular (SALLAM; LAHER, 2016). No coração, o desequilíbrio dos mecanismos redox gera a oxidação de proteínas essenciais para o mecanismo de excitação-contração dos cardiomiócitos, tais como proteínas miofibrilares, canais iônicos e de liberação de cálcio, podendo assim afetar a contratilidade do miocárdio. As proteínas estruturais também podem sofrer modificações, sendo possível alterar sua estrutura e função, aumentando a sua susceptibilidade à proteólise (MISRA et al., 2009). As ERO também têm papel modulador de vias de sinalização intracelular, visto que sua produção está associada à ativação de receptores de superfície celular, tais como receptores de citocinas e receptores acoplados à proteína G. Ambos os receptores controlam vias de apoptose e estão envolvidos diretamente em desencadear a proliferação celular de cardiomiócitos, pois sua produção atua na fase G1 do ciclo celular (KEVIN; NOVALIJA; STOWE, 2005).

O aumento da síntese de TNF-α característico do evento isquêmico também é capaz de aumentantar a produção de ERO, causando da redução do potencial de membrana mitocondrial, diminuição de transcrição gênica mitocondrial e diminuição na produção de ATP, ocasionando assim apoptose celular. Ademais, o TNF-α é capaz de interferir em mecanismos de ação antioxidante através da depleção de nicotinamida adenina dinucleotídeo (NADH), importante coenzima responsável pelo transporte de elétrons, e a partir da redução de agentes antioxidantes (FERRARI et al., 2004). O aumento de ERO a partir de situações patológicas pode desencadear a maturação das IL-1β e IL-18 a partir do acionamento do NF-κβ e consequente mobilização dos inflamassomas (INCALZA et al., 2018).

O processo de apoptose celular envolvendo o aumento de ERO está relacionado principalmente com danos ao DNA, gerando ativação da proteína poli-ADP-ribose transferase e consequente depleção de NADH, o que resultará em diminuição de síntese de ATP e morte celular. Além disso, o aumento no EO gera a ativação da proteínas responsáveis pelo ciclo celular e ativação de genes responsáveis por iniciar o processo de apoptose celular (FERRARI et al., 2004).

2.1.3.3 Alterações Hemodinâmicas

Após o infarto, o coração sofre alterações estruturais e funcionais, principalmente um remodelamento do ventrículo esquerdo (VE), podendo evoluir para um quadro de insuficiência cardíaca. Essas alterações, tanto ao nível intracelular quanto extracelular, trarão consequências para a parede da cavidade e, com o decorrer do tempo, mudarão a arquitetura do ventrículo e os padrões de volume intraventriculares (WEBB et al., 2006). As alterações hemodinâmicas que decorrem após o IAM dependerão do local da lesão, do tamanho da área lesada e da quantidade de cardiomiócitos perdidos. Ademais, a estimulação do sistema nervoso simpático, do sistema renina-angiotensina-aldosterona e da secreção do peptídeo natriurético tipo-B também irão mediar as alterações pressóricas e de volume, não só no coração, mas também na circulação sistêmica (KAIN; PRABHU; HALADE, 2014).

As mudanças que ocorrem a curto prazo, logo nas primeiras horas e se estendem por dias, vão afetar tanto a área do miocárdio infartada quanto a não-infartada. O influxo de células do sistema imune para o local irá gerar uma degradação de colágeno interfibrilar para tentar manter a pressão e volume nos padrões sistólicos e diastólicos normais. Ao mesmo tempo, fibroblastos são atraídos para a região lesada com o intuito de formar um tecido cicatricial através da deposição de colágeno no local, tornando-a mais fina e alongada (BHATT; AMBROSY; VELAZQUEZ, 2017; GALLI; LOMBARDI, 2016; PFEFFER; BRAUNWALD, 1990). Os monócitos também podem degradar a área do miocárdio remanescente, podendo alterar a geometria ventricular. Eles são capazes de gerar proteólise na matriz de colágeno do miocárdio e ativação de fibroblastos, provendo fibrose intersticial, além de secretar fatores pró-apoptóticos que irão agir nos cardiomiócitos (SWIRSKI; NAHRENDORF, 2013).

Alguns mecanismos compensatórios a curto prazo irão manter a pressão e o volume dentro dos padrões normais mesmo com uma região acinética do ventrículo. O VE se dilata como mecanismo compensatório para melhorar a função da bomba através da própria distensão da parede e do aumento da atividade cronotrópica e inotrópica a partir do acionamento do sistema adrenérgico (através do mecanismo de Frank-Starling) (PFEFFER; BRAUNWALD, 1990; WESTMAN et al., 2016).

A longo prazo, em virtude da sobrecarga pressórica na área remanescente ao evento, ocorre hipertrofia dos cardiomiócitos e consequente dilatação da cavidade do VE. Devido às alterações da arquitetura do ventrículo e à perda de cardiomiócitos, o coração não consegue de forma efetiva bombear o sangue para a circulação sistêmica, tornando-se ineficiente na função de ejeção do sangue, o que pode ocasionar o aumento do volume sistólico e diastólico final do VE e diminuição da fração de ejeção (FE) desses indivíduos (COHN; FERRARI; SHARPE, 2000; PFEFFER; BRAUNWALD, 1990).

2.2 EXERCÍCIO FÍSICO

2.2.1 Efeitos sobre o Sistema Cardiovascular

O coração é considerado uma bomba cuja função é manter o aporte de oxigênio e nutrientes para si e para o restante do corpo. Para manter sua eficiência, ele é responsável por cerca de 20% do consumo de oxigênio, cujo destino é um complexo mecanismo metabólico e contrátil responsável pela manutenção do fluxo, mesmo em condições de exercício, em que a demanda de trabalho aumenta significativamente (VEGA et al., 2017). O fluxo sanguíneo eleva durante a sessão de EF para fornecer aporte de oxigênio principalmente para a musculatura ativa e para o miocárdio, tecidos que prioritariamente necessitam de mais oxigênio (HEINONEN et al., 2014).

A aptidão cardiorrespiratória pode ser entendida como sendo a capacidade dos sistemas circulatório e respiratório de manter a oferta de oxigênio para os tecidos que participam do EF, principalmente o tecido muscular ativo (ZHANG et al., 2017).

Esse parâmetro é obtido através da medida de $\dot{V}O_{2máx}$, sendo ele fortemente relacionada com desfechos adversos nas doenças cardiovasculares, além de ser um parâmetro independente para a predição de mortalidade, pois tem uma associação inversa para o risco de morte por eventos cardíacos ou por todas as causas (KETEYIAN et al., 2008; LAUKKANEN et al., 2016; NAUMAN et al., 2016). Já o débito cardíaco (DC) é a variável mais importante para a determinação do $\dot{V}O_{2máx}$, cuja fórmula foi definida através da equação de Fick: $\dot{V}O_2$ = DC x diferença arteriovenosa de O_2. O DC aumenta durante o EF devido ao aumento da frequência cardíaca e do volume sistólico. Esse aumentado a partir do volume diastólico final do VE e da contratilidade miocárdica, explicando assim o incremento do $\dot{V}O_{2máx}$. (LAVIE et al., 2015). Outro mecanismo para a justificativa da melhora do $\dot{V}O_{2máx}$ a partir do EF é a ativação na musculatura periférica de vias de sinalização da molécula de coativador-1α do receptor ativado por proliferador de peroxissimo (PGC1-α), gerando biogênese mitocondrial. A ativação dessa via é desencadeada através da diferença de ADP/AMP associada à ativação da 5' proteína cinase ativada por AMP (AMPK) nas células musculares durante o exercício (GIBALA et al., 2012).

2.2.2 Efeitos Metabólicos Cardíacos

O coração possui grandes exigências energéticas e metabólicas, as quais ficam desreguladas a partir do evento isquêmico do IAM. A principal fonte de energia, que anteriormente era a oxidação de ácidos graxos, posterior ao infarto, torna-se predominantemente a oxidação de glicose. Cada sessão de exercício físico aeróbico é capaz de aumentar o DC em até 6 vezes, fazendo com que a demanda de ATP seja suprida pela fosforilação oxidativa no interior da mitocôndria. As sessões consecutivas de treinamento são capazes de aprimorar o manejo da glicose e ácidos graxos no metabolismo energético do coração, além de atuar na biogênese mitocondrial a partir da regulação positiva da AMPK e PGC1-α (MANN; ROSENZWEIG, 2012).

A regulação do Ca^{2+} intracelular é fundamental para a contração do cardiomiócito. A diminuição da atividade da Ca^{2+}-ATPase do retículo sarcoplasmático (SERCA) leva à redução da recaptação de Ca^{2+}, comprometendo a contratilidade cardíaca. O EF é capaz de aumentar a atividade do trocador de Na^{2+}/Ca^{2+} (proteína de membrana responsável por regular o gradiente de Ca^{2+} intracelular) e aumentar a atividade da SERCA, melhorando a contratilidade cardíaca (ADAMS et al., 2017; MANN; ROSENZWEIG, 2012).

Atualmente, estudos têm mostrado que o EF é uma ferramenta muito importante na formação de novos cardiomiócitos após lesão isquêmica (FIGUEIREDO; CORIOLANO; DUARTE, 2014; VUJIC et al., 2018), sendo capaz de ativar, mobilizar e promover a diferenciação em células tronco residentes cardíacas e células progenitoras cardíacas, atuando na formação da novos cardiomiócitos (FIGUEIREDO; CORIOLANO; DUARTE, 2014). Ademais, o EF também é capaz de modular a expressão da sirtuína-1 no coração, sendo essa responsável por ser um dos fatores de crescimento e sobrevivência do cardiomiócito; assim como a sirtuína-3, uma sirtuína mitocondrial que tem função protetora contra as ERO (MANN; ROSENZWEIG, 2012). O EF atua também no sistema ubiquitina proteassoma e na sinalização mediada por miostatina no músculo cardíaco. Ambos os mecanismos estão relacionados com a atrofia de cardiomiócitos, sendo o EF capaz de modular a ativação desses mecanismos, além de aumentar o fator de crescimento semelhante à insulina tipo 1 (IGF-1) e promover hipertrofia cardíaca através da via fosfatidilinositol-3-cinase/proteína cinase B (ADAMS et al., 2017).

2.2.3 Efeitos sobre a Inflamação

Durante o EF, a partir da contração muscular gerada por ele, há um aumento da produção de diversas miocinas, entre elas a IL-6. Essa miocina, que durante o exercício pode aumentar sua concentração em até 100 vezes, tem efeito anti-inflamatório a partir da modulação da produção de IL-10, antagonista do receptor de IL-1 (IL-1ra) e supressão de TNF-α (PEDERSEN, 2017; STEENSBERG et al., 2003; WILUND, 2007). A magnitude na qual ela é produzida irá depender de fatores associados ao EF, tais como: intensidade, duração e a dimensão da musculatura esquelética envolvida (ANTUNES et al., 2019; PEDERSEN; FISCHER, 2007). Ao contrário da produção de IL-6 que ocorre devido à ativação da via NF-κβ em monócitos, a produção de IL-6 na musculatura é regulada pela ativação de vias relacionadas com Ca^{2+}, p38 proteína cinase ativada por mitógeno (p38 MAPK) e decréscimo de glicogênio muscular (ANTUNES et al., 2019; PEDERSEN, 2011; SCHEELE; NIELSEN; PEDERSEN, 2009).

A IL-10, como já mencionado anteriormente, tem um papel muito importante no mecanismo de reparação tecidual e supressão da inflamação no local da lesão miocárdica pós-IAM. O fato dessa citocina ser produzida por diferentes grupos celulares e sua modulação estar diretamente associada ao EF demonstra seu importante papel também no ambiente anti-inflamatório sistêmico gerado pelo treinamento físico (BATISTA JÚNIOR et al., 2009). Por outro lado, citocinas com perfil pró-inflamatórios, como TNF-α e IL-1β, não aumentam durante as sessões de treinamento, demonstrando que a cascata de produção de citocinas envolvida com ação da musculatura difere da cascata gerada por eventos como o IAM (PEDERSEN, 2011; WILUND, 2007).

A partir do déficit energético gerado pelo EF e um influxo maior de Ca^{2+} intracelular, há uma ativação da MAPK e consequente fosforilação do PGC-1α. No núcleo celular, o PGC-1α age como coativador regulador de receptores nucleares envolvidos com biogênese mitocondrial, expressão de enzimas e proteínas desacopladoras na CTE (JAGER et al., 2007; LIN; HANDSCHIN; SPIEGELMAN, 2005). O PGC-1α produzido nas células musculares é capaz de inibir o NF-κβ, diminuindo assim a síntese de citocinas pró-inflamatórias (HANDSCHIN; SPIEGELMAN, 2008).

Dada a extensão da musculatura esquelética e sua função como órgão secretor de citocinas, o músculo acaba tendo grande importância sobre o funcionamento e metabolismo de outros órgãos, visto que a secreção de diversos fatores acabam sendo direcionados para a circulação (PEDERSEN, 2011). O aumento nos níveis de citocinas anti-inflamatórias após sessões agudas do EF gera um ambiente com efeitos antagônicos e de supressão à “inflamação sistêmica crônica de baixo grau” gerada a partir do IAM, o que, por fim, acaba estabelecendo um efeito anti-inflamatório crônico (BATISTA JÚNIOR et al., 2009; PEDERSEN; FISCHER, 2007).

2.2.4 Efeitos sobre o Estresse Oxidativo

O desequilíbrio entre os agentes pró-oxidantes e as defesas antioxidantes do organismo decorrem de diversos processos, tanto fisiológicos (envelhecimento e exercício) quanto patológicos (doenças cardiovasculares) (VALKO et al., 2007). O termo “hormesis” tem como significado o processo de respostas adaptativas de células ou organismos que foram expostos a determinados tipos de estresse, tornando-os mais resistentes a um subsequente evento estressor. Em resposta à esses fatores, para manter a homeostase, mecanismos celulares e de transcrição gênica são acionados (MATTSON, 2008; PEAKE et al., 2015).

O EF é considerado um grande agente causador de estresse ao indivíduo, gerando uma exposição a estresse mecânico, metabólico, termorregulatório e oxidativo. A exposição crônica ao treinamento gera mecanismos compensatórios e adaptativos, tornando o organismo mais tolerante para o evento subsequente (MERRY; RISTOW, 2016). O efeito do EF sobre a modulação redox irá depender de diversos fatores, incluindo idade, sexo, nível de treinamento do indivíduo, intensidade e duração do exercício (PINGITORE et al., 2015). A partir da realização de uma sessão de EF, há um maior acionamento da CTE para fornecimento de energia a partir da contração muscular, aumentando a necessidade de O_2, ocasionando por fim, a produção excessiva e acúmulo de ERO no tecido. Além disso, há um aumento de fontes de $O_2^{-\bullet}$ a partir da Nox e xantina oxidase (OST et al., 2016).

Uma sessão de EF gera peroxidação lipídica, dano em DNA e proteínas. No entanto, a regularidade das sessões é capaz de gerar adaptações a partir das sucessivas exposições às ERO, induzindo mecanismos de reparo e resistência ao EO, além do aumento da produção de agentes antioxidantes (JI; KANG; ZHANG, 2016; OST et al., 2016). A partir da contração muscular e dos danos em estruturas celulares, ocorre a ativação da AMPK, NF-κβ e MAPK na musculatura esquelética, aumentando a síntese de PGC-1α. A partir da ação do PGC-1α na expressão gênica, mecanismos para a biogênese mitocondrial e produção de antioxidantes ocorrem. Ademais, a contração muscular é capaz de produzir as proteínas de choque térmico (HSP), importantes proteínas com ação protetora contra lesões teciduais geradas pela exposição às ERO (MCARDLE et al., 2001; PEAKE et al., 2015).

