UNIVERSIDADE FEDERAL DE MINAS GERAIS

FACULDADE DE MEDICINA

DANIEL MASSOTE MAGALHÃES

EFEITO AGUDO DE DISTINTOS PROTOCOLOS DE EXERCÍCIO FÍSICO AERÓBICO SOBRE BIOMARCADORES PLASMÁTICOS E URINÁRIOS EM ADULTOS JOVENS SAUDÁVEIS E FISICAMENTE ATIVOS

Belo Horizonte

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Medicina Molecular da Faculdade de Medicina da Universidade Federal de Minas Gerais.

Orientadora:Prof.ª. Drª. Ana Cristina Simões e Silva.
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NOTA EXPLICATIVA

A apresentação da presente dissertação foi organizada sob a forma de artigos científicos, de acordo com a resolução 03/2010, aprovada pelo Programa de Pós-graduação da Faculdade de Medicina da Universidade Federal de Minas Gerais, disponível em http://www.medicina.ufmg.br/cpg/programas/saude_crianca/arquivos/2010/Resolucao03-2010.pdf.

Na introdução da dissertação apresentamos, de forma genérica, os biomarcadores que foram analisados nos artigos bem como a caracterização do exercício físico. O primeiro artigo consiste em uma avaliação do efeito agudo de dois protocolos de exercício físico aeróbico [(I - Exercício intensidade alta e intermitente (HIIE); II – Exercício de intensidade moderada e contínua (MICE)] sobre os níveis plasmáticos e urinários de citocinas. O segundo artigo avalia o efeito agudo dos mesmos protocolos, de exercício físico aeróbico, citados anteriormente sobre os níveis plasmáticos e urinários de irisina e adipocinas. Por fim, no terceiro artigo avaliamos o efeito agudo do exercício físico aeróbico sobre enzimas e peptídeos do sistema renina-angiotensina por meio dos referidos protocolos.

As referências bibliográficas estão dispostas ao final da cada seção (introdução e três artigos).

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LISTA DE ABREVIATURAS E SIGLAS

ACC	Carboxylase
ACE	Angiotensin converting enzyme
Ach	Acetylcholine
ACSM	American College of Sports Medicine
AHA	American Heart Association
Ang II	Angiotensin II
AT_1	Angiotensin type 1 receptor
BMI	Body mass index
BP	Blood pressure
CBA	Cytometric bead array
CHF	Chronic heart failure
CVD	Cardiovascular diseases
DP	Desvio padrão
ELISA	Enzyme - linked immunosorbent assay
FNDC5	Fibronectin domain-containing protein 5
HbA1c	Glycated hemoglobin
HIIE	High intensity interval exercise
HRp	Heart rate peak
HT	Hypertension
IFN-γ	Interferon γ
IL-10	Interleucina 10
IL-12p70	Interleucina 12p70
IL-1β	Interleucina 1β
IL-6	Interleucina 6
IL-8	Interleucina 8
IPAQ	International physical activity questionnaire
LV	Left ventricle
MAS	MAS receptor
MCP-1	Monocyte chemoattractant protein-1
MICE	Moderate intensity continuous exercise

MIG	Monokine induced by interferon gamma
NEFA	Non-ester fatty acid
PAR-Q	Physical activity readiness questionnaire
PGC-1α	Proliferator-activated receptor gamma coactivator 1-alpha
RAS	Renin-angiotensin system
SHR	Spontaneous hypertensive rats
SRA	Sistema renina-angiotensina
SSE	Steady state exercise
TNF	Tumoral necrosis factor
UFMG	Universidade Federal de Minas Gerais
WKY	Winstar kyoto

SUMÁRIO

1- RESUMO

INTRODUÇÃO: O exercício físico tem sido relacionado a adaptações fisiológicas que melhoram as condições de desempenho físico e saúde. Entre os possíveis fatores que podem contribuir para essas adaptações está a modulação de biomarcadores, como citocinas, adipocinas, miocinas e componentes do sistema renina-angiotensina. No entanto, existem poucos dados comparando protocolos de exercício físico de diferentes intensidades nesta modulação em indivíduos saudáveis ativos. Portanto, investigamos o efeito agudo de dois protocolos de exercício aeróbio, em diferentes intensidades e equalizados em trabalho total, nos níveis plasmáticos e urinários desses biomarcadores. **MÉTODOS:** Treze adultos jovens, do sexo masculino, saudáveis e fisicamente ativosforam recrutados para quatro sessões de exercício supervisionado. Foram realizadas duas sessões de avaliação para medir a composição corporal, nível de atividade física, capacidades aeróbicas e anaeróbicas anteriormente aos protocolos de (I) Exercício Intermitente de Alta Intensidade (HIIE) e (II) Exercício Contínuo de Intensidade Moderada (MICE) em um cicloergômetro. O protocolo HIIE incluiu uma fase de ativação [5 minutos a 60-70% da freqüência cardíaca pico (HRp)], seguido de 10 séries de 30 segundos (sprints acima de 90% HRp com 1 minuto de pausa ativa) e 3 minutos de volta a calma. O protocolo MICE foi realizado a uma potência constante (correspondente a 60-70% de HRp) e finalizado quando o trabalho total (Wt) se igualasse ao Wt do protocolo HIIE. As amostras de sangue e urina foram coletadas antes e após os protocolos, depois armazenadas a -80 ° C para análise posterior por imunoensaio enzimático (ELISA) para componentes RAS, irisina e adipocinas e pela técnica de Cytometric Bead Array (CBA) para citocinas. Os níveis das moléculas no plasma e urina foram medidos antes (valores basais) e após ambos os protocolos de exercício. **RESULTADOS:** A) Não encontramos diferença nos níveis pós exercício comparado com os níveis basais das citocinas no plasma e na urina. Por outro lado, encontramos uma correlação significativa e positiva entre os níveis plasmáticos de TNF e o trabalho total no protocolo HIIE; B) Irisin e adipokines: Os protocolos de exercício não alteraram significativamente os níveis de urina e plasma de irisina, leptina, resistina e níveis plasmáticos de adiponectina. No entanto, os níveis de adiponectina na urina aumentaram significativamente após o protocolo HIIE. C) Componentes RAS: Encontramos um aumento significativo nos níveis urinários de ACE e Ang (1-7) e nos níveis plasmáticos de ACE2 após o protocolo HIIE. As concentrações na urina de ACE2 e Ang- (1-7) aumentaram significativamente após o protocolo MICE.. **CONCLUSÃO:** O protocolo HIIE melhorou o perfil metabólico ao aumentar os níveis de

adiponectina. Este protocolo desencadeou resposta inflamatória em indivíduos que pedalaram em alta potência (o TNF apresentou correlação positiva com o trabalho total no HIIE). Além disso, o protocolo HIIE produziu aumento dos níveis urinários de ECA e de Ang-(1-7) e elevação dos níveis plasmáticos de ECA2. No entanto, o protocolo MICE foi superior ao HIIE no aumento dos níveis urinários de ECA2 e Ang (1-7), sugerindo um papel benéfico para as adaptações cardiovasculares e renais que dependem dessa via.

2- ABSTRACT

INTRODUCTION: Physical exercise has been related to physiological adaptations, which improve the physical performance and heathy conditions. Among the possible factors that may contribute to these adaptations is the modulation of biomarkers such as cytokines, adipokines, myokines and renin-angiotensin system components. However, there are scarce data comparing protocols of physical exercise with different intensity in this modulation in active healthy individuals. Therefore, we investigated the acute effect of two iso-work protocols of aerobic exercise in urine and plasma levels of these biomarkers. **SUBJECTS AND METHODS:** Thirteen young healthy physically active men were recruited to four supervised training sessions. Two evaluation sessions were performed to measure body composition, physical activity, aerobic and anaerobic capacities before High Intensity Interval Exercise (HIIE) and Moderated Intensity Continuous Exercise (MICE) iso-work exercise sessions on a cycle ergometer. The HIIE protocol included a 5-minute warm-up at 60-70% of heart rate peak(HRp) intensity followed by 10 sets of 30 seconds above 90% with 1 minute of recovery and 3 minutes of cool down(both at the same warm-up power). MICE protocol was performed at a constant power corresponding to 60-70% of HRp and finalized at the same total work of HIIE. Blood and urine samples were collected before and after the protocols, then stored at -80°C for further analysis by enzyme-linked immunoassay (ELISA) for RAS components, irisin and adipokines and by the technique of Cytometric Bead Array (CBA) for cytokines. Plasma and urine levels of molecules were measured before (baseline values) and after both exercise protocols. **RESULTS:** A) We did not find any difference in the comparison of baseline levels (before both exercise protocols) of all cytokines in plasma and urine. On the other hand, we found a significant and positive correlation between plasma levels of TNF and total work in HIIE protocol; B) Irisin and adipokines: Exercise protocols did not change significantly the plasma and urine levels of irisin, leptin, resistin and plasma adiponectin levels. However, urine levels of adiponectin significantly increased after HIIE

protocol. C) RAS components: We found following HIIE protocol a significant increase of ACE and Ang (1-7) urine levels and ACE2 plasma levels. Urine concentrations of ACE2 and of Ang-(1-7) significantly raised after MICE protocol. **CONCLUSIONS:** The HIIE protocol probably improved metabolic profile by increasing adiponectin levels. This protocol stimulated inflammatory response in individuals who pedaled at higher intensities (TNF had a positive correlation with total work in HIIE). In addition, HIIE protocol increased urinary levels of ACE and of Ang-(1-7) and plasma levels of ACE2. However, the MICE protocol was superior to HIIE in increasing urinary levels of ACE2 and Ang (1-7), supporting a beneficial role for cardiovascular and renal adaptations that depend on this pathway.

3- INTRODUÇÃO

O exercício físico intermitente de alta intensidade e curta duração (*High Intensity Iintermitent Exercise* - HIIE) tem sido investigado como uma alternativa ao exercício contínuo de moderada intensidade e média/longa duração (*Moderate Intensity Continuous Eexercise* - MICE), tanto por proporcionar adaptações fisiológicas iguais ou superiores ao MICE, quanto pela menor demanda de tempo para sua prática[1,2,3,4]. Estas informações tornam-se importantes uma vez que o tempo restrito tem sido relatado como um dos principais fatores que justificam o sedentarismo[5,6,7,8,9], um comportamento relacionado à inatividade física, que tem alta prevalência em todo o mundo[10]. No Brasil, o comportamento sedentário está presente em grande parte da população[10] e está diretamente associado a fatores prejudiciais à saúde, dentre os quais encontram-se obesidade, diabetes tipo 2, hipertensão arterial, acidentes vasculares cardíacos e encefálicos e depressão[11]. Quando um indivíduo apresenta tais distúrbios ou doenças, pode haver alteração na expressão de alguns marcadores biológicos, tais como quimiocinas, citocinas, adipocitocinas (adipocinas) e componentes do sistema renina-angiotensina, que são detectáveis na corrente sanguínea e na urina. Embora os mecanismos subjacentes não estejam bem esclarecidos, esses biomarcadores podem-se inter-relacionar e podem ser modulados pelo exercício físico em diversos grupos de indivíduos (doentes, sedentários, saudáveis e fisicamente ativos).