A produção de NO também aumenta com o EF a partir da ação da óxido nítrico sintase induzível (iNOS), podendo reagir com $O_2^{-\bullet}$ e gerando peroxinitrito com consequente dano celular. No entanto, a molécula de NO também é capaz de agir como vasodilatora, aumentando o fluxo sanguíneo vascular e intramuscular (JI; KANG; ZHANG, 2016).

2.3 PAPEL DO EXERCÍCIO FÍSICO NA REABILITAÇÃO CARDÍACA

A partir das altas taxas de mortalidade e hospitalizações por eventos cardiovasculares, a reabilitação cardíaca é uma ferramenta muito importante para a recuperação do indivíduo e de prevenção de um novo evento. Para isso, diversas abordagens se fazem necessárias para o paciente voltar à rotina e às atividades de vida diária, não só por meio do exercício, mas também de um programa multidisciplinar que inclui educação em saúde, orientações sobre mudanças de hábitos associados a fatores de risco e mecanismos de controle do estresse. Nos últimos anos, tem-se adotado um consenso acerca da importância do EF de diversas modalides para pacientes com doenças cardiovasculares, mesmo aqueles com alto grau de severidade e comprometimento pela doença, pois a inatividade física é altamente prejudicial ao quadro e acelera o processo de desenvolvimento da doença (DALAL; DOHERTY; TAYLOR, 2015; MCMAHON; ADES; THOMPSON, 2017). A American Heart Association classificou o sedentarismo como fator de risco com maior potencial de mudança dentro do quadro de doenças cardiovasculares (LAVIE et al., 2015). Assim, o programa de reabilitação cardíaca tem como objetivos limitar os efeitos deletérios causados pela doença cardíaca, bem como reduzir o risco de um novo evento e estabilizar o processo de aterosclerose instalado no paciente (DALAL; DOHERTY; TAYLOR, 2015; PRICE et al., 2016).

O EF pode ser encarado como um dos mecanismos centrais para a melhora da qualidade de vida desses pacientes, modificando fatores de risco e tornando esse paciente mais saudável e ativo. Dentre as melhorias funcionais atribuídas ao exercício estão a melhora da função cardíaca, o aumento da tolerância ao exercício e a diminuição dos sintomas relacionados à doença (PRICE et al., 2016).

A reabilitação cardíaca através do EF aeróbico é fortemente recomendada para pacientes com DAC e pós-IAM, sendo considerada a principal modalidade de treinamento (LAVIE; ARENA; FRANKLIN, 2016; LAWLER; FILION; EISENBERG, 2011; PARASHAR et al., 2012; PIEPOLI et al., 2016; PRICE et al., 2016) e sendo capaz de diminuir a mortalidade, a chance de um novo evento e as hospitalizações desses pacientes (ANDERSON et al., 2016; LAWLER; FILION; EISENBERG, 2011). O EF aeróbico já está bem documentado como sendo eficaz e seguro, com classe I de recomendação para esses pacientes (BALADY et al., 2011; THOMAS et al., 2007).

2.4 TREINAMENTO CONTÍNUO DE MODERADA INTENSIDADE

O treinamento contínuo de moderada intensidade é hoje o principal programa de treinamento para a reabilitação de pacientes diagnosticados com doenças cardiovasculares. Considerado um exercício seguro, de fácil controle, com resultados significativos na melhora da capacidade funcional, aptidão cardiorrespiratória e redução de sintomas, contribui significativamente para a redução de riscos e mortalidade desses pacientes (FLETCHER et al., 2013; LAVIE et al., 2015; PRICE et al., 2016). No exercício aeróbico, os sistemas cardiovascular, pulmonar e musculoesquelético trabalham juntos para gerar uma resposta adaptativa ao exercício (LAVIE et al., 2015).

Embora as últimas atualizações nas recomendações para prescrição de EF para essa população sejam de muitos anos atrás, as mesmas continuam sendo adotadas em programas de reabilitação cardíaca e estudos envolvendo EF como agente de reabilitação (BATACAN et al., 2017; CONRAADS et al., 2015; ELLINGSEN et al., 2017). A prescrição do treinamento contínuo de moderada intensidade não tem um consenso exato sobre a duração e a intensidade das sessões (PRICE et al., 2016). A European Society of Cardiology, juntamente com a European Association for Cardiovascular Prevention and Rehabilitation, preconizam 20 a 30 minutos de exercício por sessão, 3 sessões por semana (de preferência 6 a 7), com intensidade de 50 a 80% do $\dot{V}O_{2pico}$ ou da frequência cardíaca de pico (FC_{pico}), 40 a 60% da frequência cardíaca de reserva (FCR) e escala subjetiva de Borg de 10 a 14 (CORRÀ et al., 2010).

A American Heart Association juntamente com a American Association of Cardiovascular and Pulmonary Rehabilitation recomendam em torno de 20 a 60 minutos por sessão, 3 a 5 dias por semana, com intensidade de 40 a 80% do $\dot{V}O_2$ de reserva ou da FCR com valores de 11 a 16 na escala de Borg (FLETCHER et al., 2013; WILLIAMS; BALADY, 2009). O American College of Sports Medicine recomenda 20 a 60 minutos por sessão com 5 a 10 minutos de aquecimento, 3 ou mais sessões de treinamento por semana, com intensidade de 40 a 80% da FCR, $\dot{V}O_2$ de reserva, $\dot{V}O_{2pico}$, com valores de 11-16 na Escala de Borg. Os tempos e intensidades do treinamento irão variar conforme a tolerância do paciente durante a evolução do treinamento (ACSM, 2014).

2.5 TREINAMENTO INTERVALADO DE ALTA INTENSIDADE (HIIT)

O HIIT é uma modalidade de EF que tem como característica períodos curtos de trabalho com intensidade elevada (80 a 95% do $\dot{V}O_{2máx}$ ou da FCmáx) intercalados com períodos de recuperação de baixa intensidade (40 a 50% do $\dot{V}O_{2máx}$) (ROY, 2013). Inicialmente, os protocolos de HIIT foram desenvolvidos para a melhora do condicionamento cardiorrespiratório de atletas, contudo, com prescrições adequadas, a mesma forma de treinamento pode ser utilizada para pacientes com doenças cardiovasculares. Os princípios que regem a prescrição do HIIT para esses pacientes são os mesmos utilizados para populações saudáveis: períodos de baixa intensidade devem fazer com que o sistema cardiopulmonar do indivíduo se recupere parcialmente dos períodos de alta intensidade, o que permite que ele seja capaz de realizá-los novamente. O conceito fisiológico que está por trás do HIIT é a quantidade de tempo somado que o indivíduo permanece em uma intensidade mais alta do que normalmente conseguiria fazer em um treinamento contínuo, fazendo com que se consiga chegar em um $\dot{V}O_{2máx}$ mais alto, gerando adaptações no sistema cardiorrespiratório (ARENA et al., 2013). Nesses indivíduos, o trabalho pode ser realizado com caminhadas, ciclismo, remo, nado ou outras formas de exercício, sendo sempre prescrita de maneira individualizada e adequada para cada paciente (GILLEN; GIBALA, 2013).

O HIIT tem aparecido em novos consensos de programas de reabilitação cardíaca através do EF, no entanto, eles demonstram não ter evidências suficientes da sua eficácia e segurança para ser amplamente recomendado para esses pacientes (ACSM, 2014; FLETCHER et al., 2013; MEZZANI et al., 2012). A modalidade está sendo bastante discutida na literatura como sendo uma alternativa para esses pacientes, isso porque algumas meta-análises e estudos clínicos randomizados demonstram que o HIIT é superior na melhora de variáveis como $\dot{V}O_{2máx}$, função endotelial, variáveis hemodinâmicas e diminuição de riscos cardiovasculares (CARDOZO; OLIVEIRA; FARINATTI, 2015; ELLIOTT et al., 2015; HANNAN et al., 2018; HUSSAIN; MACALUSO; PEARSON, 2016; ITO; MIZOGUCHI; SAEKI, 2016; JAUREGUIZAR et al., 2016; LIOU et al., 2016; MOHOLDT et al., 2012; RAMOS et al., 2015; REED; ROSSI; CANNON, 2017; ROGNMO et al., 2004; WARBURTON et al., 2005; WESTON; WISLØFF; COOMBES, 2013; WISLØFF et al., 2007). Além disso, alguns estudos têm demonstrado maior aderência ao EF através de protocolos de HIIT, sendo uma opção considerada mais agradável do que o treinamento contínuo convencional (BARTLETT et al., 2011; GIBALA et al., 2012).

Em contrapartida, um estudo com pacientes com DAC demonstrou que ambas as modalidades de EF foram capazes de aumentar igualmente o $\dot{V}O_{2máx}$ e melhorar a função endotelial (CONRAADS et al., 2015). Ainda, o HIIT não se mostrou mais eficiente do que o treinamento contínuo no remodelamento cardíaco, melhora da função ventricular esquerda e capacidade aeróbica em pacientes com insuficiência cardíaca (ELLINGSEN et al., 2017).

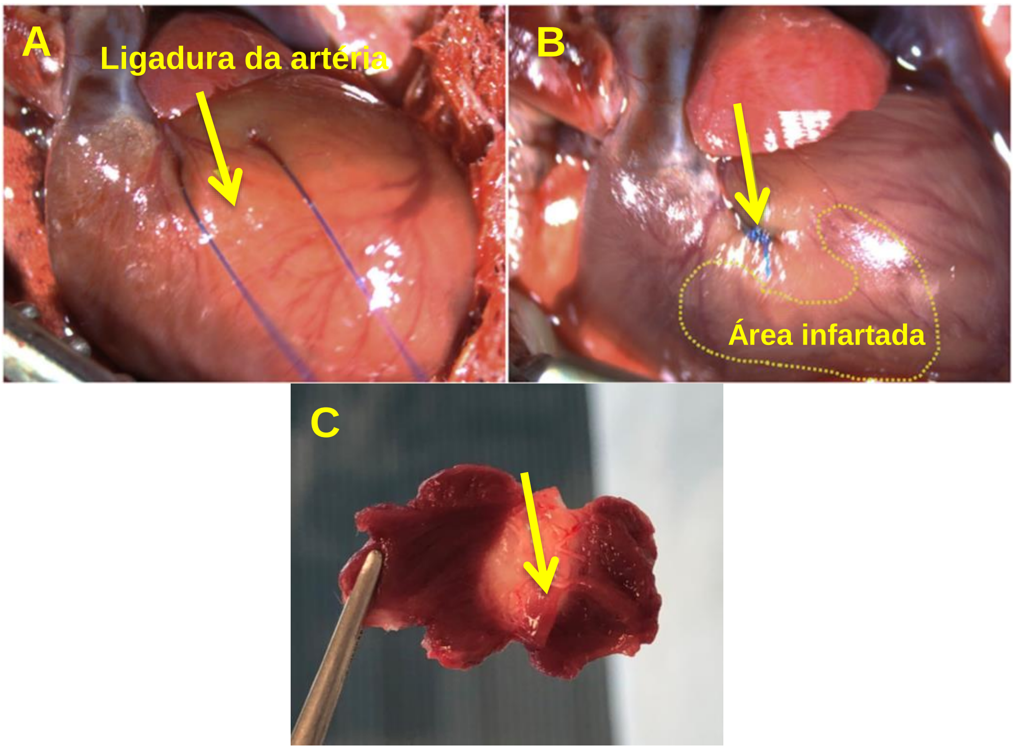
2.6 MODELO EXPERIMENTAL

As doenças cardiovasculares e suas complicações são as maiores causas de mortalidade no mundo. O uso de modelos animais para o estudo e investigação dessas doenças é uma ferramenta muito importante, pois permite compreender mecanismos etiológicos e suas implicações na fisiopatologia da doença, assim como descobrir novas estratégias de prevenção e tratamento para utilização na prática clínica (ZARAGOZA et al., 2011).

O modelo de indução do IAM mais utilizado em ratos consiste na ligadura da artéria coronária descendente esquerda. Através desse procedimento, mimetiza-se o processo de interrupção do fluxo sanguíneo através das artérias coronárias para o leito miocárdico característico da DAC (Figura 4) (JOHNS; OLSON, 1954; PFEFFER et al., 1979). Para o diagnóstico de IAM se fazem necessárias avaliações ecocardiográficas para fins de detecção de áreas com hipo ou acinesia, diâmetros de parede ventricular e função hemodinâmica, assim como o cálculo da área infartada, podendo ser mensurada através do método de planimetria a partir da dissecação do VE após a eutanásia do animal (LINDPAINTNER et al., 1993; MARTINEZ et al., 2011; MILL et al., 1990). Embora esse modelo seja o mais utilizado para induzir o quadro de lesão miocárdica, ele não expõe o animal aos mecanismos etiológicos iniciais do IAM decorrente do processo aterosclerótico. Apesar desse quadro de alterações preliminares serem de suma importância para o evento isquêmico em seres humanos, o modelo animal em ratos Wistar não possibilita a fisiologia necessária para que o IAM ocorra de forma espontânea, necessitando assim a intervenção de forma cirúrgica para que se possa estudar diferentes estratégias para o manejo de pacientes que sofreram o evento.

O EF é amplamente utilizado na prática experimental para avaliação de seus efeitos protetores em uma ampla gama de sistemas, em especial no sistema cardiovascular. O modelo animal se mostra bastante responsivo a diferentes protocolos de exercício, podendo assim, serem avaliadas possíveis modulações em diversos parâmetros relacionados com intensidade, volume, frequência e modalidades de treinamento (FENG et al., 2019).

Figura 4 – Modelo de indução do IAM através da ligadura da artéria coronária descendente esquerda (A e B) e cicatriz localizada no VE causada pelo procedimento (C).



Fonte: Adaptado de Katz et al., 2019 e arquivo pessoal.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar o efeito do HIIT e do treinamento contínuo de moderada intensidade na capacidade funcional, perfil inflamatório e marcadores de estresse oxidativo em ratos Wistar submetidos ao IAM.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar a capacidade funcional através da distância, tempo para exaustão e velocidade máxima obtidas no teste de esforço máximo no início do experimento, durante o protocolo de intervenção e ao final do experimento;
- Mensurar as concentrações das citocinas IL-10 e TNF-α no tecido muscular cardíaco e músculo gastrocnêmio;
- Mensurar os biomarcadores de dano oxidativo (concentração de malondialdeído e 2’,7’-diclorofluoresceína) no tecido muscular cardíaco e músculo gastrocnêmio;
- Mensurar a concentração de glutationa total e a atividade da enzima superóxido dismutase no tecido muscular cardíaco e músculo gastrocnêmio.

4 REFERÊNCIAS DA REVISÃO DA LITERATURA

ADAMS, V. et al. Molecular effects of exercise training in patients with cardiovascular disease: focus on skeletal muscle, endothelium, and myocardium. **American Journal of Physiology-Heart and Circulatory Physiology**, v. 313, n. 1, p. H72–H88, 2017.

ACSM. AMERICAN COLLEGE OF SPORTS AND MEDICINE. **ACSM'S guidelines for exercise testing and prescription**. 9ª ed. Philadelphia: Wolters Kluwer Health, 2014.

ANDERSON, J. L.; MORROW, D. A. Acute myocardial infarction. **New England Journal of Medicine**, v. 376, n. 21, p. 2053–64, 2017.

ANDERSON, L. et al. Exercise-based cardiac rehabilitation for coronary heart disease cochrane systematic review and meta-analysis. **Journal of the American College of Cardiology**, v. 67, n. 1, p. 1–12, 2016.