Diversas moléculas produzidas em resposta à lesões, microrganismos e outros antígenos, que medeiam e regulam reações imunológicas e inflamatórias são conhecidas genericamente como citocinas. Algumas dessas citocinas desempenham ações pró-inflamatórias e outras anti-inflamatórias. O fator de necrose tumoral (TNF) é uma citocina pró-inflamatória produzida principalmente por macrófagos infiltrados no tecido adiposo. O TNF age por meio de dois receptores solúveis, o sTNFR1 (p55) e o sTNFR2 (p75). Tais receptores ativam vias de sinalização que desencadeiam a resistência à insulina pela fosforilação do seu receptor (IR). Além disso, estimulam a lipólise no tecido adiposo e aumentam as concentrações de triacilgliceróis e acidos graxos na corrente sanguínea[12]. A interleucina-6 (IL-6) também é classicamente caracterizada como pró-inflamatória e é produzida principalmente por linfócitos T e macrófagos. No tecido adiposo, pode haver também o aumento da produção de IL-6, o que pode estimular a síntese hepática de triacilgliceróis e promover a hipertrigliceridemia[13]. Há evidências de que a IL-6 esteja relacionada à resistência à insulina e ao processo de aterosclerose[12,14]. Por outro lado, o tecido muscular esquelético também pode

produzir IL-6, conferindo uma característica modulatória a essa molécula, que se torna capaz de inibir a produção de TNF e estimular a síntese de citocinas anti-inflamatórias como antagonista do receptor de interleucina-1 (IL-1ra) e interleucina-10 (IL-10)[15]. A IL-6 também tem sido investigada como um importante mediador na inibição do crescimento de células cancerígenas em resposta ao exercício físico[16]. Além dessas características, a IL-6 secretada pelo tecido muscular pode produzir alterações metabólicas no próprio tecido aumentando a captação de glicose e oxidação lipídica, enquanto aumenta a lipólise no tecido adiposo e a produção de glicose no tecido hepático[15]. A interleucina 1 beta (IL-1β) é uma citocina pró-inflamatória produzida, principalmente, por monócitos e macrófagos. A IL-1β está associada ao aumento das moléculas de adesão e aumento da expressão de TNF, IL-6 e IL-8. A interleucina-8 (IL-8) é uma quimiocina produzida por monócitos, macrófagos, células estruturais, células endoteliais e fibroblastos. A IL-8 estimula a migração de células do sistema imune, principalmente os neutrófilos, e determina aumento da expressão de moléculas de adesão por células endoteliais. A Interleucina 12 (IL-12p70) também é uma citocina pró-inflamatória secretada pelos linfócitos B, neutrófilos, células dendríticas e macrófagos que estimula a expressão de moléculas de adesão e aumenta a produção de TNF e de interferon gama (IFN γ) por promover a diferenciação de linfócitos Th0 (células não iniciadas) em Th1(células inflamatórias)[17,18]. A IL-10, por sua vez, é uma citocina considerada anti-inflamatória por inibir a produção de IL-1α, IL-1β, TNF e IL-8, inibindo também a ativação de macrófagos e monócitos. IL-10 também estimula a expressão de IL-1ra e receptor solúvel de TNF, que são consideradas moléculas anti-inflamatórias[19].

As adipocinas são peptídeos bioativos secretados pelo tecido adiposo que influenciam uma variedade de processos fisiológicos e fisiopatológicos. A concentração plasmática de algumas dessas adipocinas, como a leptina e resistina, além de citocinas como TNF e IL-6, estão comumente associadas à massa adiposa de forma direta. Por outro lado, a adiponectina está inversamente associada a esta massa[12]. A leptina interfere na homeostase energética, inibindo o apetite e estimulando o gasto energético, além de aumentar a sensibilidade à insulina[12]. A adiponectina é secretada exclusivamente pelo tecido adiposo e possui efeito antidiabético por inibir a resistência à insulina e a produção de glicose hepática, além de aumentar a oxidação lipídica e utilização de carboidratos pelo tecido muscular. Possui também efeito anti-aterosclerótico por inibir a formação de células espumosas e efeito anti-inflamatório por inibir a expressão de TNF e IL-6 [20,21,22]. A expressão da resistina no tecido adiposo em humanos é decorrente da infiltração de macrófagos e monócitos neste tecido. A expressão de resistina é

estimulada por citocinas como o TNF e a IL-6 e sua ação está relacionada à resistência à insulina e ao desenvolvimento de aterosclerose, embora os mecanismos não estejam completamente elucidados[12,23,24]. Esta adipocina também é capaz de induzir a expressão de TNF e IL-6 no tecido adiposo e nas células mononucleares do sangue[24].

A irisina é um hormônio polipeptídico, recentemente descoberto, cuja produção decorre exclusivamente da contração muscular. Esta miocina (citocina produzida no tecido muscular esquelético)[25,26] atua de forma parácrina, autócrina e endócrina em diversos órgãos e tecidos. No tecido adiposo, a irisina aumenta a concentração da proteína desacopladora-1 ou termogenina (UCP-1) na membrana interna da mitocôndria. A UCP-1, através do acúmulo de prótons retirados das reações oxidativas do ciclo de Krebs, produz energia em forma de calor, aumentando o gasto energético e favorecendo alterações metabólicas benéficas à saúde[27,28].

O Sistema Renina-Angiotensina (SRA) influencia amplamente as funções cardiovasculares e renais por meio de múltiplos mediadores, receptores e mecanismos de sinalização intracelular[29,30,31,32,33,34]. Este sistema é composto por angiotensinogênio, uma proteína secretada pelo fígado que circula no plasma como um peptídeo biologicamente inativo. A renina, uma enzima sintetizada por células justaglomerulares dos rins, cliva a porção N-terminal do angiotensinogênio para formar o decapeptido inativo angiotensina I (Ang I). A secreção da renina é regulada por diversos fatores, como alterações na concentração de cloreto de sódio (NaCl) detectadas pela mácula densa do túbulo distal (que juntamente com as células justaglomerulares formam o aparelho justaglomerular), por mecanismo baroreceptor renal na arteríola aferente sensível a alterações da pressão de perfusão renal, pela estimulação nervosa simpática via receptores adrenérgicos β-1 ou por *feedback* negativo por ação direta da Ang I nas células justaglomerulares. A Ang I é convertida em angiotensina II (Ang II) pela enzima conversora de angiotensina (ECA), que está presente no endotélio vascular sistêmico, principalmente no endotélio vascular pulmonar. A Ang II exerce seus principais efeitos via receptor angiotensinérgico do tipo1 (AT1). Esta via formada por ECA, Ang II e receptor AT1 é considerada a via clássica e suas principais ações são vasoconstrição, disfunção endotelial, proliferação celular e hipertrofia, promoção de fibrose, efeitos trombogênicos, arritmogênicos e retenção hidrossalina[35]. No entanto, atualmente, admite-se a existência no SRA de um eixo contraregulatório intrínseco, formado pela enzima conversora de angiotensina 2 (ECA2)[36,37], que converte a Ang II em angiotensina-(1-7) [Ang (1-7)] que atua por sua ligação ao receptor MAS, deflagrando diversas ações opostas à via clássica, tais como: vasodilatação, proteção

vascular, antiproliferação e inibição do crescimento celular, efeitos antifibrinogênicos, antitrombogênicos, antiarritmogênicos e modulação renal da excreção de sódio e água[31,32]. As ações pró-inflamatórias e anti-inflamatórias dos eixos clássico e contraregulatório do SRA, respectivamente, decorrem de diversos fatores como a modulação de vias de sinalização intracelular, que influenciam a imunidade inata, a imunidade adaptativa e a inflamação[38]. Associada a esta característica, o SRA apresenta capacidade de modular a produção e liberação de diversas citocinas em condições fisiológicas e patológicas. De maneira geral, a ativação do eixo ECA/AngII/AT1 está associada ao aumento da expressão de citocinas pró-inflamatórias como TNF e IL-12, por exemplo. Em contrapartida, a ativação do eixo ECA2/Ang(1-7)/MAS pode promover o aumento na expressão de IL-10, além de reduzir a expressão de citocinas pró-inflamatórias como TNF, IL-6, IL-1β e IL-12[38,39].

Embora em algumas condições patológicas a interação entre biomarcadores esteja bem caracterizada na literatura[39-42], são muito limitadas e conflitantes as informações sobre tais moléculas em resposta ao exercício, principalmente em indivíduos fisicamente ativos[47,53,58]. Alguns estudos mostraram aumento na concentração de adiponectina e redução de leptina após uma sessão de exercício físico[43,44], enquanto outros não encontraram diferença significativa[42,43]. Os níveis de irisina apresentam aumento após uma sessão de exercício aeróbico em alguns trabalhos[27,47] e, embora seja classificada como uma miocina, seus níveis não alteraram em outros estudos[48,49]. A literatura em relação aos efeitos do exercício físico sobre as citocinas também apresenta contradições[50-54]. Algumas citocinas como TNF, IL-6 e IL-10 podem alterar[50-53] ou não[54] após exercício físico. Em revisão recente publicada pelo nosso grupo de pesquisa verificou-se que poucos estudos investigaram o efeito do exercício físico nos componentes do SRA, sendo a grande maioria deles realizados em modelos animais[55].

Parte da discrepância em relação aos efeitos do exercício físico sobre os biomarcadores pode ser justificada pela heterogeneidade das populações e modalidades de exercício, além da ampla possibilidade de variação dos componentes da carga de treinamento, principalmente volume e intensidade. Além disso, em indivíduos saudáveis e fisicamente ativos, adaptados a perturbações como as que ocorrem durante o exercício físico, o controle homeostático na corrente sanguínea ocorre de forma eficiente. Esta condição somada a curta meia-vida de algumas moléculas, dificulta a detecção de alterações desses marcadores durante o exercício. No entanto, esta limitação pode ser minimizada pela análise dos níveis urinários dessas

moléculas, pois, na urina, tais moléculas podem se apresentar em concentrações mais elevadas, refletindo elevações agudas que podem ter ocorrido na corrente sanguínea. Dessa forma, dosagens urinárias representam de forma mais acurada o período de tempo no qual foi realizado o protocole de exercício[56]. Por fim, poucos estudos[57,58,59] utilizam fatores equalizadores entre os protocolos de exercício, dificultando a comparação de resultados.

Dessa forma, o objetivo deste estudo foi avaliar o efeito agudo de dois protocolos de exercício físico aeróbico no cicloergômetro, distintos em relação à configuração da carga de treinamento e equiparados em relação ao trabalho total, sobre os níveis plasmáticos e urinários de diversas moléculas em indivíduos adultos jovens, do sexo masculino, saudáveis e fisicamente ativos.

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4- OBJETIVOS

Objetivo geral:

Avaliar o efeito agudo de distintos protocolos de exercício aeróbico (HIIE – exercício intermitente de alta intensidade e MICE exercício contínuos de intensidade moderada), equalizados pelo trabalho total, sobre níveis plasmáticos e urinários de marcadores biológicos em indivíduos adultos jovens, do sexo masculino, saudáveis e fisicamente ativos.

Objetivos específicos:

a) Verificar o nível de correlação entre marcadores biológicos, variáveis clínicas e parâmetros relacionados ao exercício físico.

b) Avaliar possíveis inter-relações entre os diversos marcadores biológicos.

5- ARTIGO 1

Changes in inflammatory molecules following moderate intensity continuous and high intensity intermittent acute exercises in young healthy physical active men.

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Abstract

INTRODUCTION AND OBJECTIVE: Currently, low-grade inflammation has been linked not only to chronic diseases but also to beneficial adaptations to physical exercise, as muscle hypertrophy[1]. In addition, recent studies showed a possible role of physical exercise in the modulation of inflammatory response. However, there is very few data evaluating different intensity iso-work protocols in regard to the effects on inflammatory molecules. Therefore, in this study we evaluated urinary and plasma levels of cytokines following high and moderate intensity protocols of physical exercise. **SUBJECTS AND METHODS:** Thirteen young healthy physically active men were recruited to four supervised training sessions. Two evaluation sessions were performed to measure body composition, physical activity, aerobic and anaerobic capacities before High Intensity Interval Exercise (HIIE) and Moderated Intensity Continuous Exercise (MICE) iso-work exercise sessions on a cycle ergometer. The HIIE protocol included a 5-minute warm-up at 60-70% of heart rate peak (HRp) intensity followed by 10 sets of 30 seconds above 90% with 1 minute of recovery and 3 minutes of cool down (both at the same warm-up power). MICE protocol was performed at a constant power corresponding to 60-70% of HRp and finalized at the same total work of HIIE. Blood and urine samples were collected before and after the protocols, then stored at -80°C for further analysis by the technique of Cytometric Bead Array (CBA). The molecules measured were Tumor Necrosis Factor (TNF), Interleukin 6 (IL-6),Interleukin 8 (IL-8), Interleukin 10 (IL-10),Interleukin 12 (IL-12p70), Interleukin 1 beta (IL-1β). **RESULTS:** We did not found any difference in the comparison of baseline levels (before both exercise protocols) of all cytokines in plasma and urine. In addition, no differences were detected in the comparison of urine and plasma levels of these molecules just after both exercise protocols. On the other hand, we found a significant and positive correlation ($r = 0.721$, $p = 0.008$) between plasma levels of TNF and total work in HIIE protocol. **CONCLUSION:** These findings showed that

young healthy physical active men did not respond acutely to aerobic exercise protocols in cytokines modulation. Moreover, individuals who made more intense sprints in HIIE protocol may increase more the TNF plasma levels.

INTRODUCTION

The knowledge about low-grade inflammation has grown rapidly in recent years, with high evidence that it is a major factor to the development of chronic diseases[1]. For example, atherosclerosis has been clearly linked to leucocyte-mediated damage in the wall of arteries after recruitment by inflammatory cytokines, such as IFN-γ and TNF[2]. Also, glucose and lipid metabolism appear to be imbalanced by the infiltration of macrophage cells into adipose tissue. This process is mediated by upregulated cytokines in obesity, leading thus to insulin resistance and type-2 diabetes[3].

Currently, it is well known that regular exercise practice promotes adaptations on cardiac and skeletal muscle and consequently increases the physical capacity[4,5]. Physical exercise also plays a central role in promoting health as a protective factor against cardiovascular and metabolic diseases[4,5]. For example, regular exercise practice was linked to a significant reduction in glycated hemoglobin (HbA1c), triglycerides, and blood pressure levels when compared to conventional pharmacologic therapies[4]. Although there is a consensus in the literature on the beneficial effects of exercise[6], the mechanisms are complex and not fully elucidated[7].

An hypothesis to explain the role of physical exercise is that it might be a modulator of circulating leucocytes and inflammatory biomarkers, such as Tumor Necrosis Factor (TNF), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 10 (IL-10), Interleukin 12 (IL-12p70), Interleukin 1 beta (IL-1β)[8]. These markers have already been implicated in the onset of chronic diseases, since the unbalance between inflammatory and anti-inflammatory molecules has been considered a triggering factor of chronic degenerative diseases. Therefore, the evaluation of the influence of exercise on plasma and urine concentrations of cytokines could help understanding the role of exercise. In addition, the comparison between different protocols of exercise may be associated to different results in inflammatory profile[8,9].

In this context, it is important to investigate whether there is some benefit in reducing the time and increasing the intensity of physical exercise, such as occurs in the High Intensity Interval Exercise (HIIE)[10]. Although this training method is used for decades[11,12], recently it

has been gaining strength in the social media as an better method than Moderate Intensity Continuous Exercise (MICE) for adaptations related to performance and energy expenditure parameters[10,13]. In this study, we aimed to evaluate urinary and plasma levels of cytokines following high and moderate intensity protocols of physical exercise in physically active men.

Material and methods

Participants

Thirteen physically active volunteers were submitted to four supervised exercise sessions conducted with at least 7 days of interval from each other. Participants were recruited from fitness centers in Belo Horizonte (Brazil) from July to December 2016 and included in the study protocol if they: i) were 20-25 year-old males; ii) did not present any contraindication to moderate or high intensity exercises; and iii) were physically active based on the International Physical Activity Questionnaire (IPAQ) criteria[14]. Participants were excluded if they had any chronic clinical condition including orthopedic, cardiovascular, metabolic, renal, pulmonary, oncologic or hematologic disorder or any acute infectious or allergic disease during the previous four weeks of study protocol. In addition, cyclists and individuals who have used CNS-stimulant drugs, anabolic steroids, corticosteroids, anti-inflammatories or antibiotics in the past four weeks prior to the study were excluded. All subjects were informed about the risks, discomforts and benefits associated with the protocol and provided written informed consent before admission to the study. The Research Ethics Committee of the *Universidade Federal de Ouro Preto*, Brazil approved this study under the protocol (60070016.5.0000.5150).

Exercise Protocols

In the **first session**, participants were asked to inform about general health conditions and to complete the Physical Activity Readiness Questionnaire (PAR-Q)[15], a short seven-question form that assesses readiness to exercise,and the International Physical Activity Questionnaire IPAQ, that quantifies the physical activity performed by the volunteer according to the type, frequency, volume and intensity. After, they were submitted to the Wingate test[16]to estimate their anaerobic power and capacity. In this test, the volunteer has to pedal for 30 seconds at maximum speed against a fixed resistance (7.5% of body mass) in order to generate the highest possible power peak and to avoid the decline of the power curve (in the power X time graphic) in that period of time.

The anthropometric characteristics were obtained in the **second session**. Body circumferences were obtained using an anthropometric tape and the body fat was calculated by the seven skinfold method according to Jackson and Pollock (1978)[17] using a skinfold caliper (Lange ®). The Body Mass Index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (Kg/m^2). The aerobic capacity (VO_{2peak}) was measured by a cycle-ergometer progressive exercise-to-fatigue test[18]. The test started at 25 W, and the workload increased at a rate of 25W every 2 min, when the rating of perceived exertion (RPE)[19] was evaluated. The cadence was maintained at 50 rpm. The ventilation variables were measured, using a gas analyzer (BIOPAC®) calibrated before each test according the manufacturer's recommendations. The maximal heart rate measured on this test (HR_{peak}) was registered by monitor Polar (Polar Team System, Finland) to use as a control parameter of intensity in the exercise protocols. The test was interrupted when the volunteers did not maintain the cadence (50 rpm), configuring fatigue.

The HIIE protocol was performed in the **third session** (Figure 1). This protocol included a 5-minute warm-up at a constant power corresponding to 60-70% of the HR_{peak}, followed by 10 bouts of 30 seconds above 90% HR_{peak}. The exercise was then finalized with 1 minute of active recovery and 3 minutes of cool down, both at the same constant warm-up power [watts - W], registered by MCE® software (Staniak, Polônia). The total work [Kilo Joules – KJ] of the HIIE protocol was calculated (the sum of work values in each stage of HIIE, informed by software) and registered to be equalized in the MICE protocol.

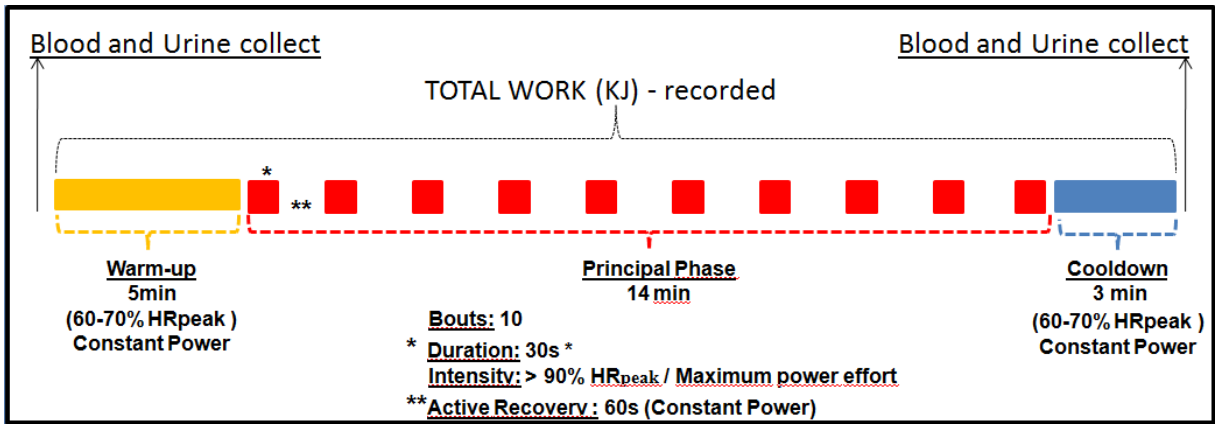

Figure 1 – *Schematic view of High Intensity Intermittent Exercise (HIIE).*

The MICE iso-work protocol was performed in the **fourth session**, when the volunteers cycled at a constant power corresponding to 60-70% of the HR_{peak} and finalized when the total work obtained in the HIIE was achieved.

Collection of biological samples

Biological samples were collected immediately before and after the exercise protocols. Ten milliliters of blood were drawn by venipuncture in vacuum tubes containing heparin. Urine samples were collected at the same time points of peripheral blood draw. Participants were instructed to obtain midstream clean catch specimens using 20 mL Global Plastic sterile tubes. Biological samples were kept on ice and processed within 30 minutes after being obtained.

Blood samples were centrifuged at 1,800*g* for 10 min, 4 °C, twice for plasma obtaining. The plasma was collected, aliquoted and stored at −80 °C until assayed.

Urine samples were transferred to 15mL plastic tubes and immediately centrifuged (1,800*g*, 5 minutes, room temperature). The cell-free supernatant was collected, aliquoted and stored at a -80 ºC freezer until analysis.

In order to rule out any confounding factors caused by circadian rhythm, all samples were collected at the same time of the day (between 8 – 11h).

Assessment of plasma/urinary levels of proteins

Cytokines [TNF, IL-6, IL-12p70, IL-8, IL-1β and IL-10] were measured by flow cytometry using the Cytometric Bead Array (CBA) Human Cytokines Kit (BD Biosciences, San Jose, CA, USA), following the manufacturer's instructions.

Acquisition was performed using a FACSCanto II flow cytometer (BD Biosciences). The instrument was checked for sensitivity and overall performance with Cytometer Setup & Tracking beads (BD Biosciences) prior to data acquisition. Quantitative results were generated using FCAP Array v3.0 software (Soft Flow Inc., Pecs, Hungary). The technicians responsible for the measurements were blind to the clinical status of the subjects.