ANTUNES, B. M. et al. Anti-inflammatory response to acute exercise is related with intensity and physical fitness. **Journal of Cellular Biochemistry**, v. 120, n. 4, p. 5333–42, 2019.

ARENA, R. et al. Should high-intensity-aerobic interval training become the clinical standard in heart failure? **Heart Failure Reviews**, v. 18, n. 1, p. 95–105, 2013.

BAGATINI, M. D. et al. Oxidative stress versus antioxidant defenses in patients with acute myocardial infarction. **Heart and Vessels**, v. 26, n. 1, p. 55–63, 2011.

BALADY, G. J. et al. Referral, enrollment, and delivery of cardiac rehabilitation/secondary prevention programs at clinical centers and beyond. **Circulation**, v. 124, n. 25, p. 2951–60, 2011.

BARTLETT, J. D. et al. High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. **Journal of Sports Sciences**, v. 29, n. 6, p. 547–53, 2011.

BATACAN, R. B. et al. Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies. **British Journal of Sports Medicine**, v. 51, p. 494–503, 2017.

BATISTA JÚNIOR, M. L. et al. Efeito anti-inflamatório do treinamento físico na insuficiência cardíaca: papel do TNF-α e da IL-10. **Arquivos Brasileiros de Cardiologia**, v. 93, n. 6, p. 692–700, 2009.

BENJAMIN, E. J. et al. Heart disease and stroke statistics—2019 update: a report from the American Heart Association. **Circulation**, v. 139, n. 10, p. e56–e528, 2019.

BHATT, A. S.; AMBROSY, A. P.; VELAZQUEZ, E. J. Adverse remodeling and reverse remodeling after myocardial infarction. **Current Cardiology Reports**, v. 19, n. 71, p. 1-10, 2017.

BOATENG, S.; SANBORN, T. Acute myocardial infarct. **Disease-a-Month**, v. 59, n. 1, p. 83–96, 2013.

CARDOZO, G. G.; OLIVEIRA, R. B.; FARINATTI, P. T. V. Effects of high intensity interval versus moderate continuous training on markers of ventilatory and cardiac efficiency in coronary heart disease patients. **Scientific World Journal**, p. 1–8, 2015.

CHEN, Q. M.; MALTAGLIATI, A. J. Nrf2 at the heart of oxidative stress and cardiac protection. **Physiological Genomics**, v. 50, n. 2, p. 77–97, 2018.

COHN, J. N.; FERRARI, R.; SHARPE, N. Breather-induced anomalous charge diffusion. **Journal of the American College of Cardiology**, v. 35, n. 3, p. 569–82, 2000.

CONRAADS, V. M. et al. Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: the SAINTEX-CAD study. **International Journal of Cardiology**, v. 179, p. 203–10, 2015.

CORRÀ, U. et al. Secondary prevention through cardiac rehabilitation: physical activity counselling and exercise training: key components of the position paper from the cardiac rehabilitation section of the European Association of Cardiovascular Prevention and Rehabilitat. **European Heart Journal**, v. 31, n. 16, p. 1967–74, 2010.

DALAL, H. M.; DOHERTY, P.; TAYLOR, R. S. Cardiac Rehabilitation. **BMJ**, v. 351, p. 1–8, 2015.

EELEN, G. et al. Endothelial cell metabolism. **Physiological Reviews**, v. 98, n. 1, p. 3–58, 2018.

ELLINGSEN, Ø. et al. High-intensity interval training in patients with heart failure with reduced ejection fraction. **Circulation**, v. 135, n. 9, p. 839–49, 2017.

ELLIOTT, A. D. et al. Interval training versus continuous exercise in patients with coronary artery disease: a meta-analysis. **Heart Lung and Circulation**, v. 24, n. 2, p. 149–57, 2015.

ESPINOSA-DIEZ, C. et al. Antioxidant responses and cellular adjustments to oxidative stress. **Redox Biology**, v. 6, p. 183–97, 2015.

FELDMAN, A. M. et al. The role of tumor necrosis factor in the pathophysiology of heart failure. **Journal of the American College of Cardiology**, v. 35, n. 3, p. 537–44, 2000.

FENG, R. et al. A systematic comparison of exercise training protocols on animal models of cardiovascular capacity. **Life Sciences**, v. 24, n. 8, p. 1246–56, 2019.

FERRARI, R. et al. Oxidative stress during myocardial ischaemia and heart failure. **Current Pharmaceutical Design**, v. 10, n. 14, p. 1699–711, 2004.

FIGUEIREDO, P. A.; CORIOLANO, H. J.; DUARTE, J. A. Cardiac regeneration and cellular therapy: Is there a benefit of exercise? **International Journal of Sports Medicine**, v. 35, n. 3, p. 181–90, 2014.

FLETCHER, G. F. et al. Exercise standards for testing and training. **Circulation**, v. 128, n. 8, p. 873–934, 2013.

GALLI, A.; LOMBARDI, F. Postinfarct left ventricular remodelling: a prevailing cause of heart failure. **Cardiology Research and Practice**, v. 2016, p. 1–12, 2016.

GIBALA, M. J. et al. Physiological adaptations to low-volume, high-intensity interval training in health and disease. **Journal of Physiology**, v. 590, n. 5, p. 1077–84, 2012.

GILLEN, J. B.; GIBALA, M. J. Is high-intensity interval training a time-efficient exercise strategy to improve health and fitness? **Applied Physiology, Nutrition, and Metabolism**, v. 39, n. 3, p. 409–12, 2013.

GIMBRONE, M. A.; GARCÍA-CARDEÑA, G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. **Cardiovascular Pathology**, v. 22, n. 1, p. 9–15, 2013.

HANDSCHIN, C.; SPIEGELMAN, B. M. The role of exercise and PGC1α in inflammation and chronic disease. **Nature**, v. 454, n. 7203, p. 463–9, 2008.

HANNAN, A. et al. High-intensity interval training versus moderate-intensity continuous training within cardiac rehabilitation: a systematic review and meta-analysis. **Open Access Journal of Sports Medicine**, v. 9, p. 1–17, 2018.

HEINONEN, I. et al. Organ-specific physiological responses to acute physical exercise and long-term training in humans. **Physiology**, v. 29, n. 6, p. 421–36, 2014.

HEUSCH, G. et al. Cardiovascular remodelling in coronary artery disease and heart failure. **The Lancet**, v. 383, n. 9932, p. 1933–43, 2014.

HEUSCH, G.; GERSH, B. J. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. **European Heart Journal**, v. 38, n. 11, p. 774–84, 2017.

HUSSAIN, S. R.; MACALUSO, A.; PEARSON, S. J. High-intensity interval training versus moderate-intensity continuous training in the prevention/management of cardiovascular disease. **Cardiology in Review**, v. 24, n. 6, p. 273–81, 2016.

IDE, T. et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. **Circulation Research**, v. 88, n. 5, p. 529–35, 2012.

IGHODARO, O. M.; AKINLOYE, O. A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. **Alexandria Journal of Medicine**, v. 54, n. 4, p. 287–93, 2018.

INCALZA, M. A. et al. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. **Vascular Pharmacology**, v. 100, n. June 2017, p. 1–19, 2018.

ITO, S.; MIZOGUCHI, T.; SAEKI, T. Review of high-intensity interval training in cardiac rehabilitation. **Internal Medicine**, v. 55, n. 17, p. 2329–36, 2016.

JAGER, S. et al. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 . **Proceedings of the National Academy of Sciences**, v. 104, n. 29, p. 12017–22, 2007.

JAUREGUIZAR, K. et al. Effect of high-intensity interval versus continuous exercise training on functional capacity and quality of life in patients with coronary artery disease: a randomized clinical trial. **Journal of Cardiopulmonary Rehabilitation and Prevention**, v. 36, p. 96–105, 2016.

JI, L. L.; KANG, C.; ZHANG, Y. Exercise-induced hormesis and skeletal muscle health. **Free Radical Biology and Medicine**, v. 98, p. 113–122, 2016.

JOHNS, T.; OLSON, B. Experimental myocardial infarction: I. A method of coronary occlusion in small animals. **Annals of Surgery**, v. 140, n. 5, p. 675–82, 1954.

KAIN, V.; PRABHU, S. D.; HALADE, G. V. Inflammation revisited: inflammation versus resolution of inflammation following myocardial infarction. **Basic Research in Cardiology**, v. 109, n. 444, p. 1–17, 2014.

KATZ, M. G. et al. Surgical and physiological challenges in the development of left and right heart failure in rat models heart failure. **Heart Failure Reviews**, p. 1–19, 2019.

KETEYIAN, S. J. et al. Peak aerobic capacity predicts prognosis in patients with coronary heart disease. **American Heart Journal**, v. 156, n. 2, p. 292–300, 2008.

KEVIN, L. G.; NOVALIJA, E.; STOWE, D. F. Reactive oxygen species is mediators of cardiac injury and protection: the relevance to anesthesia practice. **Anesthesia and Analgesia**, v. 101, n. 5, p. 1275–87, 2005.

KIYUNA, L. et al. Targeting mitochondrial dysfunction and oxidative stress in heart failure: challenges and opportunities. **Free Radical Biology and Medicine**, v. 129, p. 155–68, 2018.

LATET, S. C. et al. The cellular immune system in the post-myocardial infarction repair process. **International Journal of Cardiology**, v. 179, p. 240–7, 2015.

LAUKKANEN, J. A. et al. Long-term change in cardiorespiratory fitness and all-cause mortality: a population-based follow-up study. **Mayo Clinic Proceedings**, v. 91, n. 9, p. 1183–8, 2016.

LAVIE, C. J. et al. Exercise and the cardiovascular system: clinical science and cardiovascular outcomes. **Circulation Research**, v. 117, n. 2, p. 207–19, 2015.

LAVIE, C. J.; ARENA, R.; FRANKLIN, B. A. Cardiac rehabilitation and healthy life-style interventions. **Journal of the American College of Cardiology**, v. 67, n. 1, p. 13–5, 2016.

LAWLER, P. R.; FILION, K. B.; EISENBERG, M. J. Efficacy of exercise-based cardiac rehabilitation post-myocardial infarction: a systematic review and meta-analysis of randomized controlled trials. **American Heart Journal**, v. 162, n. 4, p. 571-84, 2011.

LIBBY, P.; NAHRENDORF, M.; SWIRSKI, F. K. Leukocytes link local and systemic inflammation in ischemic cardiovascular disease an expanded cardiovascular continuum. **Journal of the American College of Cardiology**, v. 67, n. 9, p. 1091–103, 2016.

LIN, J.; HANDSCHIN, C.; SPIEGELMAN, B. M. Metabolic control through the PGC-1 family of transcription coactivators. **Cell Metabolism**, v. 1, n. 6, p. 361–70, 2005.

LINDPAINTNER, K. et al. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling in the rat. **Journal of Molecular and Cellular Cardiology**, v. 25, p. 133–43, 1993.

LIOU, K. et al. High intensity interval versus moderate intensity continuous training in patients with coronary artery disease: a meta-analysis of physiological and clinical parameters. **Heart Lung and Circulation**, v. 1920, p. 1–9, 2016.

LIU, T. et al. NF-κB signaling in inflammation. **Signal Transduction and Targeted Therapy**, v. 2, n. April, p. 1–9, 2017.

MACK, M.; GOPAL, A. Epidemiology, traditional and novel risk factors in coronary artery disease. **Cardiology Clinics**, v. 32, n. 3, p. 323–32, 2016.

MANN, N.; ROSENZWEIG, A. Can exercise teach us how to treat heart disease? **Circulation**, v. 126, n. 22, p. 2625–35, 2012.

MARTINEZ, P. F. et al. Echocardiographic detection of congestive heart failure in postinfarction rats. **Journal of Applied Physiology**, v. 111, n. 2, p. 543–51, 2011.

MATSUZAWA, Y. et al. Treating coronary disease and the impact of endothelial dysfunction. **Progress in Cardiovascular Diseases**, v. 57, n. 5, p. 431–42, 2015.

MATTSON, M. P. Hormesis defined. **Ageing Research Reviews**, v. 7, n. 1, p. 1–7, 2008.

MCALINDON, E. et al. Infarct size reduction in acute myocardial infarction. **Heart**, v. 101, n. 2, p. 155–60, 2014.

MCARDLE, A. et al. Contractile activity-induced oxidative stress: cellular origin and adaptive responses. **American Journal of Physiology - Cell Physiology**, v. 280, n. 3, p. 621-7, 2001.

MCMAHON, S. R.; ADES, P. A.; THOMPSON, P. D. The role of cardiac rehabilitation in patients with heart disease. **Trends in Cardiovascular Medicine**, v. 27, n. 6, p. 420–25, 2017.

MERRY, T. L.; RISTOW, M. Mitohormesis in exercise training. **Free Radical Biology and Medicine**, v. 98, p. 123–30, 2016.

MEZZANI, A. et al. Aerobic exercise intensity assessment and prescription in cardiac rehabilitation: A joint position statement of the European Association for Cardiovascular Prevention and Rehabilitation, the American Association of Cardiovascular and Pulmonary Rehabilitation. **European Journal of Preventive Cardiology**, v. 20, n. 3, p. 442–67, 2012.

MILL, J. G. et al. Changes in performance of the surviving myocardium after left ventricular infarction in rats. **Cardiovascular Research**, v. 24, n. 9, p. 748–53, 1990.

MISRA, M. K. et al. Oxidative stress and ischemic myocardial syndromes. **Medical Science Monitor**, v. 15, n. 10, p. 209-19, 2009.

MOHOLDT, T. et al. Aerobic interval training increases peak oxygen uptake more than usual care exercise training in myocardial infarction patients: a randomized controlled study. **Clinical Rehabilitation**, v. 26, n. 1, p. 33–44, 2012.

MÜNZEL, T. et al. Impact of oxidative stress on the heart and vasculature: part 2 of a 3 - part series. **Journal of the American College of Cardiology**, v. 70, n. 2, p. 212–29, 2017.

NABEL, E. G.; BRAUNWALD, E. A tale of coronary artery disease and myocardial infarction. **The New England Journal of Medicine**, v. 366, p. 54–63, 2012.

NAHRENDORF, M.; PITTET, M. J.; SWIRSKI, F. K. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. **Circulation**, v. 121, n. 22, p. 2437–45, 2010.

NAUMAN, J. et al. Prediction of cardiovascular mortality by estimated cardiorespiratory fitness independent of traditional risk factors: the HUNT study. **Mayo Clinic Proceedings**, v. 92, n. 2, p. 218–27, 2016.

ONG, S. B. et al. Inflammation following acute myocardial infarction: multiple players, dynamic roles, and novel therapeutic opportunities. **Pharmacology and Therapeutics**, v. 186, p. 73–87, 2018.

OST, M. et al. Regulation of myokine expression: role of exercise and cellular stress. **Free Radical Biology and Medicine**, v. 98, p. 78–89, 2016.

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

PARASHAR, S. et al. Predictors of early and late enrollment in cardiac rehabilitation, among those referred, after acute myocardial infarction. **Circulation**, v. 126, n. 13, p. 1587–95, 2012.

PEAKE, J. M. et al. Modulating exercise-induced hormesis: does less equal more? **Journal of Applied Physiology**, v. 119, n. 3, p. 172–89, 2015.

PEDERSEN, B. K. Exercise-induced myokines and their role in chronic diseases. **Brain, Behavior, and Immunity**, v. 25, n. 5, p. 811–16, 2011.

PEDERSEN, B. K. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. **European Journal of Clinical Investigation**, v. 47, n. 8, p. 600–11, 2017.