Statistical analysis

All variables were tested for Gaussian distribution by the Shapiro-Wilk normality test. The effects of different exercise protocols (HIIE *vs.* MICE) in two different times (before *vs.* after

the exercise) on the levels of cytokines were compared using repeated-measures two-way ANOVA followed by the Bonferroni post hoc test. Spearman's correlation analyses were performed to examine the relationship between biomarkers changes (i.e., the difference between the biomarkers levels obtained after and before the exercises) and age, BMI, body fat, Wingate data (maximum power, average power and total work), VO_{2peak}, glucose, arterial pressure average and exercise total work. All statistical tests were two-tailed and were performed using a significance level of α=0.05. Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA), as well as GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, EUA).

Results

The physical and performance parameters of the volunteers are shown in Table 1 and Table 2 respectively

Table 1 – General physical characteristics of the participants.

Variable	Mean (Standard Deviation)
Age (years)	21.92 (±1.75)
Weight (Kg)	74.60 (±8.80)
BMI	24.13 (±1.95)
Fat (%)	10.27 (±3.50)

BMI – Body Mass Index.

Table 2 – Physical performance characteristics of the participants.

Variable	Mean (±Standard Deviation)
$VO_{2\ (peak)}$ (mL/Kg/min)	44.22 (±5.48)
Anaerobic Power $_{(peak)}$ (W/Kg)	9.33 (±0.72)
Anaerobic Power $_{(average)}$ (W/Kg)	6.95 (±0.64)
Fatigue Index (%)	28.51 (±5.57)
Total Work (KJ)	208.69 (±19.21)

$VO_{2\ (peak)}$ wasmeasured by a cycle-ergometer progressive exercise-to-fatigue test. The remaining variables (Anaerobic Power $_{(peak)}$, Anaerobic Power $_{(average)}$,Fatigue index and Total work were measured by the Wingate test.

To confirm the homogeneity between the exercise protocols, we found no difference on the total work [HIIE = 141.5 KJ (±18.3) and MICE = 142.8 KJ (±18.4), p=0,114, as shown in figure 2A. As expected, protocols duration (figure 2B) [HIIE = 21.69 (±1.1) and MICE = 38.62 (±4.4) p=0.0015;] and intensity [Heart Rate - HIIE = 164.7bpm (±14.1) and MICE = 110.5bpm (±11.6) p=0,002/ Power - HIIE = 306.2W (±54.7) and MICE = 62.8W (±0.03) p=0,001] were considerably different.

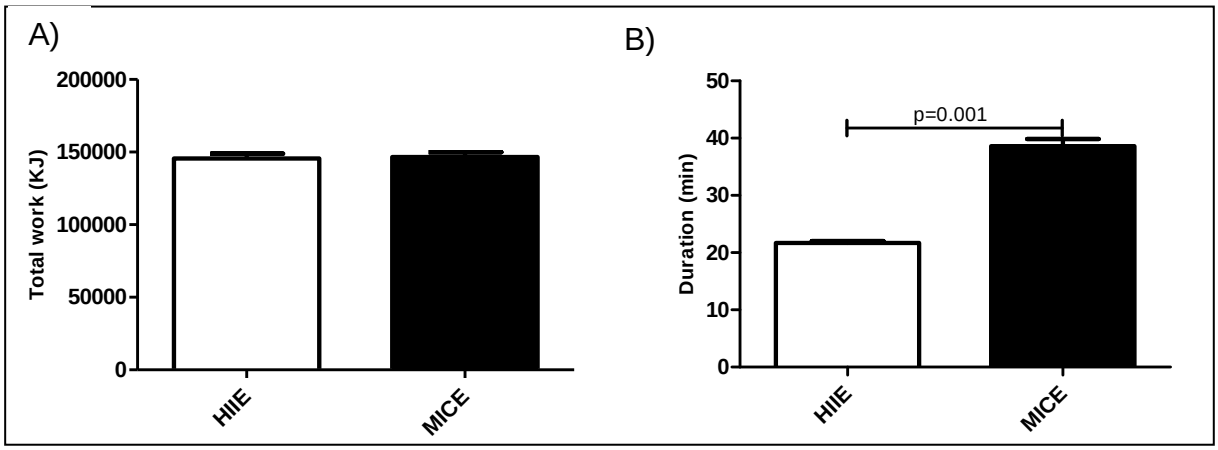

Figure 2 - *Characterization of exercise protocols.*The total work in HIIE and MICE was very similar. Significantly differences were detectedin the duration of exercise sessions between HIIE and MICE. p=0.001 (Wilcoxon signed rank test).

Plasma and Urinary levels of Cytokines

We did not find any difference in the comparison of baseline levels (before both exercise protocols) of all cytokines in plasma and urine sample, table 3. However, we found a significant and positive correlation (r =0.721, p=0.008) between TNF levels and total work in plasma samples of HIIE protocol as shown in figure 3. We did not find correlation between another parameters.

Table 3– Plasma and urine levels of cytokines before and after exercise protocols.

Variable	Exercise Protocol	Plasma		Urine	
		Before	After	Before	After
IL-12p70	HIIE	1.51	1.68	6.46	4.50
	MICE	1.54	1.57	5.60	7.01
TNF	HIIE	3.35	3.16	5.20	4.14
	MICE	8.76	3.77	4.55	5.75
IL-10	HIIE	2.63	2.77	5.46	4.40
	MICE	2.54	2.66	4.69	5.45
IL-6	HIIE	2.47	2.94	10.90	11.72
	MICE	2.60	2.76	24.88	17.31
IL-1β	HIIE	7.56	7.69	14.36	13.30
	MICE	6.52	7.14	13.57	17.58
IL-8	HIIE	10.58	11.00	11.42	12.60
	MICE	9.89	6.09	14.33	15.34

HIIE (High Intensity Interval Exercise); MICE (Moderate Intensity Continuous Exercise); IL (Interleukin); TNF (Tumor Necrosis Factor

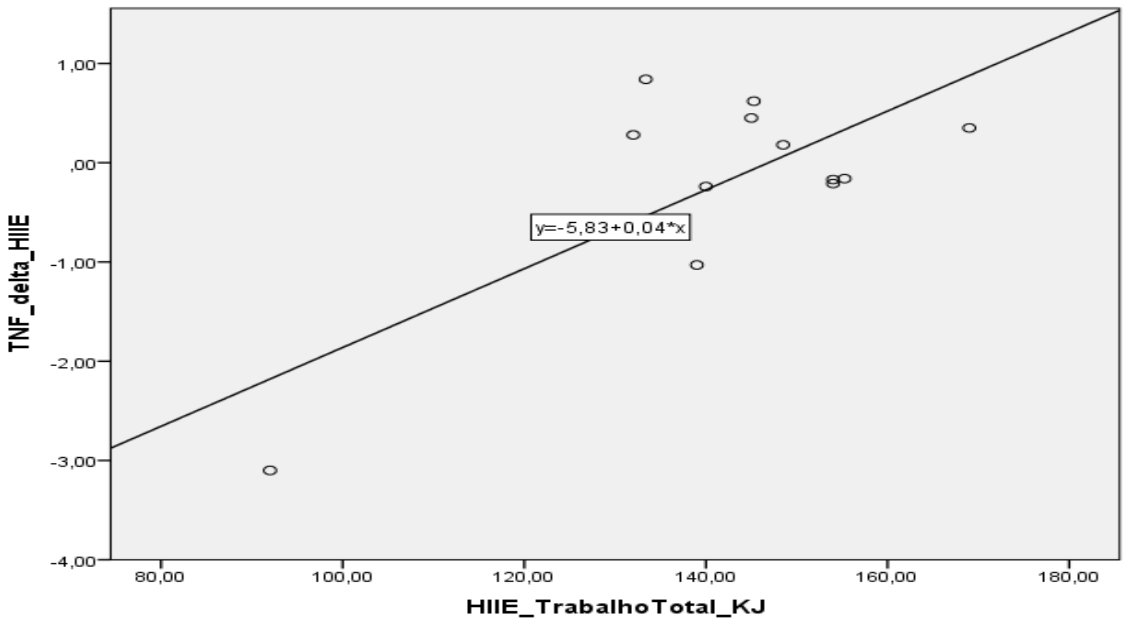

Figure 3:*Correlation between the variation of plasma TNF and total work in High Intensity Intermittent Exercise (HIIE) protocol*. Positive correlation between TNF delta (plasma levels after minus before exercise protocol) and total work in HIIE protocol was observed (r =0.721, p=0.008).

Discussion

In the present study, we did not find alterations in plasma and urinary levels of cytokines according to exercise protocols. In contrast with our results, previous studies have shown some changes in plasma levels of cytokines following exercise [9,20] and alterations in the ratio between IL-10 and TNF [9,21]. These differences may be attributed to in variable exercise protocols, time of sampling and methods of measurement.

On the other hand, our study clearly showed that the variation of plasma levels of TNF (after minus before exercise) significantly and positively correlated with total work produced by volunteers in the HIIE protocol. We did not find studies that measured work and reported association of this parameter with TNF modulation. Although this correlation has no cause and effect relationship, some studies that found increased post-exercise TNF may help us, in theory, explain our finding

TNF is an important mediator of inflammation that activates leukocytes, enhances adherence of neutrophils and monocytes to endothelium, promotes inflammatory cells migration into the intercellular matrix, stimulates fibroblast proliferation, and triggers local release of other inflammatory cytokines. During the early phases of infection, invasion, neoplastic transformation, injury, or wound healing, these inflammatory actions are thought to contribute to localization and infection walling off, cytotoxicity against malignant cells and facilitation of tissue remodeling[22]. In addition, this cytokine exerts effects on glucose and lipid metabolism by stimulating the lipolysis and glycogenolysis increase, thus mobilizing an energy substrate for skeletal muscle and other tissues after exercise[9].

TNF is produced by several cells, including macrophages, lymphocytes, polymorphonuclear leukocytes, eosinophils, astrocytes, Langerhans cells and Kupffer cells. In addition, TNF can be produced and released in adipose tissue and skeletal muscle. In adipose tissue, TNF is produced mainly by infiltrated macrophages and acts through two soluble receptors, sTNFR1 (p55) and sTNFR2 (p75). The binding of TNF to these receptors activates pathways responsible for insulin resistance and for other obesity-related complications[23]. In skeletal muscle, increased TNF expression has been detected in type II fibers and associated with the metabolic demand of muscle, and also with glycogen muscle replenishment after exhaustive exercise[20,24]. TNF is also associated with proteolysis and remodeling of skeletal muscle by promoting tissue accumulation of neutrophils and macrophages and by inducing the

production of reactive oxygen species from adherent neutrophils. These processes may contribute to muscle injury[20,25,26, 27,28]. An increase in contraction intensity also results in increased recruitment of type II muscle fibers, which is more recruited in HIIE than MICE[29,30,31]. Consequently, the recruitment of type II muscle fibers and the increased expression of TNF expression were more pronounced following HIIE protocol. This fact is in accordance with our finding that plasma levels of TNF were positively correlated with total work in HIIE protocol.

Previous Studies have shown increase in TNF after high intensity or longer duration exercise sessions[27,32,33]. The study of Starkier (2001)[32] showed a significant increase in plasma levels of TNF after prolonged running (marathon) and 2 hours post exercise. The authors also observed an increase in plasma concentrations of creatine kinase following exercise session (2 hours and 24 hours), suggesting that TNF release in response to exercise is related to muscle damage[32]. The 1.75-h cycling bout at 60% $watts_{max}$ combined with a 10km cycling time trial induced significant inflammation in a heterogeneous group of trained cyclists, but the magnitude was widely varied between subjects. Plasma levels of TNF increased after exercise and remained elevated at 1h post exercise. Exercise intensity (both $\%HR_{max}$ and RPE) was the best predictor of acute inflammatory response and correlated with plasma levels of cytokines, including TNF[27].

One session of cycle ergometer with high intensity acutely induced low-grade inflammation in young, recreationally active man[33]. HIIE promoted significant increases in TNF levels and two weeks of HIIE training did not alter this response. The authors hypothetized that this increase in TNF may be related to muscle proteolysis[33]. Cabral-Santos (2015)[9] and co-workers compared the effect of HIIE (5 Km run) versus volume matched steady state exercise (SSE) on inflammatory and metabolic responses in young men. The results showed that both exercise protocols promote different metabolic responses but similar inflammatory profiles. TNF was increased after both exercise protocols, while, in SSE protocol, high cortisol levels and increased in non-ester fatty acid (NEFA) concentrations were also detected, suggesting a lipolysis process after exercise[21]. Plasma levels of IL-10 were also increased immediately after exercise and remained elevated for 30 and 60 minutes after both protocols, while TNF concentrations reduced 30 minutes after both exercise protocols and continued stable at 60 minutes[21].

In conclusion, this homogeneous group of young healthy physical active men did not respond acutely to aerobic exercise protocols in cytokines modulation. This find shows that the protocols probable did not disturb the immunological homeostasis of these individuals. However, subjects who made more intense sprints in HIIE protocol may increase more the TNF plasma levels.

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6- ARTIGO 2

Adipokines and irisin levels in response to different intensity physical exercise protocols in young healthy men.

Daniel Massote Magalhães et al [1].

INTRODUCTION: Adipose tissue and skeletal muscle have been identified as endocrine organs due to their capacity to produce and release hormones, named adipokines and myokines, respectively. There are three classical adipokines, adiponectin, leptin and resistin, which, respectively, exert anti-inflammatory actions, food intake control and pro-inflammatory effects. Irisin is a myokine, released in response to muscle contraction that is involved in energy expenditure by heat production in adipose tissue. Currently, the modulatory effects of physical exercise on adipokines and irisin levels are not fully understood. Moreover, there are scarce data comparing protocols of physical exercise with different intensity in this process. Therefore, we assessed the acute effect of high and moderate intensity protocols of physical exercise on plasma and urine levels of adipokines and irisin. **METHODS:** Nine young healthy physically active men were recruited to four supervised training sessions. Two evaluation sessions were performed to measure body composition, physical activity, aerobic and anaerobic capacities before High Intensity Interval Exercise (HIIE) and Moderated Intensity Continuous Exercise (MICE) isowork exercise sessions on a cycle ergometer. The HIIE protocol invcluded a 5-minute warm-up at 60-70% of heart rate peak (HRp) intensity followed by 10 sets of 30 seconds above 90% with 1 minute of recovery and 3 minutes of cool down (both at the same warm-up power). MICE protocol was performed at a constant power corresponding to 60-70% of HRp and finalized at the same total work of HIIE. Blood and urine samples were collected before and after the protocols, then stored at -80°C for further analysis of irisin concentrations byenzyme-linked immunoassay (ELISA). **RESULTS:** Plasma levels of adipokines and of irisin did not differ in the comparison between baseline concentrations (before exercise protocols) and levels after both exercise protocols. Urine concentrations of resistin, leptin and irisin also did not differ at baseline and after both exercise protocols. However, urine levels of adiponectin significantly increased after HIIE protocol (p=0.0005). In addition, the concentrations of adiponectin in urine were significantly higher following HIIE than after MICE (p=0.0039).

CONCLUSION: Our study showed that the HIIE protocol induced a more intense increase in urine levels of adiponectin in comparison to MICE. This result suggests that HIIE may be an interesting training intervention in order to improve metabolic profile.

INTRODUCTION

Adipose tissue has several functions as energy storage, thermal insulation, and mechanical protection. However, this tissue is also considered an endocrine organ, which secretes bioactive peptides involved in autocrine, paracrine and endocrine functions, named as "adipokines"[1]. There are three classical adipokines: adiponectin, leptin and resistin, which, respectively, exert anti-inflammatory, anti-atherosclerotic and anti-diabetic actions; food intake and metabolism control; pro-inflammatory effects and increase in insulin resistance[2].

Skeletal muscle also has been identified as an endocrine organ due to its capacity to produce and release hormones, named "myokines", in response to contraction[3,4]. Irisin is a myokine formed by the cleavage of fibronectin domain-containing protein 5 (FNDC5), a membrane protein that is produced as a consequence of the increase in both peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)[5] and white adipose tissue[6]. Irisin has been recognized for important actions in adipose tissue by increasing the fat metabolism in response to physical exercise.

Currently, the modulatory effects of physical exercise have been linked with health improvement and prevention of type 2 diabetes and cardiovascular diseases[7]. However, the effect of exercise on adipokine and irisin levels has been contradictory in the literature[8,9,10]. Furthermore, there is scarce data comparing different intensities protocols of exercise. Therefore, we assessed the acute effect of high and moderate intensity protocols of physical exercise on plasma and urine levels of adipokines and irisin.

Methods

Participants

All participants (n=13) were adults (20-25 years old), healthy, of male gender and physically active based on the International Physical Activity Questionnaire (IPAQ) criteria.[11] Contraindications for physical exercise practices in moderate and high intensity were ruled out by anamnesis and by the Physical Activity Readiness Questionnaire (PAR-Q)[12]. Cyclists and individuals who had used CNS-stimulant drugs, anabolic steroids, corticosteroids, anti-

inflammatories or antibiotics in the four weeks prior to the study were ineligible. All volunteers included responded and signed the informed consent form. The study was approved by the Ethical Committee of our institution under the protocol (60070016.5.0000.5150).

Exercise Protocols

Preliminary measurements

- *Body composition*

Body circumferences were measured by anthropometric tape and the body fat was calculated by Jackson and Pollock (1978)[13] protocol with skinfold caliper (Lange ®). The Body Mass Index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (Kg/m^2).

- *Performance test*

The individuals were submitted to the Wingate test[14]to estimate their anaerobic power and capacity and to the VO_2 test - a cycle-ergometer progressive exercise-to-fatigue test[15] -to measure the aerobic capacity (VO_{2peak}) using a gas analyzer (BIOPAC®). In both tests the heart rate was measured by monitor Polar (Polar Team System, Finland) and the maximal heart rate in VO_2test (HR_{peak}) was registered. In VO_2test, the maximal power reached by volunteers was registered.

Exercise protocol

The exercise protocols were performed at the same time of the day (between 8 – 11h), the temperature and humid was maintained between 21°C – 24°C and 58% - 75% respectively. The protocols were equalized in amount of work [Kilo Joules – KJ]. The power [watts – W] and work was measured by MCE® software (Staniak, Polônia). Parameters of volume, intensity and total work are demonstrate in figure 1.

- *High Intensity Intermittent Exercise (HIIE)*

This protocol was composed by: I - Warm-up (5-minute at a constant power corresponding to 60-70% of the HR_{peak},); II – Principal phase (10 sets of 30 seconds above 90% HR_{peak} + 1 minute of active recovery at the same warm-up power); III - Cool down (3 minutes at the same constant warm-up power).

- *Moderate Intensity Continuous Exercise (MICE)*

The MICE protocol was performed at a constant power corresponding to 60-70% of the HR_{peak}.

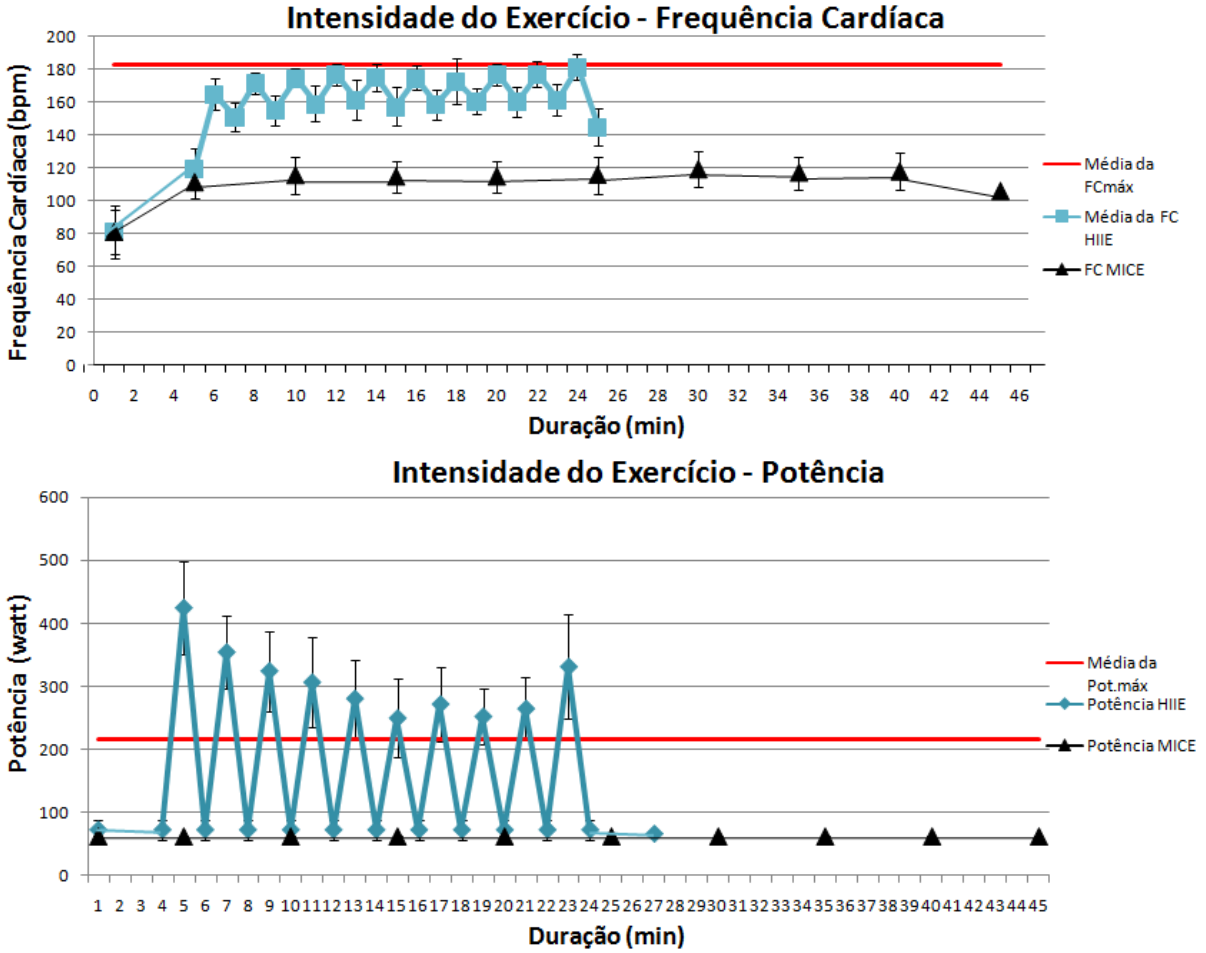

Figure 1- *Heart rate and power representative graphic of a volunteer*. Each parameter was measured in three situations: HIIE protocol, MICE protocol and VO_2test. MAX_1 is the mean of maximal heart rate measured at VO_2test and MAX_2 is the mean of maximal power measured at VO_2test.