PEDERSEN, B. K.; FISCHER, C. P. Beneficial health effects of exercise – the role of IL-6 as a myokine. **TRENDS in Pharmacological Sciences**, v. 28, n. 4, p. 2–6, 2007.

PFEFFER, M. A. et al. Myocardial infarct size and ventricular function in rats. **Circulation Research**, v. 44, n. 4, p. 503–12, 1979.

PFEFFER, M.; BRAUNWALD, E. EResearch advances series ventricular remodeling after myocardial infarction. **Circulation**, v. 81, p. 1161–72, 1990.

PIEPOLI, M. F. et al. Secondary prevention in the clinical management of patients with cardiovascular diseases. Core components, standards and outcome measures for referral and delivery. **European Journal of Preventive Cardiology**, v. 21, n. 6, p. 664–81, 2014.

PIEPOLI, M. F. et al. Challenges in secondary prevention after acute myocardial infarction: a call for action. **European Journal of Preventive Cardiology**, v. 23, n. 18, p. 1994–2006, 2016.

PINGITORE, A. et al. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. **Nutrition**, v. 31, n. 7–8, p. 916–22, 2015.

PRABHU, S. D.; FRANGOGIANNIS, N. G. The biological basis for cardiac repair after myocardial infarction. **Circulation Research**, v. 119, n. 1, p. 91–112, 2016.

PRICE, K. J. et al. A review of guidelines for cardiac rehabilitation exercise programmes: is there an international consensus? **European Journal of Preventive Cardiology**, v. 23, n. 16, p. 1715–33, 2016.

QUINDRY, J. C. et al. Benefits and risks of high-intensity interval training in patients with coronary artery disease. **The American Journal of Cardiology**, v. 123, n. 8, p. 1370–7, 2019.

RAMOS, J. S. et al. The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. **Sports Medicine**, v. 45, n. 5, p. 679–92, 2015.

RAY, P. D.; HUANG, B.-W.; TSUJI, Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. **Cellular signalling**, v. 24, n. 5, p. 981–90, 2012.

REED, G. W.; ROSSI, J. rey E.; CANNON, C. P. Acute myocardial infarction. **The Lancet**, v. 389, p. 197–210, 2017.

ROGNMO, Ø. et al. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. **European Journal of Cardiovascular Prevention and Rehabilitation**, v. 11, n. 3, p. 216–22, 2004.

ROY, B. A. High-intensity interval training: efficient, effective, and a fun way to exercise brought. **ACSM's Health and Fitness Journal**, v. 17, n. 3, p. 3, 2013.

SACK, M. N. et al. Basic biology of oxidative stress and the cardiovascular system: part 1 of 3 - part series. **Journal of the American College of Cardiology**, v. 70, n. 2, p. 196–211, 2018.

SALLAM, N.; LAHER, I. Exercise modulates oxidative stress and inflammation in aging and cardiovascular diseases. **Oxidative Medicine and Cellular Longevity**, v. 2016, p. 46–54, 2016.

SANTOS-GALLEGO, C. G.; PICATOSTE, B.; BADIMÓN, J. J. Pathophysiology of acute coronary syndromes. **Current Atherosclerosis Reports**, v. 16, n. 401, p. 1–9, 2014.

SBC. SOCIEDADE BRASILEIRA DE CARDIOLOGIA. **Cardiômetro**. 2019. Disponível em: <http://www.cardiometro.com.br/anteriores.asp>. Acesso em: 6 jul. 2019.

SCHEELE, C.; NIELSEN, S.; PEDERSEN, B. K. ROS and myokines promote muscle adaptation to exercise. **Trends in Endocrinology and Metabolism**, v. 20, n. 3, p. 95–99, 2009.

SHINDE, A. V.; FRANGOGIANNIS, N. G. Fibroblasts in myocardial infarction: a role in inflammation and repair. **Journal of Molecular and Cellular Cardiology**, v. 70, p. 74–82, 2014.

SIES, H. **Oxidative Stress**. Londres: Academic Press Inc, 1985.

SIES, H. On the history of oxidative stress: Concept and some aspects of current development. **Current Opinion in Toxicology**, v. 7, p. 122–6, 2018.

SIES, H.; BERNDT, C.; JONES, D. P. Oxidative Stress. **Annual Review of Biochemistry**, v. 86, p. 715–48, 2017.

STEENSBERG, A. et al. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. **American Journal of Physiology-Endocrinology and Metabolism**, v. 285, n. 2, p. 433–7, 2003.

SWIRSKI, F. K.; NAHRENDORF, M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. **Science**, v. 339, n. 6116, p. 161–6, 2013.

THOMAS, R. J. et al. AACVPR/ACC/AHA 2007 performance measures on cardiac rehabilitation for referral to and delivery of cardiac rehabilitation/secondary prevention services. **Circulation**, v. 116, n. 14, p. 1611–42, 2007.

THYGESEN, K. et al. Universal definition of myocardial infarction. **European Heart Journal**, v. 40, p. 237–69, 2019.

TOWNSEND, N. et al. Cardiovascular disease in Europe - epidemiological update 2015. **European Heart Journal**, v. 36, n. 40, p. 2696–705, 2015.

TSUTSUI, H.; KINUGAWA, S.; MATSUSHIMA, S. Oxidative stress and heart failure. **American Journal of Physiology - Heart and Circulatory Physiology**, v. 301, p. 2181–90, 2011.

VAFAIE, M. State-of-the-art diagnosis of myocardial infarction. **Diagnosis**, v. 3, n. 4, p. 137–42, 2016.

VALKO, M. et al. Free radicals and antioxidants in normal physiological functions and human disease. **International Journal of Biochemistry and Cell Biology**, v. 39, n. 1, p. 44–84, 2007.

VEGA, R. B. et al. Molecular mechanisms underlying cardiac adaptation to exercise. **Cell Metabolism**, v. 25, n. 5, p. 1012–26, 2017.

VUJIC, A. et al. Exercise induces new cardiomyocyte generation in the adult mammalian heart. **Nature Communications**, v. 9, n. 165, p. 1–9, 2018.

WARBURTON, D. E. R. et al. Effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease. **American Journal of Cardiology**, v. 95, n. 9, p. 1080–4, 2005.

WEBB, C. S. et al. Specific temporal profile of matrix metalloproteinase release occurs in patients after myocardial infarction: relation to left ventricular remodeling. **Circulation**, v. 114, n. 10, p. 1020–7, 2006.

WESTMAN, P. C. et al. Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction. **Journal of the American College of Cardiology**, v. 67, n. 17, p. 2050–60, 2016.

WESTON, K. S.; WISLØFF, U.; COOMBES, J. S. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. **British Journal of Sports Medicine**, v. 48, n. 16, p. 1227–34, 2013.

WHO. WORLD HEALTH ORGANIZATION. **Cardiovascular Diseases (CVDs)**. 2016. Disponível em: https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Acesso em: 6 jul. 2019.

WILLIAMS, M. A.; BALADY, G. J. Cardiac rehabilitation and secondary prevention programs. In: FUSTER, V. **The AHA Guidelines and Scientific Statements Handbook**. Wiley-Blackwell, 2009.

WILUND, K. R. Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? **Clinical Science**, v. 112, n. 11, p. 543–55, 2007.

WISLØFF, U. et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. **Circulation**, v. 115, n. 24, p. 3086–94, 2007.

ZARAGOZA, C. et al. Animal models of cardiovascular diseases. **Journal of Biomedicine and Biotechnology**, v. 2011, p. 335–69, 2011.

ZHANG, Y. et al. Nonexercise estimated cardiorespiratory fitness and mortality due to all causes and cardiovascular disease. **Mayo Clinic Proceedings: Innovations, Quality & Outcomes**, v. 1, n. 1, p. 16–25, 2017.

5 ARTIGO CIENTÍFICO

Impact of High Intensity Interval Training and Moderate Intensity Continuous Training on Functional Capacity, Inflammatory Profile and Oxidative Stress markers of Myocardial-Infarcted Rats

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ABSTRACT

Purpose: To investigate the effect of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on functional capacity, tissue cytokines levels and redox state in a rat model of myocardial infarction. **Methods:** Eighteen male Wistar rats were allocated to the acute sedentary group (SED, n=6), MICT (n=6), and HIIT (n=6). Exercise training was performed 5×/week for eight weeks. **Results:** Both trained groups presented differences compared to the SED group in the distance, time to exhaustion, and maximal velocity in the eighth week. In the left ventricle, the IL-10/TNF-α ratio was higher in the MICT group when compared with the SED group. In gastrocnemius muscle, both groups were effective in improving IL-10, TNF-α, and IL-10/TNF-α ratio. Total glutathione concentration was higher in both trained groups in gastrocnemius and cardiac tissue when compared with the SED group. The total reactive oxygen species (ROS) cardiac level was lower in the HIIT group when compared to the SED group. Also, the DCF gastrocnemius level was lower in the HIIT group when compared to the MICT group. The cardiac lipoperoxidation level was lower in both trained groups when compared with the SED group. **Conclusion:** Eight weeks of exercise training increased functional capacity. However, the MICT protocol seemed to be superior to HIIT. Both protocols could not have the ability to modulate the inflammatory profile in cardiac tissue, although this effect occurred in gastrocnemius muscle. Both protocols improved oxidative stress parameters, although the HIIT protocol seemed to be more effective in decrease oxidative tissue damage.

KEYWORDS: Myocardial Infarction. Physical Exercise. HIIT. Inflammation. Oxidative Stress.

ABBREVIATIONS

HIIT – High Intensity Interval Training; IL-10 - Interleukin 10; IL-6 - Interleukin 6; MI – Myocardial Infarction; MICT – Moderate Intensity Continuous Training; OS – Oxidative Stress; ROS – Reactive Oxygen Species; SED – Sedentary; TNF-α - Tumor Necrosis Factor-alpha.

INTRODUCTION

Myocardial infarction (MI) is one of the main causes of cardiovascular deaths in the world, being a severe problem of public health (World Health Organization 2016). MI generates interruption of blood flow through vessel lumen blockage, causing cellular apoptosis and cardiomyocytes death (Thygesen et al. 2019). Furthermore, MI triggers the activation of intracellular cascade of proinflammatory cytokines expression, mainly tumor necrosis factor-alpha (TNF-α) (Westman et al. 2016; Ong et al. 2018) and increases the generation of reactive oxygen species (ROS) in cardiac tissue (Misra et al. 2009; Münzel et al. 2017). Commonly, the damage to the cellular components, are related to an imbalance between pro and anti-oxidant mediators (Kiyuna et al. 2018) and its interaction with macromolecules such as lipids, proteins and DNA, causing cell damage, energy deficit and cell death (Kevin et al. 2005; Bagatini et al. 2011; Tsutsui et al. 2011).

Previous studies evaluated the protective effect of exercise training on several parameters of hemodynamic, metabolic and functional variables of cardiovascular, respiratory and muscle tissues in cardiovascular diseases (Giallauria et al. 2009; Currie et al. 2013; Conraads et al. 2015). Furthermore, the molecular basis of rehabilitation now recognize the effect physical exercise as an adjuvant treatment for patients with cardiovascular diseases through the modulation of inflammation parameters (Adamopoulos et al. 2002; Goldhammer et al. 2005; Ribeiro et al. 2012) and oxidative stress (Edwards et al. 2004; Linke et al. 2005; Meirelles et al. 2014). Continuous moderate-intensity aerobic training is the primary training program recommendation in several guidelines for the rehabilitation of patients diagnosed with cardiovascular diseases. (Fletcher et al. 2013; Lavie et al. 2015; Price et al. 2016). However, high-intensity interval training (HIIT) has appeared as new consensus for rehabilitation programs (Mezzani et al. 2012; Fletcher et al. 2013; ACSM, 2014) as an alternative for these patients, once that shows to be superior in the improvement of maximal oxygen uptake, hemodynamic variables and reduction of cardiovascular risks (Rognmo et al. 2004; Warburton et al. 2005; Wisløff et al. 2007; Moholdt et al. 2012; Cardozo et al. 2015; Jaureguizar et al. 2016).

It is now well recognized that the intrinsic relationship between inflammation and ROS generation plays a pivotal role in the damaged heart tissue after ischemic events (Ong et al. 2018), affecting not only the heart but also skeletal muscle by decreasing local perfusion (Lunde et al. 2001). On the other hand, exercise is considered as non-pharmacological and low-price rehabilitation treatment with an anti-inflammatory agent, since from the muscle contraction generated by it, there is an increase in the production of several cytokines with greater immunomodulatory and metabolic effects (Pedersen 2017). Furthermore, physical exercise also can be an anti-oxidant agent, generating positive adaptations in both non-enzymatic and enzymatic anti-oxidant agents from successive exposures to ROS, inducing mechanisms of repair and resistance to oxidative stress (OS) (Ji et al. 2016; Ost et al. 2016). However, to the best of our knowledge, no previous studies evaluated the impact of HIIT or MCIT on inflammatory and redox state of metabolically active tissues that are impacted by acute myocardial infarction.

Therefore, the purpose of this study was to evaluate the impact of eight weeks of HIIT and MICT protocols on functional capacity with inflammatory profile, oxidative stress in cardiac muscle, and gastrocnemius muscle of infarcted rats.

METHODS

Animals and Ethical Approval

Male Wistar rats (280g to 350g; ≈ 90 days old) from the animal room of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) were kept in cages under conditions of food and water *ad libitum*, temperature between 22 and 24°C, and a light-dark cycle of 12 hours. Experimental protocols followed the Guide for the Care and Use of Experimental Animals published by the US National Institutes of Health (NIH, 2011), the National Laws of Care and Ethics in Scientific Experimentation (11.794/2008) and Resolution nº 39/2018 of National Council for Control of Animal Experimentation (CONCEA). The research protocols were approved by the Ethics Committee on the Use of Animals of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) (213/17).

Myocardial Infarction Surgery

The animals were anesthetized with isoflurane 2-3% associated with a concentration of 100% oxygen. The anesthetic depth level was conferred through eyelid reflexes, interdigital and caudal reflexes, and muscle tone. A catheter was used to perform endotracheal intubation, connecting the rats to a mechanical ventilator with a breathing rate of 60 breaths/min. After procedures, an incision was made among the 4th and 5th intercostal space previously trichotomized, the heart was exposed and was made the left descending coronary artery ligation with a 6-0 mono nylon suture. The thoracic north was closed with a 3-0 mono nylon suture, and the pneumothorax removal procedure was done with a 2-3 ml syringe, and the skin was sutured with a 3-0 mono nylon suture. Mechanical ventilation was withdrawn to check spontaneous breathing movements (Capalonga et al. 2016).

Experimental Design

Five weeks after MI, eighteen animals were allocated in three groups: sedentary (SED, n=6), intensity continuous training (MICT, n=6), and high-intensity interval training (HIIT, n=6).

Maximal Treadmill Exercise Test

Five weeks after the MI surgery, the animals were familiarized with the treadmill in three settings (starting at 10m/min with increase of 5 m/min, totalizing 3 minutes and maximal speed at 20 m/min). The maximal exercise test protocol was based on increases in speed until exhaustion. The protocol started with 10m/min, and treadmill velocity was increased 5 m/min every 3 minutes until animal exhaustion (Nunes et al. 2015). Records were performed before, in the fourth week and after exercise protocols.