Blood and urine sampling

Biological samples were collected immediately before and after the exercise protocols. Ten milliliters of blood were drawn by venipuncture in vacuum tubes containing heparin. Urine samples were collected at the same time points of peripheral blood draw. Participants were instructed to obtain midstream clean catch specimens using 20 mL Global Plastic sterile tubes. Biological samples were kept on ice and processed within 30 minutes after being obtained.

Blood samples were centrifuged at 1,800*g* for 10 min, 4 °C, twice for plasma obtaining. The plasma was collected, aliquoted and stored at −80 °C until assayed.

Urine samples were transferred to 15mL plastic tubes and immediately centrifuged (1,800*g*, 5 minutes, room temperature). The cell-free supernatant was collected, aliquoted and stored at a -80 ºC freezer until analysis.

In order to rule out any confounding factors caused by circadian rhythm, all samples were collected at the same time of the day (between 8 – 11h).

Assessment of plasma/urinary levels of proteins

Plasma and urine samples were thawed and the levels of adiponectin, leptin, resistin and irisin were measured by Enzyme-Linked Immunosorbent Assay (ELISA), according to the procedures supplied by the manufacturer (MyBioSource, San Diego, CA, USA). All kits applied the sandwich ELISA technique. Concentrations were expressed as pg/mL. The sensitivity of the asseys was 3,12 ng/ml for Irisin; 0,079-0,891 ng/mL for adiponectin; 7,8 pg/mL for leptina and 0.026 ng/mL for resistin. Experiments were performed blinded regarding exercise protocols.

Statistical analysis

All variables were tested for Gaussian distribution by the Shapiro-Wilk normality test. The effects of different exercise protocols (HIIE *vs*. MICE) in two different times (before *vs.* after the exercise) on the levels of adipokines and irisin were compared using repeated-measures two-way ANOVA followed by the Bonferroni post hoc test Spearman's correlation analyses were performed to examine the relationship between biomarkers changes (i.e., the difference between the biomarkers levels obtained after and before the exercises) and age, BMI, body fat, Wingate data (maximum power, average power and total work), VO_{2peak}, glucose, arterial pressure average and exercise total work. All statistical tests were two-tailed and were performed using a significance level of α=0.05. Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA), as well as GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, EUA).

Results

General physical characteristics and exercise performance parameters of the volunteers showed the homogeneity of the group. Physical characteristic [Age – 21.92 years (±1.75); Body mass – 74.60Kg (±8.80); Body Mass Index (BMI) – 24.13 (±1.95) and Body fat – 10.27% (±3.50)] and performance parameters of VO_2test [$VO_{2\ (peak)}$ – 44.22mL/Kg/min

(±5.48)] and Wingate test [Anaerobic Power $_{(peak)}$ – 9.33 W/Kg (±0.72); Anaerobic Power $_{(average)}$- 6.95 W/Kg (±0.64); Fatigue Index – 28.51 % (±5.57); Total Work – 208.69 KJ (±19.21)] did not significantly differ.

Both exercise protocols were homogeneous in regard to total work [HIIE = 141,5 (±18,33) and MICE = 142,8 (±18,45); p=0,114]. As expected, the duration [HIIE = 21,69 (±1,11) and MICE = 38,62 (±4,41) p=0,001;] and the intensity [Heart Rate - HIIE = 164.7bpm (±14.14) and MICE = 110.5bpm (±11.6) p=0,002/ Power - HIIE = 306.2W (±54.7) and MICE = 62.8W (±0.03) p=0,001;] were considerably different.

Levels of Biomarkers

We did not find significant difference in the irisin, leptin and resistin levels in plasma and urine samples as shown in Table 1. However, urine levels of adiponectin significantly increased after HIIE protocol (Figure 2).

Table 1 – Plasma and urine levels of irisin and adipokines before and after exercise protocols.

Variable	**Exercise**	**Plasma**		**Urine**	
	Protocol	Before	After	Before	After
Irisin	HIIE	14.61	11.61	706.72	404.10
	MICE	11.10	12.39	276.01	808.52
Adiponectin	HIIE	31461.13	37689.60	2003.63	39101.79*
	MICE	31776.71	38052.32	1522.07	4436.93
Leptin	HIIE	920.80	935.76	#	#
	MICE	935.00	907.33	#	#
Resistin	HIIE	8637.57	8995.74	4566.60	9214.38
	MICE	10438.80	9737.05	4849.80	6394.89

HIIE (High Intensity Interval Exercise); MICE (Moderate Intensity Continuous Exercise);
*P<0.001 relative to before exercise (Bonferroni posttests); # undetectable

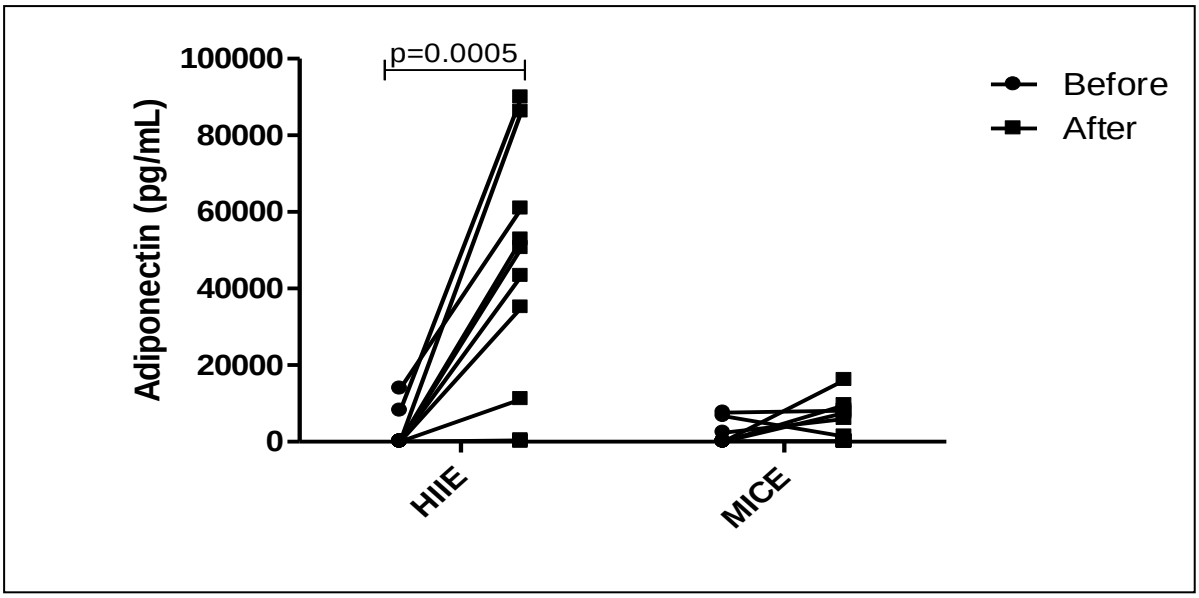

Figure 2: *Urinary levels of adiponectin according to exercise protocols.*High intensity interval exercise (HIIE) protocol significantly increased urinary levels of adiponectin (p=0.0005, Two-way ANOVA followed by Bonferroni test). Moderate intensity continuous exercise (MICE) did not change urinary levels of adiponectin (p>0.05, Two-way ANOVA followed by Bonferroni test).

Discussion

The aim of the present study was to evaluate the acute effect of a single bout of HIIE and MICE aerobic exercise on plasma and urinary levels of irisin and adipokines. We showed the absence of significant changes in irisin, leptin and resistin levels after both exercise protocols. However, we found a significant increase in urinary levels of adiponectin after HIIE protocol. To the best of our knowledge this is the first study that evaluated the role of exercise protocols in urinary levels of adipokines. It should be pointed that adipokines have been previously measured both in plasma and urine samples[17,18].

Adiponectin is secreted by adipose tissue and has anti-inflammatory, anti-atherosclerotic and anti-diabetic effects[19,20,21]. In macrophages, adiponectin inhibits the expression and secretion of TNF and increases the production of the anti-inflammatory cytokine IL-10[22,23]. This adipokine also reduces lipid accumulation in macrophage, thus inhibiting the foam cells formation and the development of atherosclerotic lesions[24]. Besides that, adiponectin activates 5-AMP-activated protein kinase (AMPK) and stimulates phosphorylation of acetyl coenzyme A carboxylase (ACC), fatty-acid oxidation, glucose uptake and lactate production in myocytes. The activation of AMPK can directly regulate glucose metabolism and insulin

sensitivity. The phosphorylation of ACC reduces molecules involved in gluconeogenesis in the liver, thus decreasing glucose levels[25,26].

Although it had been previously believed that adiponectin is produced exclusively by adipocytes, recent evidence indicated that adiponectin is also actively secreted by myocytes[27,28]. Moreover, Punyadeera et al (2005)[29] detected the presence of adiponectin by histochemical techniques in the sarcolemmas of skeletal muscle fibers after exercise and showed an abundant expression of adiponectin mRNA in muscle tissue[29].

The modulation exerted by physical exercise on adiponectin is still a matter of debate. Several studies have shown no significant changes in plasma levels of adiponectin after a single exercise session [30,31,32]. Other studies have shown variations in this protein levels in overweight or obese individuals [33,34]. We also found some studies[35,36,37]showing elevation of adiponectin levels after exercise in healthy individuals. However, in only two studies, this increase was maintained after correction for plasma volume, which expands after exercise. Jrime et al (2004)[36] investigated plasma adiponectin response to acute exercise in highly trained male rowers who performed a maximal 6,000-m rowing ergometer test. Adiponectin was decreased immediately after the exercise and was significantly increased above the resting value after 30 min of recovery. In another study by the same authors, the acute effects of volume-extended rowing training produced significant increase in plasma levels of adiponectin in athletes selected for a national team both immediately and 30 min post-exercise[37]. In contrast, no changes in adiponectin levels were detected in athletes of lower categories not selected for national team[37]. In both studies, the authors associated the increase in adiponectin levels to its potential role in energy homeostasis and muscle recovery[37,38]. This is a plausible hypothesis since adiponectin has potent effects on carbohydrate and lipid metabolism in skeletal muscle[38]. In addition, the authors related the differences between groups to the physical performance of the athletes (better physical condition group increases adiponectin). Furthermore, they speculated that the amount of muscle mass involved in exercise rowing (70% of the whole-body muscle mass) is greater than some modalities (running and cycling) assessed in others studies, which justifies the difference in their findings[37,38].

Although in the present study the group of volunteers was homogeneous and the physical performance were relatively similar, the amount of muscle mass involved in exercise can

justify the difference showed between HIIE and MICE, since both the activation of motor units and muscle mobilization during exercise increase along with the intensity[39,40,41].

In conclusion, our study showed that the HIIE protocol induced a more intense increase in urine levels of adiponectin in comparison to MICE. This result suggests that HIIE may be an interesting training intervention in order to improve metabolic profile.

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7- ARTIGO 3

Moderate intensity continuous and high intensity intermittent aerobic exercise acutely modulate ACE2-Ang(1-7)-MAS axis of the renin-angiotensin system in healthy subjects

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Abstract

INTRODUCTION: The renin-angiotensin system (RAS) is considered a dual acting system with two opposite arms: the classical one, formed by angiotensin converting enzyme(ACE), Angiotensin II (Ang II) and angiotensin type 1 (AT1) receptor that exerts vasoconstriction and pro-inflammatory actions, and the counter-regulatory, composed by ACE2, Angiotensin-(1-7) [Ang-(1-7)] and Mas receptor, which elicits vasodilation and anti-inflammatory effects. In this regard, experimental studies have suggested that changes in both RAS axesmay contribute to the beneficial role of physical exercise in chronic diseases related to inflammation[2]. However, there is no data comparing the effects of different intensity protocols of exercise on both RAS axes in healthy individuals. Therefore, we investigated the acute effect of two protocols of physical exercise in urine and plasma levels of RAS components. **SUBJECTS AND METHODS:** Teen young healthy physically active men were recruited to four supervised training sessions. Two evaluation sessions were performed to measure body composition, physical activity, aerobic and anaerobic capacities before High Intensity Interval Exercise(HIIE) and Moderated Intensity Continuous Exercise(MICE) iso-work exercise sessions on a cycle ergometer. The HIIE protocol included a 5-minute warm-up at 60-70% of heart rate peak (HRp) intensity followed by 10 sets of 30 seconds above 90% with 1 minute of recovery and 3 minutes of cool down (both at the same warm-up power). MICE protocol was performed at a constant power corresponding to 60-70% of HRp and finalized at the same total work of HIIE. Blood and urine samples were collected before and after the protocols, then stored at -80°C for further analysis by enzyme-linked immunoassay (ELISA). Plasma and urine levels of ACE, ACE2, Ang-(1-7) and Ang II were measured before(baseline values) and after both exercise protocols. **RESULTS:** We found following HIIE protocol a

significant increase of urine levels of and of plasma levels of ACE2 (p=0.0098 and p=0.0161, respectively). Urine concentrations of ACE2 and of Ang-(1-7) significantly raised after MICE protocol(p=0.0184 and p<0.0001, respectively). When comparing these variations in RAS components between both exercise protocols, a more intensereduction of plasma and of urine levels of ACE(p=0.0144andp=0.0042, respectively) was in line witha greater increase ofurine concentrations of Ang-(1-7)(p=0,0059)in MICE protocol. **CONCLUSIONS:**Larger decrease of ACE and higher increase of ACE2 and Ang-(1-7) following MICE protocol may indicate more intense stimulation of the counter-regulatory RAS axis in response to moderate intensity exercise. On the other hand, HIIE protocol exerted more intense effects on plasma levels of ACE, thus interfering with the classical RAS axis. Further studies are needed to elucidate the precise meaning of these acute changes in both RAS axes following exercise.

Introduction

The last 3 decades were marked by accumulated knowledge regarding the functions of physical exercise in health promotion and in the treatment of chronic diseases[1,2]. There is a worldwide consensus that the physical exercise practice presents a wide range of beneficial effects in major chronic disorders. Physical exercise helps in controlling hypertension (HT) and reduces the risk of cardiovascular diseases (CVD)[2,3,4,5,6,7]. Currently, it is known that aerobic exercise aids the blood pressure (BP) control even in cases of HT resistant to pharmacological treatment[8]. Hence, the American College of Sports Medicine (ACSM) and American Heart Association (AHA) recommend regular physical exercise for both prevention and treatment of HT and CVD[9].

In addition to controlling water and sodium homeostasis, the Renin-Angiotensin System (RAS) is an important regulator of inflammation, cardiovascular and renal functions, thus being tightly linked to HT and CVD[10]. To sum up, the RAS exerts its physiological responses by two opposite arms: (1) a classical one, composed by the angiotensin converting enzyme (ACE), angiotensin II (Ang II) and angiotensin type 1 (AT_1) receptor; and (2) a counter-regulatory arm, comprising an ACE homologue enzyme named ACE2[10,11], the heptapeptide angiotensin-(1-7) [Ang-(1-7)] and its G-protein coupled receptor, the Mas receptor[12].

Based on the physiological functions influenced by both physical exercise and RAS, it is tempting to hypothesize that they act together in cardiovascular system balance, improving some biological features as the blood pressure (BP). Therefore, preclinical studies have

evaluated physical exercise-induced changes on classical RAS axis components (i.e., ACE, AngII and AT_1 receptor). These changes were associated with a decrease in BP, increase in fractional urinary sodium excretion, decrease in creatinine clearance in addition to preventing left ventricle (LV) hypertrophy[13,14,15,16]. Authors have linked these outcomes with the downregulation of ACE, Ang II and AT_1 receptor induced by physical exercise[13,14,17]. Despite the interesting results obtained from animal studies, data from human samples are still scarce[18,19].

In addition to downregulating the classical RAS axis, physical exercise also resulted in upregulation of the counter-regulatory axis components (i.e., ACE2/ Ang(1-7) /Mas receptor). Recently, our group published a review showing the lack of information about exercise and its modulatory effect on counter-regulatory axis components in human sample[20]. Here again, conclusions came most from experimental data using mice and rats[21,22,23,24].

Among the great number of physical exercises types, we are interested in High Intensity Interval Training (HIIT), which is based on low volume protocols and 'near maximal' efforts generally performed at an intensity that elicits 80% (but often 85–95%) of maximal heart rate compared with Moderate Intensity Continuous Training (MICT). Some studies have highlighted the beneficial effects of HIIT, such as the increase in mitochondrial content, aerobic capacity and blood glucose regulation[25,26,27]. So far, no study has been conducted to evaluate the effects of HIIE and MICE on the RAS or investigated which training method is more efficient in RAS components modulation. Hence, this study was designed to investigate the acute effects of physical exercise on RAS components and to improve our understanding on how they can interact together to improve cardiovascular function. We assessed plasma and urinary concentrations of RAS components right before and after two different protocols of physical exercise, HIIE and MICE in healthy individuals.

Material and methods

Participants

Thirteen healthy physically active (IPAQ criteria)[28] non-cyclist men [Age = 21.92 year (±1.75); Weight = 74.60 Kg (±8.80); Body Mass Index = 24.13 (±1.95); Body Fat = 10.27% (±3.50] did 4 sessions of physical exercise. All volunteers responded and signed the informed consent after understanding about the risks, discomforts and benefits for participle in the

present study. The study was approved by Research Ethics Committee of the *Universidade Federal de Ouro Preto*, Brazil under the protocol (60070016.5.0000.5150).

Exercise Protocols

Session 1. The subjects answered: I-IPAQ[28] quantifies the physical activity; II-Anamnesis (general health conditions); III- Physical Activity Readiness Questionnaire (PAR-Q)[29] for assess readiness to exercise. Finally, they did the Wingate test[30]to estimate their anaerobic power and capacity.

Session 2. Body circumferences and the body fat [according to Jackson and Pollock (1978)[31]using a skinfold caliper (Lange®)] were measured. In addition the Body Mass Index (BMI) (Kg/m^2) was calculated. The VO_{2peak} was measured by a cycle-ergometer progressive exercise-to-fatigue test according to Balke & Ware et al (1959)[32]. The maximal heart rate measured on this test (HR_{peak}) was registered by monitor Polar (Polar Team System, Finland)..

Session 3. The HIIE protocol was performed. [5 minutes warm-up (60-70% HR_{peak}) + 16 minutes principal phase (10 sets 30 > 90% HR_{peak} + 1 minute of active recovery after each set) + 3 minutes cool down (the same power of warm-up). We registred the power (W) and the work (KJ) by MCE®software (Staniak, Polônia). The total work obtained in the HIIE protocol was the same obtained in the MICE protocol.

Session 4. The MICE protocol (constant power corresponding to 60-70% of the HR_{peak}) was performed and finalized when the total work obtained in the HIIE was achieved.

Biological samples obtaining

Biological samples were collected immediately before and after the exercise protocols. Ten milliliters of blood were drawn by venipuncture in vacuum tubes containing heparin. Urine samples were collected at the same time points of peripheral blood draw. Participants were instructed to obtain midstream clean catch specimens using 20 mL Global Plasticsterile tubes. Biological samples were kept on ice and processed within 30 minutes after being obtained.

Blood samples were centrifuged at 1,800*g* for 10 min, 4 °C, twice for plasma obtaining. The plasma was collected, aliquoted and stored at −80 °C until assayed.

Urine samples were transferred to 15mL plastic tubes and immediately centrifuged (1,800*g*, 5 minutes, room temperature). The cell-free supernatant was collected, aliquoted and stored at a -80 ºC freezer until analysis.

In order to rule out any confounding factors caused by circadian rhythm, all samples were collected at the same time of the day (between 8 – 11h).

Assessment of plasma/urinary levels of proteins related to the RAS

Plasma and urine samples were thawed and the levels of Ang II, Ang-(1-7), ACE and ACE2 were measured by Enzyme-Linked Immunosorbent Assay (ELISA), according to the procedures supplied by the manufacturer (MyBioSource, San Diego, CA, USA). All kits applied the sandwich ELISA technique, except for ACE measurement whose kit applied the competitive ELISA method. Concentrations were expressed as pg/mL. The sensitivity of the assays was 1.0 pg/mL for ACE and ACE2; 2.0 pg/mL for Ang-(1-7); and 18.75 pg/mL for Ang II. Experiments were performed blinded regarding exercise protocols.

Statistical analysis

All variables were tested for Gaussian distribution by the Shapiro-Wilk normality test. The effects of different exercise protocols (HIIE *vs.* MICE) in two different times (before *vs.* after the exercise) on the levels of RAS proteins were compared using repeated-measures two-way ANOVA followed by the Bonferroni post hoc test**.** We also calculated the ratios of molecules representing the counter-regulatory/classical arms of the RAS [i.e. ACE2/ACE and Ang(1-7)/AngII ratios]. The differences between the ratios were calculated using the paired t-test or the Wilcoxon signed rank test when the variables were normally or non-normally distribute, respectively. Spearman's correlation analyses were performed to examine the relationship between biomarkers changes (i.e., the difference between the biomarkers levels obtained after and before the exercises) and age, BMI, body fat, Wingate data (maximum power, average power and total work), VO_{2peak}, glucose, arterial pressure average and exercise total work. All statistical tests were two-tailed and were performed using a significance level of α=0.05. Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA), as well as GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, EUA).