Exercise Protocol

In the sixth week after the surgery, after the adaptation period, the animals started with de protocols of HIIT and MICT. Protocols were performed five times a week for eight weeks. HIIT protocol consisted of eight min of warming-up (10m/min) and ten 1 min sprints (85-90% of the maximal speed given in the test) interleaved with 2 min of recovery (50-60% of the maximal speed given in the test), totalizing 38 min of exercise. MICT protocol consisted five min of warming-up, and 45 min of running (50-60% of maximal speed given in the test), totalizing 50 min of exercise. In the fourth week, another test was realized, and the exercise speeds were adapted according to the maximal speed found in the test. The protocols were adapted according to the previous study from our laboratory that improved cardiorespiratory fitness in heart failure rats (Nunes et al. 2015).

Tissue collection, morphological measures, pulmonary and hepatic congestion

Forty-eight hours after the last exercise bout, the animals were sedated with isoflurane 3-4% and posteriorly were sacrificed by decapitation. Gastrocnemius, liver, and lung tissues were removed and weighed. The heart was separated in the left and the right ventricles and weighed. Infarct area was measured by three different evaluators and analyzed by planimetry with Image J Software (Mill et al. 1990; Alves et al. 2014). Left and right ventricular hypertrophy were measured by the ratio between the ventricle mass-to-body (LV/BM and RV/BM) (Stefani et al. 2018). After the protocols, the material was stored at – 80°C to posterior analyzes.

The animals' lungs and livers were dehydrated at the stove for 48h and weighed again to determine the congestion through the water percentage (Hentschke et al. 2017b).

Tissue homogenization

The left ventricles and gastrocnemius samples were homogenized in phosphate-buffered saline (pH 7.4) with 1:10 (w/v) tissue proportion. The homogenized were centrifuged at 3000 rpm for 10 min at 6°C, and the supernatant was used as a sample (Barcelos et al. 2017).

Cytokines

The TNF-α and IL-10 levels in gastrocnemius and left ventricle were determined by ELISA Kit Rat TNF-α (E-EL-R0019, Elabscience®, Texas, EUA) and ELISA Kit Rat IL-10 (E-EL-R0016, Elabscience®, Texas, EUA) according to the manufacturer's instructions. Values were reported in picograms per milligrams of protein.

Oxidative Stress

The protein concentration of the homogenate was determined with the method previously described (Lowry et al. 1951) using serum bovine albumin as the standard. The values are expressed in milligrams per microliters.

The ROS production was assessed using the H2DCF-DA marker, which is enzymatically hydrolyzed by intracellular esterases to form non-fluorescent H2DCF that is rapidly oxidized to a highly fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of ROS. The fluorescence intensity was measured in SpectraMax1 M2e Microplate Reader (Molecular Devices, MDS Analytical Technologies, Sunnyvale, California) using excitation and emission wavelengths of 480 and 535 nm, respectively (LeBel et al. 1992). The units of oxidant production are expressed in fluorescence units per milligrams of protein.

The malondialdehyde (MDA) concentration was determined by the thiobarbituric acid reactive substances test (TBARS) (Ohkawa et al. 1979). Briefly, 0.2 mL of homogenate was added to sodium dodecyl sulfate (8.1%, 0.05 ml), acetic acid (0.2 ml, 2.5 M, pH 3.4), and 0.8% thiobarbituric acid (0.25 ml, Sigma, USA) and heated in boiling water (90°C) for 60 min. After the supernatants were transferred to 96 wells microplate and the absorbance was read at 532 nm (EZReader, EUA). The values are expressed in nanomol per milligrams of protein.

The total glutathione level was measured using Elhman's reagent, as previously described (Tietze 1969). The values are expressed as micromol per milligrams of protein.

Superoxide dismutase (SOD) activity was measured spectrophotometrically at 480nm to Misra and Fridovich (1972). This method is based on the inhibition of adrenaline autoxidation in alkaline medium by the enzyme. Results are reported as units/mg of protein.

Statistical analysis

The data were presented in means ± standard deviations. The Shapiro-Wilk test was performed to evaluate the normal distribution of data. For parametric data, it was used the one-way analysis of variance (ANOVA) test followed by post-hoc Newman-Keuls to compare measures among the groups and the two-way analysis of variance (ANOVA) test followed by post-hoc Newman-Keuls to compare measures among the groups in different settings. For nonparametric data, was used the Kruskal-Wallis test followed by post-hoc Dunn to compare measures among the groups. It was considered P values <0.05. The statistical analyses and graphics were made in GraphPad Prism software version 7.0 for Windows (San Diego, California, USA).

RESULTS

Mortality, exclusion and adverse effects

The mortality by MI surgery induction was ~52% and 13 animals were excluded for presenting infarction small areas (<10%). No deaths occurred during tests and exercise protocols.

Infarct size, cardiac hypertrophy, pulmonary and hepatic congestion

Table 1 shows the infarcted area, cardiac hypertrophy, pulmonary, and hepatic congestion. The infarcted area was similar in all groups, suggesting an equal pathological condition. Also, left and right ventricle hypertrophy, pulmonary, and hepatic congestion did not present differences among the groups (P>0.05).

Functional capacity

Figure 1 shows the running distance (Fig. 1A), time to exhaustion (Fig. 1B), and the maximal velocity (Fig. 1C) in SED, MICT and HIIT acute myocardial infarction rats, during the maximal treadmill exercise testing. At the baseline, there were no differences between groups (P>0.05). After 4 weeks, the MICT, and the HIIT groups had a significant increase in the distance (HIIT, P<0.01; MICT, P<0.0001), the time to exhaustion (HIIT, P<0.001; MICT, P<0.0001) and the maximal velocity (HIIT, P<0.001; MICT, P<0.0001) when compared to the SED group during the maximal treadmill exercise testing. At the end of training protocols, both groups that performed exercise shown differences compared with the SED group in the distance (HIIT, P<0.001; MICT, P<0.0001), time to exhaustion (HIIT, P<0.001; MICT, P<0.0001) and maximal velocity (HIIT, P<0.0001; MICT, P<0.0001).

However, the MICT showed an increase in distance (*P*<0.01), time to exhaustion (*P*<0.05) and maximal velocity (*P*<0.05) when compared with the HIIT group.

TNF-α and IL-10 levels

Inflammation profile is showed in the Figure 2. The IL-10 (Figure 2A) and TNF-α (Figure 2B) concentration in left ventricle did not present difference among groups. However, IL-10/TNF-α ratio (Figure 2C) level in the left ventricle was higher in the MICT group when compared to the SED group (*P*<0.01). In gastrocnemius, the IL-10 level was higher in exercise groups (HIIT, *P*<0.01; MICT, *P*<0.01) when compared with the SED group. In the case of TNF-α, there was a lower level in the HIIT group (*P*<0.01) and the MICT group (*P*<0.05) when compared with the SED group. Besides, when analyzed the IL-10/TNF-α ratio, the level of both exercise group was higher when compared to the SED group (HIIT. *P*<0.001; MICT. *P*<0.0001).

Oxidative Stress

Figure 3 shows oxidative stress markers in the left ventricle. The value of DCF found in heart (Figure 3A) was lower in the HIIT group when compared with the SED group (*P*<0.05). On the other hand, the MDA value (Figure 3B) was lower in both exercise protocols, HIIT (*P*<0.01) and in MICT (*P*<0.05), when compared with the control group. Concentration of total glutathione (Figure 3C) was higher in both trained groups when compared with the control group (*P*<0.05). The SOD values (Figure 3D) did not present difference among groups. Figure 4 shows oxidative stress parameters in the gastrocnemius muscle. The DCF level (Figure 4A) was lower just in the HIIT group when compared with the MICT group (*P*<0.05). No significant difference was observed in the MDA value (Figure 4B) among experimental groups. However, the total glutathione (Figure 4C) concentration was higher in the HIIT and the MICT group when compared with the SED group (HIIT, *P*<0.001; MICT, *P*<0.01). The SOD values (Figure 4D) did not present difference among groups.

DISCUSSION

The present study investigated the impact of HIIT and MICT on functional capacity, inflammatory profile, and oxidative stress markers of infarcted rats. To our knowledge, it is the first study to investigate the HIIT and MICT effects in these parameters, showing that both exercise programs can improve functional capacity through the distance, time to exhaustion, and maximum speed after exercise protocols. Moreover, both programs of training improve the inflammatory profile and oxidative stress markers. The results of our study reinforce the anti-inflammatory and anti-oxidative effects of physical exercise, also your role as a non-pharmacological treatment in cardiac rehabilitation.

Low cardiorespiratory fitness is strongly related to adverse outcomes in cardiovascular disease (Nauman et al. 2016). Our study demonstrated that the functional capacity improved through total distance, time to exhaustion, and maximum speed results during treadmill maximal exercise testing, after eight weeks of training. This improvement may occur through several mechanisms, for example, by the cardiorespiratory fitness increase, improving the ability of the circulatory and respiratory systems to maintain oxygen supply to exercise tissues, especially to skeletal muscle (Zhang et al. 2017). Many studies have shown that HIIT is superior in improving variables such as maximal oxygen uptake, ejection fraction, and hemodynamic variables in post-infarction and coronary artery diseases, as clinical practice (Rognmo et al. 2004; Wisløff et al. 2007; Moholdt et al. 2012; Angadi et al. 2015; Jaureguizar et al. 2016) or experimental animal models (Moreira et al. 2013; Lu et al. 2015; Nunes et al. 2015). In contrast, few studies with cardiovascular disease patients found that there were no differences in left ventricular remodeling, aerobic capacity and endothelial function between the groups, demonstrating that both protocols were equally effective (Conraads et al. 2015; Ellingsen et al. 2017). In this sense, Barcelos et al. (2017) found that HIIT was not superior to the MICT in heart failure rats. Interestingly, our study showed that the MICT protocol is superior to the HIIT protocol in improving functional capacity. The physiological concept behind the superiority of HIIT in increasing cardiorespiratory function is the increased time that the individual remains at a higher intensity than they would normally be able to do in continuous training, increasing maximal oxygen uptake and resulting in cardiorespiratory system adaptations (Arena et al. 2013). However, our study showed that exercise high intensities were not able to increase functional capacity more than exercise moderate intensity in animals submitted to infarction. Nevertheless, we believed that the animals that made the HIIT protocol went into an overreaching state, a state of stagnation the adaptations in functional capacity (Meeusen et al. 2013). Running demands at the high intensities (70–90% of maximum oxygen consumption) can produce neural and endocrine responses, indicating physical stress. The training overload associated with the inadequate recovery and psychological stress combination can stagnate or decrease functional capacity improvements (Moraska et al. 2000), found from the training's fourth week in the HIIT group. However, even so, the functional capacity was improved when compared with the sedentary group, demonstrating that this training model can improve variables associated with cardiorespiratory fitness, a great mortality predictor in cardiovascular diseases (Laukkanen et al. 2016).

MI is associated with increased proinflammatory cytokine concentrations (Westman et al. 2016). The results of our study demonstrated that both physical exercise protocols realized were not able to change IL-10 and TNF-α levels in cardiac tissue. However, the MICT group established an environment with an anti-inflammatory pattern as visualized by the changes in IL-10/TNF-α ratio when compared to the HIIT and the SED groups. In this sense, Rodrigues et al. (2014) found that continuous aerobic exercise can reduce TNF-α but not increase IL-10 values in infarcted rats left ventricle. Feriani et al. (2017) showed that physical exercise was efficient to reduce TNF-α and increase IL-10 levels in infarcted rats. Nonetheless, infarcted area values found in both studies were relatively higher (about 40%) when compared to our study. After the MI, inflammatory cascade is initiated at the site, increasing several pro-inflammatory interleukins and cytokines, such as TNF-α, that mediates necrosis of the cardiac tissue (Libby et al. 2016), ceasing production after one week (Nian et al. 2004). The late production, after 20 weeks, is correlated with the increase of right ventricular end-diastolic diameter (Nian et al. 2004). A previous study demonstrated that TNF-α level in rat ventricles after 20 weeks of MI remained unchanged in the non-infarcted area, whereas in the infarcted area the values decreased dramatically from the first to the eighth week (Ono et al. 1998). Increased infarct area is associated with ventricular dysfunction and cardiac remodeling (Pfeffer et al. 1979; Pfeffer and Braunwald 1990) and consequent increase in TNF-α production (Bryant et al. 1998). Based on these observations, our data demonstrating that exercise does not seem to be able to improve the inflammatory profile in the cardiac tissue of rats with the small infarcted area.

Regarding the gastrocnemius muscle, our study showed that the IL-10 level was higher, and the TNF-α level was lower in both exercised MI rats. Subiela et al. (2018) demonstrated that physical exercise could reduce muscle TNF-α production in post-acute myocardial infarction patients. Another study showed that the IL-10 level was lower, just as TNF-α level was higher in rats submitted to infarction (Lopes et al. 2010). In accordance to the present report, aerobic exercise can modulate IL-10 and TNF-α production in peripheral muscles (Nunes et al. 2013; Calegari et al. 2017; Feriani et al. 2017), as well increase the IL-10/TNF-α ratio in animals submitted to MI (Jr et al. 2010). These findings corroborate with several studies that showed that the action of muscle contraction can generate an increase in myokines, which will act on the muscle creating an anti-inflammatory pattern (Scheele et al. 2009; Brandt and Pedersen 2010; Pedersen 2017) through the increase in peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) produced in muscle cells, inhibiting NF-κβ and decreasing proinflammatory cytokine synthesis (Handschin and Spiegelman 2008). Furthermore, exercise training reduces muscle inflammation through diminished macrophage infiltration, modulating the immune-metabolic crosstalk in muscle tissue (Samaan et al. 2014).

The heart is the largest source of ROS production after MI, causing deleterious effects on biological systems (Misra et al. 2009). However, physical exercise can be an antioxidant agent thought repeated exposure to acute bouts of physical stress, turning the organism more tolerant to new exposures by compensatory and adaptive mechanisms generation (Pingitore et al. 2015; Merry and Ristow 2016). In our study, only the HIIT group had lower left ventricle DCF levels when compared to the SED group. These levels may be justified by the balance between ROS production and antioxidant enzymes that were not measured, whereas glutathione is only one of the antioxidant agents. In contrast, both protocols were able to decrease lipid peroxidation values when compared to the control group. Likewise, both groups that performed exercise have higher total glutathione levels, indicating an improvement in scavenging capacity and redox balance in exercise groups. Similarly, with our results, a previous study compared the effect of MICT and HIIT on left ventricular oxidative stress in infarcted rats and found that both trainings were able to decrease lipid peroxidation and to increase antioxidant production (Lu et al. 2015). Jiang et al. (2014) and Afousi et al. (2019) demonstrated that HIIT in infarcted rats was able to decrease ROS production and increase ventricular antioxidant output when compared to a sedentary group. These data corroborate with the increase of cardioprotective effects through physical exercise.

In skeletal muscle, the lipid peroxidation result demonstrated that both protocols were not able to modulate the MDA concentration positively when compared to the sedentary group. However, gastrocnemius DCF level was lower just in HIIT rats when compared with the MICT group. On the other hand, total glutathione results were improved in trained groups, suggesting a redox balance in exercise groups. In contrast with our reports, Nunes et al. (2008) found that heart failure rats that realized swimming protocol had decrease lipoperoxidation values when compared with the sedentary group. Moreira et al. (2013) found that MICT and HIIT protocols increased glutathione concentrations in skeletal muscle in infarcted rats. In agreement, Cai et al. (2018) demonstrated that both training protocols were able to improve redox balance through decreased total ROS and increased glutathione reductase in the soleus muscle of infarcted rats. Our report suggests that ROS increase generated in the gastrocnemius muscles by muscle contraction in acute exercise sessions have triggered antioxidant mechanisms (Peake et al. 2015), making the tissue more resistant to damage from exposure to ROS with time. Based on these observations, our study reinforces that physical exercise can generate adaptations from successive exposures to ROS, inducing mechanisms of repair and resistance to oxidative stress, in addition to increased production of antioxidant agents.

CONCLUSIONS

In conclusion, the present study showed that eight weeks of exercise training could increase functional capacity by increasing the distance, time to exhaust, and maximal velocities reached in the test. However, the MICT protocol seems to be superior when compared to HIIT. Physical exercise, protocol-independent, does not seem to have the ability to modulate the inflammatory profile positively in cardiac tissue, although this effect occurs in gastrocnemius muscle. Both protocols can improve oxidative stress parameters, although the HIIT protocol seems to be more effective in decrease oxidative tissue damage.

LIMITATIONS

Despite the results of the present study, some limitations were significant and should be considered for the data interpretation. The systemic exercise effects involve several regulatory pathways that could interfere with the found results. Analysis of IL-6, an important cytokine secreted by skeletal muscle, would help to clarify the results of IL-10 modulation occurred in heart tissue, improving understanding about the role of exercise in this marker.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Methodology: Bruna Luvizon Martinelli, Lucas Capalonga, Giuseppe Potrick Stefani, Pedro Dal Lago; Formal analysis and investigation: Bruna Luvizon Martinelli, Lucas Capalonga, Giuseppe Potrick Stefani, Gilson Pires Dorneles, Fernanda Loro; Writing - original draft preparation: Bruna Luvizon Martinelli; Lucas Capalonga, Giuseppe Potrick Stefani, Gilson Pires Dorneles, Pedro Dal Lago Writing – review and editing: Bruna Luvizon Martinelli, Lucas Capalonga, Giuseppe Potrick Stefani, Gilson Pires Dorneles, Pedro Dal Lago; Supervision: Pedro Dal Lago.

REFERENCES

Adamopoulos S, Parissis J, Karatzas D, et al (2002) Physical training modulates proinflammatory cytokines and the soluble Fas/soluble Fas ligand system in patients with chronic heart failure. J Am Coll Cardiol 39:653–663. doi: 10.1016/S0735-1097(01)01795-8

Alves JP, Nunes RB, Stefani GP, Dal Lago P (2014) Resistance training improves hemodynamic function, collagen deposition and inflammatory profiles: Experimental model of heart failure. PLoS One 9:. doi: 10.1371/journal.pone.0110317

American College of Sports and Medicine (2014) ACSM'S Guidelines for Exercise Testing and Prescription, Ninth. Philadelphia, PA

Angadi SS, Mookadam F, Lee CD, et al (2015) High-intensity interval training vs. moderate-intensity continuous exercise training in heart failure with preserved ejection fraction: a pilot study. J Appl Physiol 119:753–758. doi: 10.1152/japplphysiol.00518.2014

Arena R, Myers J, Forman DE, et al (2013) Should high-intensity-aerobic interval training become the clinical standard in heart failure? Heart Fail Rev 18:95–105. doi: 10.1007/s10741-012-9333-z

Bagatini MD, Martins CC, Battisti V, et al (2011) Oxidative stress versus antioxidant defenses in patients with acute myocardial infarction. Heart Vessels 26:55–63. doi: 10.1007/s00380-010-0029-9

Barcelos GT, Rossato DD, Perini JL, et al (2017) Effects of L-arginine supplementation associated with continuous or interval aerobic training on chronic heart failure rats. Metabolism 76:1–10. doi: 10.1016/j.metabol.2017.06.009

Brandt C, Pedersen BK (2010) The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. J Biomed Biotechnol. doi: 10.1155/2010/520258

Bryant D, Becker L, Richardson J, et al (1998) Cardiac Failure in Transgenic Mice With Myocardial Expression of Tumor Necrosis Factor-α. Circulation 97:1375–1381

Cai M, Wang Q, Liu Z, et al (2018) Effects of different types of exercise on skeletal muscle atrophy, antioxidant capacity and growth factors expression following myocardial infarction. Life Sci 213:40–49. doi: 10.1016/j.lfs.2018.10.015

Calegari L, Nunes RB, Mozzaquattro BB, et al (2017) Exercise training improves the IL-10/TNF-α cytokine balance in the gastrocnemius of rats with heart failure. Brazilian J Phys Ther 72:1–7. doi: 10.1016/j.bjpt.2017.09.004

Capalonga L, Karsten M, Hentschke VS, et al (2016) Light-emitting diode therapy (LEDT) improves functional capacity in rats with heart failure. Lasers Med Sci 31:937–944. doi: 10.1007/s10103-016-1922-y

Cardozo GG, Oliveira RB, Farinatti PTV (2015) Effects of high intensity interval versus moderate continuous training on markers of ventilatory and cardiac efficiency in coronary heart disease patients. Sci World J 1–8. doi: 10.1155/2015/192479

Chen QM, Maltagliati AJ (2018) Nrf2 at the Heart of Oxidative Stress and Cardiac Protection. Physiol Genomics 50:77–97. doi: 10.1152/physiolgenomics.00041.2017

Conraads VM, Pattyn N, De Maeyer C, et al (2015) Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: The SAINTEX-CAD study. Int J Cardiol 179:203–210. doi: 10.1016/j.ijcard.2014.10.155

Currie KD, Dubberley JB, McKelvie RS, Macdonald MJ (2013) Low-volume, high-intensity interval training in patients with CAD. Med Sci Sports Exerc 45:1436–1442. doi: 10.1249/MSS.0b013e31828bbbd4

Edwards DG, Schofield RS, Lennon SL, et al (2004) Effect of exercise training on endothelial function in men with coronary artery disease. Am J Cardiol 93:617–620. doi: 10.1016/j.amjcard.2003.11.032

Ellingsen Ø, Halle M, Conraads V, et al (2017) High-Intensity Interval Training in Patients With Heart Failure With Reduced Ejection Fraction. Circulation 135:839–849. doi: 10.1161/CIRCULATIONAHA.116.022924

Espinosa-Diez C, Miguel V, Mennerich D, et al (2015) Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol 6:183–197. doi: 10.1016/j.redox.2015.07.008

Feriani DJ, Souza GIH, Carrozzi NM, et al (2017) Impact of exercise training associated to pyridostigmine treatment on autonomic function and inflammatory profile after myocardial infarction in rats. Int J Cardiol 227:757–765. doi: 10.1016/j.ijcard.2016.10.061

Fletcher GF, Ades PA, Kligfield P, et al (2013) Exercise Standards for Testing and Training. Circulation 128:873–934. doi: 10.1161/CIR.0b013e31829b5b44

Ghardashi Afousi A, Gaeini A, Rakhshan K, et al (2019) Targeting necroptotic cell death pathway by high-intensity interval training (HIIT) decreases development of post-ischemic adverse remodelling after myocardial ischemia / reperfusion injury. J Cell Commun Signal 13:255–267. doi: 10.1007/s12079-018-0481-3

Giallauria F, Galizia G, Lucci R, et al (2009) Favourable effects of exercise-based Cardiac Rehabilitation after acute myocardial infarction on left atrial remodeling. Int J Cardiol 136:300–306. doi: 10.1016/j.ijcard.2008.05.026

Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y (2005) Exercise training modulates cytokines activity in coronary heart disease patients. 100:93–99. doi: 10.1016/j.ijcard.2004.08.073

Handschin C, Spiegelman BM (2008) The role of exercise and PGC1α in inflammation and chronic disease. Nature 454:463–469. doi: 10.1038/nature07206

Hentschke VS, Capalonga L, Rossato DD, et al (2017a) Maximal oxygen uptake and exercise tolerance are improved in rats with heart failure subjected to low-level laser therapy associated with resistance training. Lasers Med Sci 32:73–85. doi: 10.1007/s10103-016-2088-3

Hentschke VS, Capalonga L, Rossato DD, et al (2017b) Functional capacity in a rat model of heart failure: impact of myocardial infarct size. Exp Physiol 102:1448–1458. doi: 10.1113/EP086076

Jaureguizar K, Vicente-Campos D, Bautista L, et al (2016) Effect of High-Intensity Interval Versus Continuous Exercise Training on Functional Capacity and Quality of Life in Patients with Coronary Artery Disease: A randomized clinical trial. J Cardiopulm Rehabil Prev 36:96–105. doi: 10.1097/HCR.0000000000000156

Ji LL, Kang C, Zhang Y (2016) Exercise-induced hormesis and skeletal muscle health. Free Radic Biol Med 98:113–122. doi: 10.1016/j.freeradbiomed.2016.02.025

Jiang HK, Miao Y, Wang YH, et al (2014) Aerobic interval training protects against myocardial infarction-induced oxidative injury by enhancing antioxidase system and mitochondrial biosynthesis. Clin Exp Pharmacol Physiol 41:192–201. doi: 10.1111/1440-1681.12211

Jr MLB, Rosa JC, Lopes RD, et al (2010) Cytokine Exercise training changes IL-10 / TNF- a ratio in the skeletal muscle of post-MI rats. Cytokine 49:102–108. doi: 10.1016/j.cyto.2009.10.007

Kevin LG, Novalija E, Stowe DF (2005) Reactive oxygen species is mediators of cardiac injury and protection: The relevance to anesthesia practice. Anesth Analg 101:1275–1287. doi: 10.1213/01.ANE.0000180999.81013.D0

Kiyuna L, Albuquerque R, Chen C-H, Ferreira JCB (2018) Targeting mitochondrial dysfunction and oxidative stress in heart failure: challenges and opportunities. Free Radic Biol Med 129:155–168. doi: 10.1038/nm.2451.A

Laukkanen JA, Zaccardi F, Khan H, et al (2016) Long-term Change in Cardiorespiratory Fitness and All-Cause Mortality: A Population-Based Follow-up Study. Mayo Clin Proc 91:1183–1188. doi: 10.1016/j.mayocp.2016.05.014

Lavie CJ, Arena R, Swift DL, et al (2015) Exercise and the cardiovascular system: Clinical science and cardiovascular outcomes. Circ Res 117:207–219. doi: 10.1161/CIRCRESAHA.117.305205

LeBel CP, Ischiropoulos H, Bondy SC (1992) Evaluation of the Probe 2′,7′-Dichlorofluorescin as an Indicator of Reactive Oxygen Species Formation and Oxidative Stress. Chem Res Toxicol 5:227–231. doi: 10.1021/tx00026a012

Libby P, Nahrendorf M, Swirski FK (2016) Leukocytes link local and systemic inflammation in ischemic cardiovascular disease an expanded cardiovascular continuum. J Am Coll Cardiol 67:1091–1103. doi: 10.1016/j.jacc.2015.12.048

Linke A, Adams V, Schulze PC, et al (2005) Antioxidative effects of exercise training in patients with chronic heart failure: Increase in radical scavenger enzyme activity in skeletal muscle. Circulation 111:1763–1770. doi: 10.1161/01.CIR.0000165503.08661.E5

Lopes RD, Batista Júnior ML, Rosa JC, et al (2010) Alterações na produção de IL-10 e TNF-α no músculo esquelético em ratos com insuficiência cardíaca secundária ao infarto do miocárdio. Arq Bras Cardiol 94:313–320. doi: 10.1590/s0066-782x2010000300006

Lowry OH, Rosebrough N, Farr L, Randall RJ (1951) Protein Measurement with the Folin Phenol Reagent. J Biol Chem 193:265–275

Lu K, Wang L, Wang C, et al (2015) Effects of high-intensity interval versus continuous moderate-intensity aerobic exercise on apoptosis, oxidative stress and metabolism of the infarcted myocardium in a rat model. Mol Med Rep 12:2374–2382. doi: 10.3892/mmr.2015.3669

Lunde PK, Sjaastad I., Thorud S, Sejersted OM (2001) Skeletal muscle disorders in heart failure. Acta Physiol Scand 171:277–294. doi: 10.1016/0140-6736(93)92662-D

Meeusen R, Duclos M, Foster C, et al (2013) Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine. Med Sci Sports Exerc 45:186–205. doi: 10.1249/MSS.0b013e318279a10a

Meirelles LR De, Matsuura C, Resende ADC, et al (2014) Chronic exercise leads to antiaggregant, antioxidant and anti-inflammatory effects in heart failure patients. Eur J Prev Cardiol 21:1225–1232. doi: 10.1177/2047487313491662

Merry TL, Ristow M (2016) Mitohormesis in exercise training. Free Radic Biol Med 98:123–130. doi: 10.1016/j.freeradbiomed.2015.11.032

Mezzani A, Hamm LF, Jones AM, et al (2012) Aerobic exercise intensity assessment and prescription in cardiac rehabilitation: A joint position statement of the European Association for Cardiovascular Prevention and Rehabilitation, the American Association of Cardiovascular and Pulmonary Rehabilitat. Eur J Prev Cardiol 20:442–467. doi: 10.1177/2047487312460484

Mill JG, Stefanon I, Leite CM, Vassallo D V. (1990) Changes in performance of the surviving myocardium after left ventricular infarction in rats. Cardiovasc Res 24:748–753. doi: 10.1093/cvr/24.9.748

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170-3175

Misra MK, Sarwat M, Bhakuni P, et al (2009) Oxidative Stress and Ischemic Myocardial Syndromes. Med Sci Monit 15:RA209-219

Moholdt T, Aamot IL, Granøien I, et al (2012) Aerobic interval training increases peak oxygen uptake more than usual care exercise training in myocardial infarction patients: A randomized controlled study. Clin Rehabil 26:33–44. doi: 10.1177/0269215511405229

Moraska A, Deak T, Spencer RL, et al (2000) Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. Am J Physiol Integr Comp Physiol 279:R1321–R1329. doi: 10.1152/ajpregu.2000.279.4.r1321

Moreira JBN, Bechara LRG, Bozi LHM, et al (2013) High- versus moderate-intensity aerobic exercise training effects on skeletal muscle of infarcted rats. J Appl Physiol 114:1029–1041. doi: 10.1152/japplphysiol.00760.2012

Münzel T, Camici GG, Maack C, et al (2017) Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3 - Part Series. J Am Coll Cardiol 70:212–229. doi: 10.1016/j.jacc.2017.05.035

Nauman J, Nes BM, Lavie CJ, et al (2016) Prediction of Cardiovascular Mortality by Estimated Cardiorespiratory Fitness Independent of Traditional Risk Factors: The HUNT Study. Mayo Clin Proc 92:218–227. doi: 10.1016/j.mayocp.2016.10.007

Nian M, Lee P, Khaper N, Liu P (2004) Inflammatory Cytokines and Postmyocardial Infarction Remodeling. Circ Res 94:1543–1553. doi: 10.1161/01.RES.0000130526.20854.fa

Nunes RB, Alves JP, Kessler LP (2015) Interval and continuous exercise enhances aerobic capacity and hemodynamic function in CHF rats. 19:257–263

Nunes RB, Alves JP, Kessler P, Lago PD (2013) Aerobic exercise improves the inflammatory profile correlated with cardiac remodeling and function in chronic heart failure rats. Clinics 68:876–882. doi: 10.6061/clinics/2013(06)24

Nunes RB, Tonetto M, Machado N, et al (2008) Physical exercise improves plasmatic levels of IL-10, left ventricular end-diastolic pressure, and muscle lipid peroxidation in chronic heart failure rats. J Appl Physiol 104:1641–1647. doi: 10.1152/japplphysiol.00062.2008

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. doi: 10.1016/0003-2697(79)90738-3

Ong SB, Hernández-Reséndiz S, Crespo-Avilan GE, et al (2018) Inflammation following acute myocardial infarction: Multiple players, dynamic roles, and novel therapeutic opportunities. Pharmacol Ther 186:73–87. doi: 10.1016/j.pharmthera.2018.01.001

Ono K, Matsumori A, Shioi T, et al (1998) Cytokine Gene Expression After Myocardial Infarction in Rat Hearts. Circulation 98:149–156. doi: 10.1161/01.cir.98.2.149

Ost M, Coleman V, Kasch J, Klaus S (2016) Regulation of myokine expression: Role of exercise and cellular stress. Free Radic Biol Med 98:78–89. doi: 10.1016/j.freeradbiomed.2016.02.018

Peake JM, Markworth JF, Nosaka K, et al (2015) Modulating exercise-induced hormesis: Does less equal more? J Appl Physiol 119:172–189. doi: 10.1152/japplphysiol.01055.2014

Pedersen BK (2017) Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest 47:600–611. doi: 10.1111/eci.12781

Pfeffer MA, Braunwald E (1990) Ventricular Remodeling After Myocardial Infarction: Experimental Observations and Clinical Implications. Circulation 81:1161–1172

Pfeffer MA, Pfeffer JM, Fishbein MC, et al (1979) Myocardial Infarct Size and Ventricular Function in Rats. Circ Res 44:503–512

Pingitore A, Lima GPP, Mastorci F, et al (2015) Exercise and oxidative stress: Potential effects of antioxidant dietary strategies in sports. Nutrition 31:916–922. doi: 10.1016/j.nut.2015.02.005

Price KJ, Gordon BA, Bird SR, Benson AC (2016) A review of guidelines for cardiac rehabilitation exercise programmes: Is there an international consensus? Eur J Prev Cardiol 23:1715–1733. doi: 10.1177/2047487316657669

Ribeiro F, Alves AJ, Teixeira M, et al (2012) Exercise Training Increases Interleukin-10 after an Acute Myocardial Infarction : A Randomised Clinical. Int J Sport Med 33:192–198

Rodrigues B, Santana AA, Santamarina AB, et al (2014) Role of Training and Detraining on Inflammatory and Metabolic Profile in Infarcted Rats : Influences of Cardiovascular Autonomic Nervous System. Mediators Inflamm 2014:1–13

Rognmo Ø, Hetland E, Helgerud J, et al (2004) High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. Eur J Cardiovasc Prev Rehabil 11:216–222. doi: 10.1097/01.hjr.0000131677.96762.0c

Samaan MC, Marcinko K, Sikkema S, et al (2014) Endurance interval training in obese mice reduces muscle inflammation and macrophage content independently of weight loss. Physiol Rep 2:. doi: 10.14814/phy2.12012

Scheele C, Nielsen S, Pedersen BK (2009) ROS and myokines promote muscle adaptation to exercise. Trends Endocrinol Metab 20:95–99. doi: 10.1016/j.tem.2008.12.002

Sies H (2018) On the history of oxidative stress: Concept and some aspects of current development. Curr Opin Toxicol 7:122–126. doi: 10.1016/j.cotox.2018.01.002

Stefani GP, Marmett B, Alves JP, et al (2018) Resistance training and L-arginine supplementation are determinant in genomic stability, cardiac contractility and muscle mass development in rats. PLoS One 13:1–15. doi: 10.1371/journal.pone.0204858

Subiela J V., Torres SH, De Sanctis JB, Hernández N (2018) Cardiorespiratory responses, nitric oxide production and inflammatory factors in patients with myocardial infarction after rehabilitation. Nitric Oxide - Biol Chem 76:87–96. doi: 10.1016/j.niox.2018.03.006

Thygesen K, Alpert JS, Jaffe AS, et al (2019) Universal definition of myocardial infarction. Eur Heart J 40:237–269. doi: 10.1161/CIRCULATIONAHA.107.187397

Tietze F (1969) Enzymic Method for Quantitative Determination of Nanogram Amounts of Total Glutathione: Applications to Mammalian Blood and Other Tissues. Anal Biochem 27:502–522

Tsutsui H, Kinugawa S, Matsushima S (2011) Oxidative Stress and Heart Failure. Am J Physiol - Hear Circ Physiol 301:H2181–H2190. doi: 10.1016/B978-0-12-801238-3.65249-2

Warburton DER, McKenzie DC, Haykowsky MJ, et al (2005) Effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease. Am J Cardiol 95:1080–1084. doi: 10.1016/j.amjcard.2004.12.063

Westman PC, Lipinski MJ, Luger D, et al (2016) Inflammation as a Driver of Adverse Left Ventricular Remodeling after Acute Myocardial Infarction. J Am Coll Cardiol 67:2050–60. doi: 10.1016/j.jacc.2016.01.073

Wisløff U, Støylen A, Loennechen JP, et al (2007) Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: A randomized study. Circulation 115:3086–3094. doi: 10.1161/CIRCULATIONAHA.106.675041

World Health Organization (2016) Cardiovascular Diseases (CVDs). https://www.who.int/en/news-room/fact-sheets/detai. Accessed 6 Jul 2019

Zhang Y, Zhang J, Zhou J, et al (2017) Nonexercise Estimated Cardiorespiratory Fitness and Mortality Due to All Causes and Cardiovascular Disease. Mayo Clin Proc Innov Qual Outcomes 1:16–25. doi: 10.1016/j.mayocpiqo.2017.04.007

Tables

Table 1 Infarct area, cardiac hypertrophy, pulmonary and hepatic congestion

	SED (n=6)	MICT (n=6)	HIIT (n=6)	*P* value
Infarct Area (%)	16.0 ± 7.6	19.4 ± 9.7	22.6 ± 5.8	0.15
LV/BW (mg/g)	1.9 ± 0.3	2.1 ± 0.2	2.1 ± 0.1	0.44
RV/BW (mg/g)	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.89
Pulmonary Congestion (%H_2O)	78.3 ± 2.6	77.2 ± 5.6	79.0 ± 3.4	0.75
Hepatic Congestion (%H_2O)	67.8 ± 7.1	61.8 ± 16.9	68.6 ± 6.1	0.71

Values are means ± SD. SED, sedentary group; MICT, moderate-intensity continuous training group; HIIT, high-intensity interval training group; LV/BW, left ventricular-to-body weight ratio; RV/BW, right ventricular-to-body weight ratio

Figure Captions

Fig 1 Values of maximal treadmill exercise test in baseline, 4 weeks and 8 weeks. A) Distance. B) Time to exhaustion. C) Maximal velocity. SED, sedentary group; MICT, moderate-intensity continuous training group; HIIT, high-intensity interval training group. Values are means ± SD. * P<0.05 vs. SED. # P<0.05 vs. HIIT

Fig 2 Values of IL-10, TNF-α and IL-10/TNF-α ratio. A) IL-10 in the left ventricle. B) TNF-α in the left ventricle. C) IL-10/TNF-α in the left ventricle. D) IL-10 in the gastrocnemius muscle. E) TNF-α in the gastrocnemius muscle. F) IL-10/TNF-α in the gastrocnemius muscle. SED, sedentary group; MICT, moderate-intensity continuous training group; HIIT, high-intensity interval training group. Values are means ± SD. * P<0.05 vs. SED. # P<0.05 vs. HIIT

Fig 3 Values of oxidative stress in the left ventricle. A) DCF. B) MDA. C) Total glutathione D) SOD. SED, sedentary group; MICT, moderate-intensity continuous training group; HIIT, high-intensity interval training group. Values are means ± SD. * P<0.05 vs. SED

Fig 4 Values of oxidative stress in the gastrocnemius muscle. A) DCF. B) MDA. C) Total glutathione D) SOD. SED, sedentary group; MICT, moderate-intensity continuous training group; HIIT, high-intensity interval training group. Values are means ± SD. * P<0.05 vs. SED. # P<0.05 vs. MICT

Fig 1

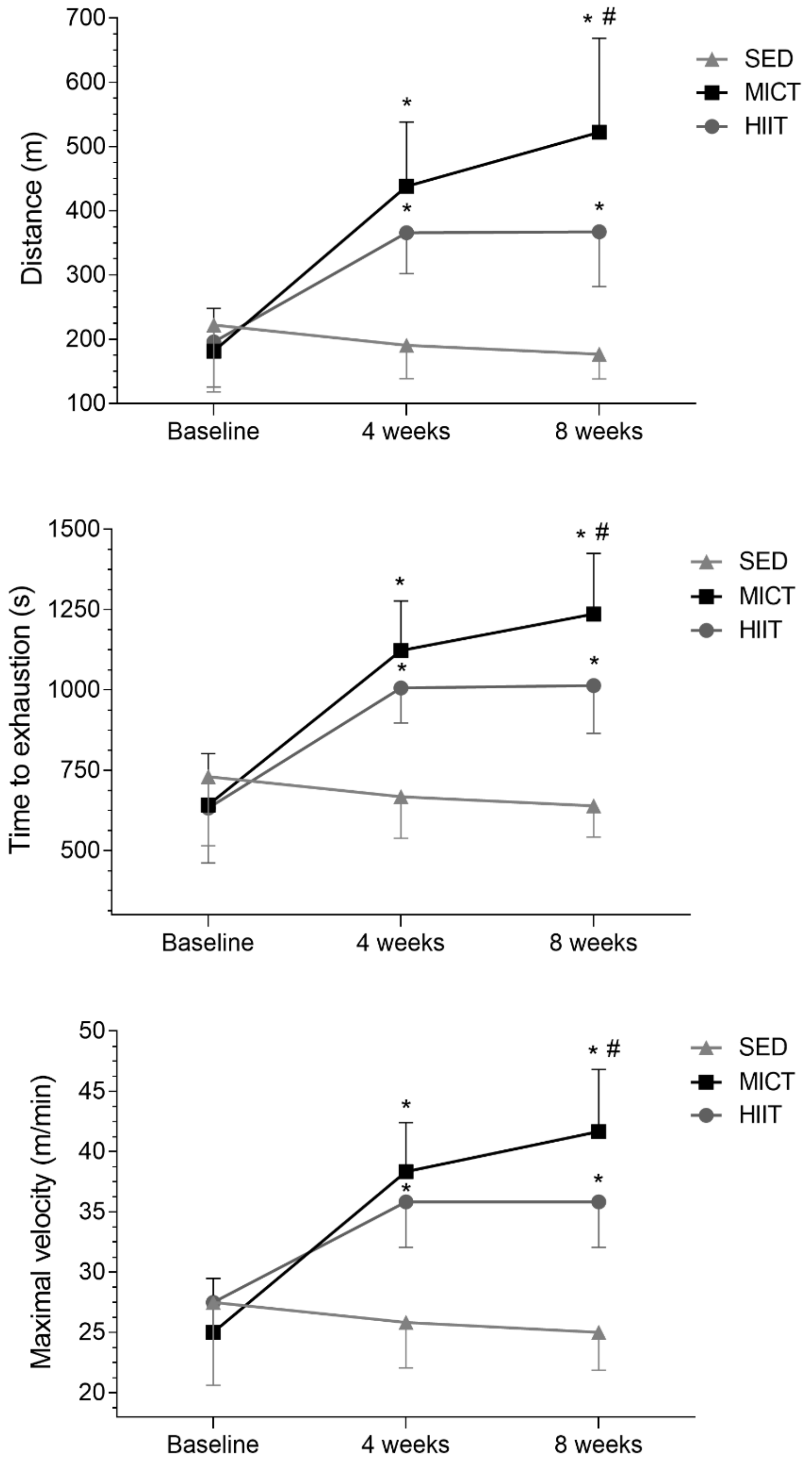

700
600
500
400
300
200
100
Distance (m)
Baseline
4 weeks
8 weeks
* #
*
*
*
SED
MICT
HIIT
1500
1250
1000
750
500
Time to exhaustion (s)
Baseline
4 weeks
8 weeks
SED
MICT
HIIT
50
45
40
35
30
25
20
Maximal velocity (m/min)
Baseline
4 weeks
8 weeks
SED
MICT
HIIT

Fig 2

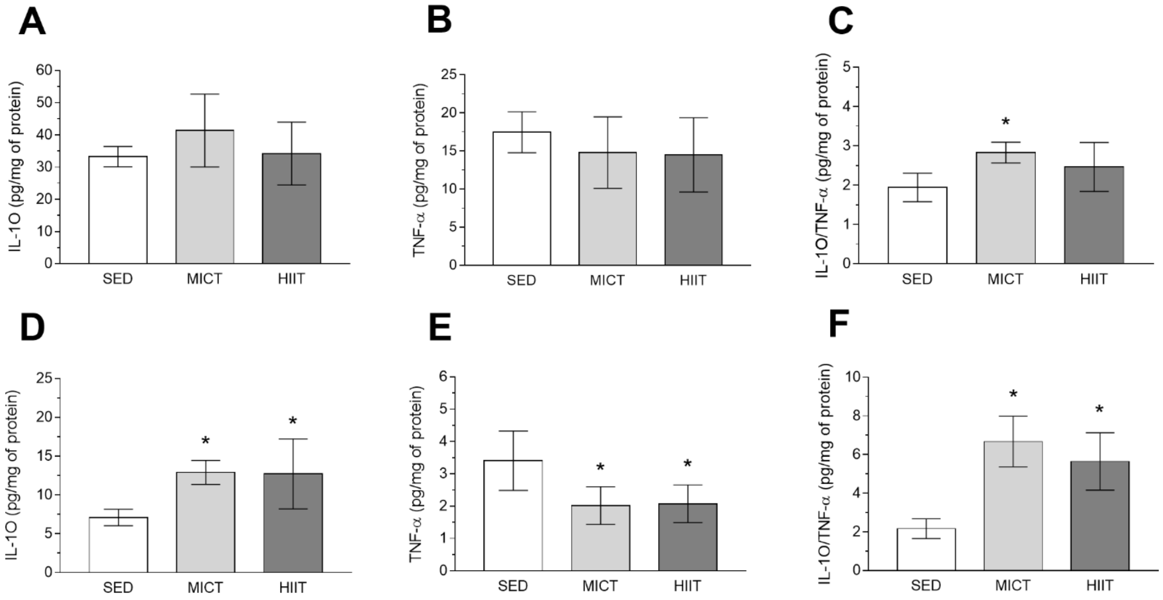

A
IL-1O (pg/mg of protein)
60
50
40
30
20
10
0
SED
MICT
HIIT
B
TNF-α (pg/mg of protein)
25
20
15
10
5
0
SED
MICT
HIIT
C
IL-1O/TNF-α (pg/mg of protein)
5
4
3
2
1
0
*
SED
MICT
HIIT
D
IL-1O (pg/mg of protein)
25
20
15
10
5
0
*
*
SED
MICT
HIIT
E
TNF-α (pg/mg of protein)
6
5
4
3
2
1
0
*
*
SED
MICT
HIIT
F
IL-1O/TNF-α (pg/mg of protein)
10
8
6
4
2
0
*
*
SED
MICT
HIIT

Fig 3

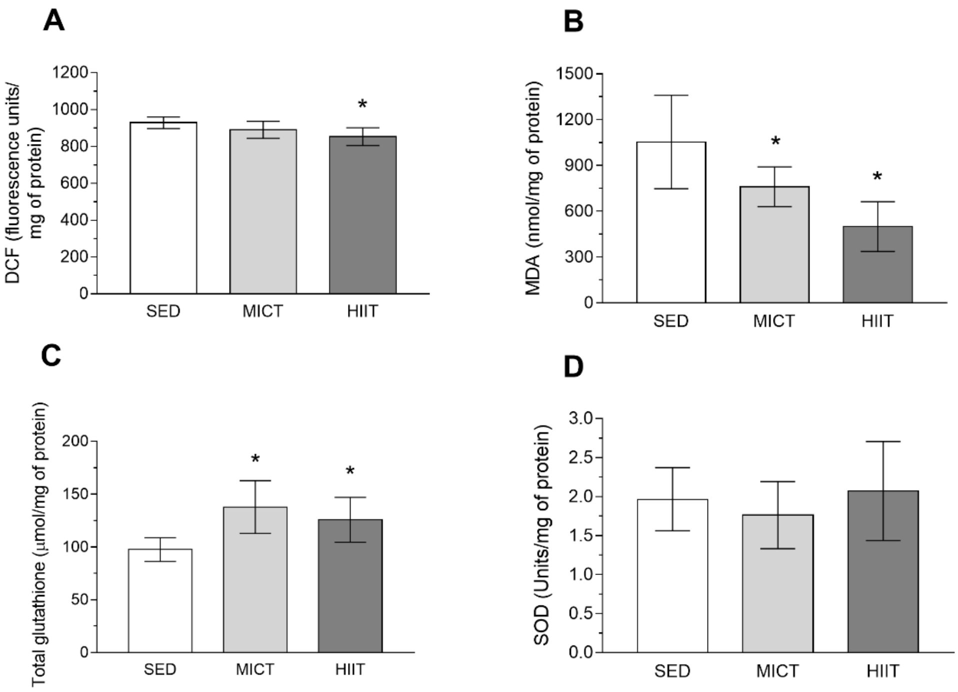
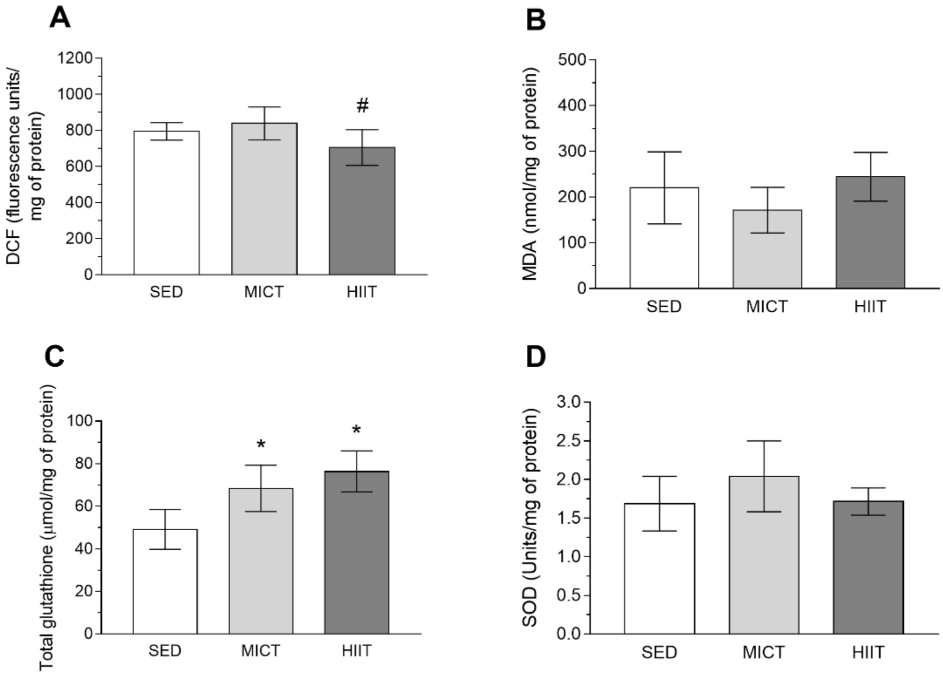

A
DCF (fluorescence units/ mg of protein)
1200
1000
800
600
400
200
0
*
SED
MICT
HIIT
B
MDA (nmol/mg of protein)
1500
1200
900
600
300
0
*
*
SED
MICT
HIIT
C
Total glutathione (μmol/mg of protein)
200
150
100
50
0
*
*
SED
MICT
HIIT
D
SOD (Units/mg of protein)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SED
MICT
HIIT

Fig 4


A
DCF (fluorescence units/ mg of protein)
1200
1000
800
600
400
200
0
#
SED
MICT
HIIT
B
MDA (nmol/mg of protein)
500
400
300
200
100
0
SED
MICT
HIIT
C
Total glutathione (μmol/mg of protein)
100
80
60
40
20
0
*
*
SED
MICT
HIIT
D
SOD (Units/mg of protein)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SED
MICT
HIIT

6 CONCLUSÃO GERAL

O exercício físico tem sido utilizado como ferramenta não-farmacológica adjuvante no tratamento de diversas doenças cardiovasculares, principalmente após o evento do IAM. Seu papel como agente anti-inflamatório e antioxidante, associado ao benefício de melhora da capacidade funcional, confere a ele um importante papel na reabilitação desses pacientes. O treinamento intervalado de alta intensidade tem surgido como alternativa ao treinamento contínuo de moderada intensidade dentro de programas de reabilitação cardíaca, demonstrando ser superior na melhora de parâmetros funcionais e cardiometabólicos.

Dessa forma, os dados desse estudo demonstraram que tanto o treinamento intervalado de alta intensidade quanto o treinamento contínuo de moderada intensidade foram efetivos em aumentar a capacidade funcional através da distância, tempo para exaustão e velocidade máxima atingida no teste de esforço máximo após 8 semanas de treinamento. No entanto, o treinamento contínuo se mostrou mais efetivo em melhorar essas variáveis.

Com relação à modulação do perfil inflamatório, ambos os treinamentos não foram capazes de modular positivamente a expressão de citocinas no músculo cardíaco, embora esse efeito tenha ocorrido à nível periférico no músculo gastrocnêmio. Ambos os treinamentos exercerem efeito antioxidante tanto no músculo cardíaco quanto no músculo gastrocnêmio através de concentrações maiores de glutationa total. Entretanto, o treinamento intervalado de alta intensidade demonstrou valores de biomarcadores de dano oxidativo mais baixos tanto no músculo esquelético quanto no músculo cardíaco comparado ao treinamento contínuo de moderada intensidade.

A partir desses resultados, se reforça o papel do exercício físico como estratégia de tratamento não-farmacológico para pacientes pós-IAM. No entanto, faz-se necessários estudos futuros para a identificação de mecanismos celulares pelos quais essas mudanças ocorrem, para que assim, o treinamento intervalado de alta intensidade seja incorporado de forma mais segura e efetiva na prática clínica de programas de reabilitação cardiovascular.

ANEXO A – PARECER DE APROVAÇÃO DA CEUA

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 213/17 Parecer 533/17

2) DATA DO PARECER: 08/11/2017

3) TÍTULO DO PROJETO:

Efeito de dois protocolos de exercício físico na modulação do perfil inflamatório, estresse oxidativo e capacidade funcional em ratos com Insuficiência Cardíaca (IC).

4) PESQUISADOR RESPONSÁVEL:

Pedro Dal Lago

5) RESUMO DO PROJETO:

O projeto pretende avaliar o efeito de duas modalidades de exercício em ratos submetidos a insuficiência cardíaca para verificar se o efeito anti-inflamatório relatado na literatura referente a modalidade de exercício de alta intensidade é estimulado neste modelo experimental.

6) OBJETIVOS DO PROJETO:

O objetivo principal do projeto é avaliar o efeito do treinamento intervalado de alta intensidade e treinamento aeróbico contínuo de moderada intensidade no perfil inflamatório, estresse oxidativo e capacidade funcional de ratos com IC.

7) FINALIDADE DO PROJETO: [ ] Ensino [X] Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título [X] Adequado [ ] Comentários

Introdução	X	Adequada		Comentários
Objetivos	X	Adequados		Comentários
Relevância e Justificativa	X	Adequados		Comentários
Materiais e Métodos	X	Adequados		Comentários
Cronograma para execução da pesquisa	X	Adequado		Comentários
Orçamento e fonte financiadora	X	Adequados		Comentários
Referências Bibliográficas	X	Adequadas		Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:

X Sim ☐ Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: **B** **C** ☐ **D** ☐ **E** ☐

Justifique:

Espécie: Ratos Wistar **Número Amostral:** 45

Redução Amostral: ☐ Sim X Não

Justifique:

Substituição de Metodologia: [ ] Sim [X] Não

Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia: [ ] Sim [X] Não

Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Acomodação e manutenção dos animais: [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Anestesia dos animais (se aplicável): [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Eutanásia dos animais (se aplicável): [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Local de Realização (Biotério/Labotarório):

Outra instituição. Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade

12) RECOMENDAÇÃO: As pendências deverão ser respondidas em uma carta, indicando as páginas do projeto que foram alteradas (nova versão), assinadas pelo pesquisador responsável.

X	Aprovado
	Com Pendência
	Não aprovado

Data de Término31/12/2018

Comentários gerais sobre o projeto:

ANEXO B – INSTRUÇÕES PARA AUTORES

Springer

European Journal of Applied Physiology

Editors-in-Chief: H. **Westerblad** ; K.R. **Westerterp**

ISSN: 1439-6319 (print version)
ISSN: 1439-6327 (Electronic version)

Journal no. 421

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The European Journal of Applied Physiology (EJAP) now publishes Invited Reviews only. The journal welcomes topics from physiological mechanics, biochemistry, endocrinology, ergonomics, immunology, motor control, neuroscience and nutrition.

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- Maximum manuscript size is 15 printed pages (4500-5000 characters/page)
- Colored illustrations highlighting critical concepts are strongly encouraged
- no limitation to number of references
- Abstracts should aim for 150-250 words
- Abstracts should contain the need for the review, primary objectives and logical conclusions
- Abstracts must not contain undefined abbreviations or references

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In addition to manuscripts, letters or comments discussing an article published in the past six months in EJAP are welcome and may appear in print in one of the following issues. Each comment will be sent to the corresponding author of the discussed article together with an invitation to respond. The comment and the reply may be sent to an independent referee. However, the editor-in-chief will make the final decision whether the correspondence will be published. EJAP allows the original author a deadline of two weeks to reply. If no answer is received within this timeframe, the editor-in-chief will decide if the letter or comment will be published without response. Letters and comments may be edited and shortened for reasons of clarity and space. In order to avoid long disputes, only the original comment together with the author's response will be published. Any additional comments or remarks should be sent to the authors directly and will not further be handled by EJAP.

- Maximum manuscript size is 1000 words
- maximum number of references is 5
- Figures can be added to clarify the written content
- Please include a separate page with a short title, the writer's name, affiliations and the exactcitation of the article commented on

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To facilitate rapid publication manuscripts should be prepared carefully in accordance with the following requirements.

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• Contributions that are part of a Special Issue must include the following footnote on the title page: "This article is published as part of the Special Issue on [title of the Special Issue]"

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Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Author Contribution Statement

Authors must provide a short description of the contributions made by each listed author (please use initials). This will be published in a separate section in front of the Acknowledgments.

For example: AM and DB conceived and designed research. AM and BB conducted experiments. GR contributed new reagents or analytical tools. AM and GR analyzed data. AM wrote the manuscript. All authors read and approved the manuscript.

TITLE PAGE

Title Page

The title page should include:

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- A concise and informative title
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Methods
Results
Conclusion

Keywords

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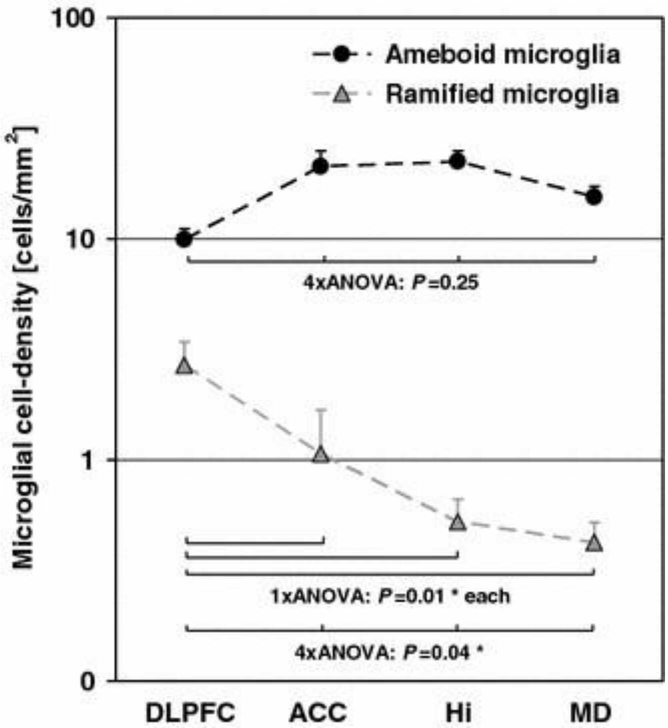

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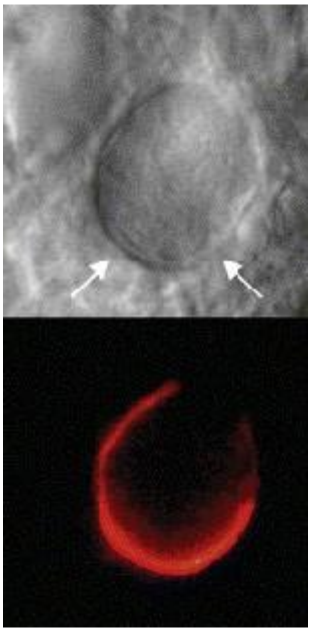

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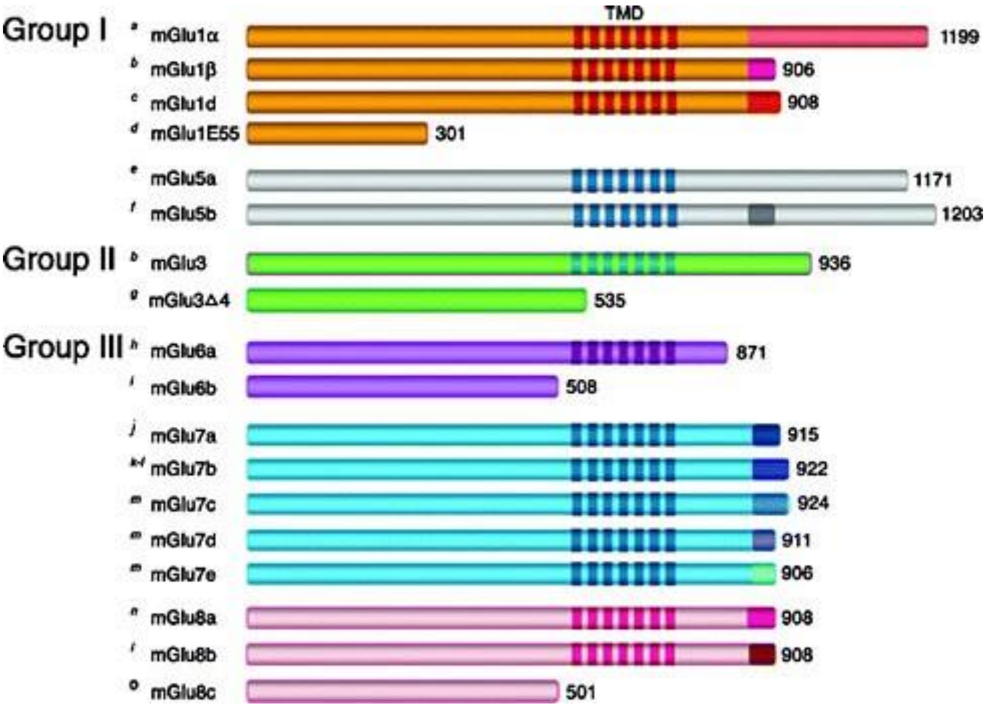

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Research Data Policy

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Research Data Policy Frequently Asked Questions

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