Results

Physical performance parameters of the volunteers did not differ. The mean and standard deviation of parameters were: VO_2test [$VO_{2\ (peak)}$ – 44.22mL/Kg/min (±5.48)] and Wingate test [Anaerobic Power $_{(peak)}$ – 9.33 W/Kg (±0.72); Anaerobic Power $_{(average)}$- 6.95 W/Kg (±0.64); Fatigue Index – 28.51 % (±5.57); Total Work – 208.69 KJ (±19.21)]. Total work was homogeneous in both exercise protocols [HIIE = 141.5 ± 18.33 KJ and MICE = 142.8KJ ±18.45 KJ; p=0.023]. As expected, protocols duration [HIIE = 21.69 ± 1.11 min and MICE = 38.62 ± 4.41 min; p=0.001 and intensity [Heart Rate - HIIE = 164.74 ± 14.14 bpm and MICE = 110.5 ± 11.60 bpm; p=0,002; Power - HIIE = 306.22 ± 54.71 W and MICE = 62.8 ± 0.03 W; p=0.001] were significantly different. We did not find any difference between both exercise protocols regarding the mean arterial pressure.

Plasma levels of RAS components

The MICE protocol resulted in a decrease in plasma levels of ACE [(Figure 1; p<0.05, Two-way ANOVA followed by Bonferroni test). On the other hand, the HIIE protocol resulted in a significant increase in plasma levels of ACE2 [(Figure 1; Two-way ANOVA followed by Bonferroni test). Plasma levels of Ang II and Ang-(1-7) were not influenced by HIIE or MICE protocols (no significant results were obtained in the two-way ANOVA tests). The ratio ACE2/ACE also was significantly altered in MICE protocol [(Figure 2) before = 0.63 ± 0.41 and after = 0.91 ± 0.60; p<0,05; paired t-test].

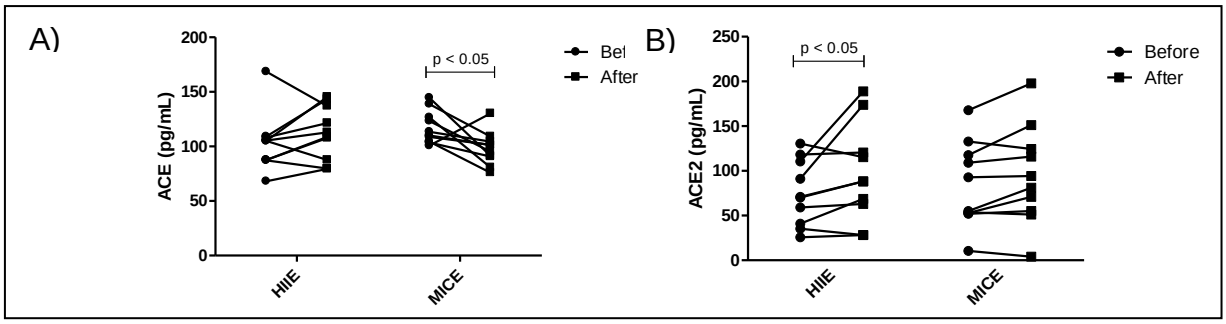

Figure 1- *Plasma levels of renin-angiotensin components before and after each exercise.*Panel A shows an decrease of plasma ACE in MICE protocol and panel B displays an increase of plasma ACE2 after HIIE protocol.

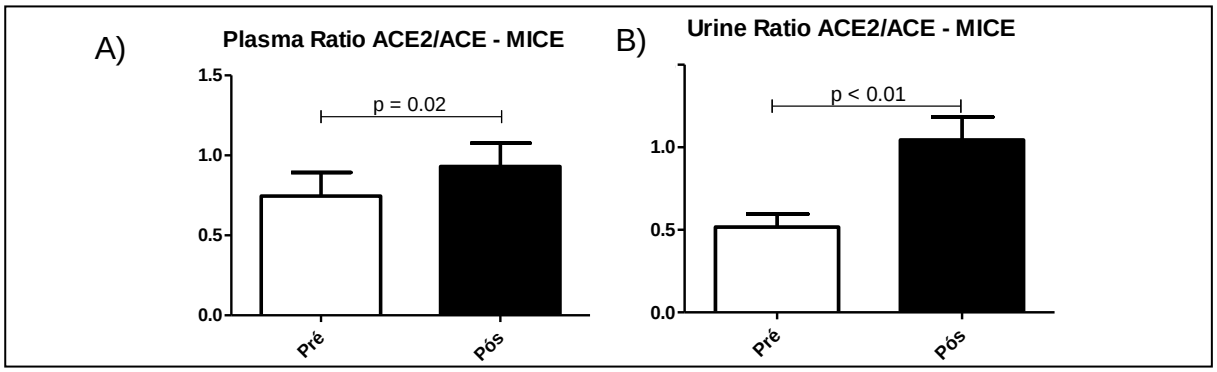

Figure 2 - *The ratio between angiotensin converting enzyme (ACE) 2 and ACE of moderate intensity continuous exercise (MICE).* Panel A shows an increase in the ACE2/ACE ratio after (Ps) in comparison to baseline (Pre) values in plasma sample. Panel B show an increase in the ACE2/ACE ratio after (Ps) in comparison to baseline (Pre) values in urine sample.

Urinary levels of RAS components

The urine analyses showed an increase in ACE levels following the HIIE (Figure 3; $p<0.05$ Two-way ANOVA followed by Bonferroni test). Urinary levels of ACE2 were increased after MICE protocol (Figure 3; $p<0.05$ Two-way ANOVA followed by Bonferroni test). The effect of MICE protocol on RAS enzymes was also confirmed by the analyses of the ratios between ACE2 and ACE as shown in Figure 2. Urinary levels of AngII were very low in all individuals and in both exercise protocols. No differences were found between times and/or exercise protocols. On the other hand, both protocols increased urinary levels of Ang-(1-7)(Figure 3; $p<0.05$ and $p<0.001$ for HIIE and MICE, respectively, Two-way ANOVA followed by Bonferroni test).

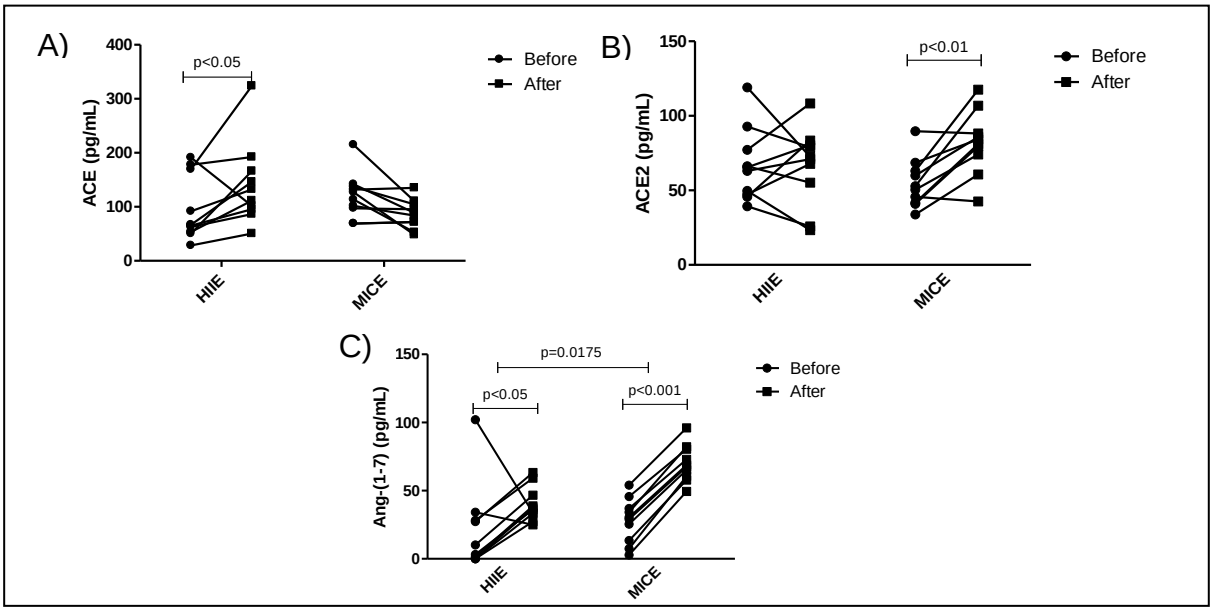

Figure 3 - *Urinary levels of renin-angiotensin components before and after each exercise*.Panel A shows an increase of urinary levels of ACE in HIIE protocol.Panel B shows an increase of urinary ACE2 levels after MICE protocol. Panel C shows an increase of urinary Ang-(1-7)levels in both exercise protocols.

Discussion

To the best of our knowledge, this is the first study to evaluate the acute effect of aerobic exercise protocols on RAS biomarkers levels in human samples. We found that the HIIE protocol increased ACE2 levels in plasma and ACE and Ang-(1-7) levels in urine. Differently, the MICE protocol decreased plasma levels of ACE and increased both ACE2 and Ang-(1-7) levels in urine,proving that the aerobic exercises can modulate the RAS, especially the country-regulatory axis of this system. The counter-regulatory arm of RAS has several beneficial effects like anti-inflammatory, vasodilation, antiproliferation, anti-hypertrophy, cardioprotective, neuromodulatory and renoprotective actions[34].In addition, the stimulation of ACE2/Ang-(1-7)/Mas axis seems to prevent the occurrence of diabetes and improves metabolic profile[34]. Although our study did not evaluate any tissue adaptation, there are some animal studies that showed an increase in the activity of the counter-regulatory RAS axis in line with an inhibition of the classical RASaxis improved cardiovascular function[16,21,22,35-44].

Some studies have shown that physical exercise promoted physiological hypertrophy while inhibited pathological hypertrophy (i.e., when myocardial hypertrophy is accompanied by fibrosis and impairment in cardiac function)[35,36]. These adaptations reduced or prevented

the increase of ACE activity[16,21,37], ACE expression[21,37] and Ang II levels[16,21,37,38.] Furthermore, pre-clinical studies showed that physical exercise increases ACE2 activity[21,37], ACE2 expression[16,21,37], Ang-(1-7) levels[21,39,40] and Ang-(1-7) expression[38].

In regard to tissue remodeling, two authors investigated RAS components after physical training and its association with arterial and pulmonary remodeling. In the study of Gu et al. (2014)[41], the expression of ACE and Ang II levels suffered a reduction, whereas the expression of ACE2 and Ang-(1-7) levels increased after exercise in arterial tissue of spontaneous hypertensive rats (SHR). These changes were associated with a reduction of BP and a decrease in aortic weight/length, wall thickness, and aortic levels of elastin and hydroxyproline[41]. Moreover, Prata et al. (2016)[22] showed that physical training associated with the administration of an ACE2 activator significantly reduced pulmonary fibrosis and type I collagen induced by bleomycin when compared with the non-trained groups.

The arterial vasodilation capacity and the reduction of BP are important cardiovascular effects that can be influenced by physical exercise and by the modulation RAS components. In this regard, Silva et al. (2011)[42] showed an increase in thevasodilator effect of Ang-(1–7), added to the endothelium of the aorta of SHR after swimmingtraining. The vasodilator effect involved nitric oxide and prostacyclinrelease and did not occur in response to acetylcholine (Ach)[42]. This effect was reduced by the selective Ang-(1–7) receptor antagonist, the compound A-779[42]. Silva Jr et al. (2015)[43] concluded in a study on running training that physical exercise downregulated Ang II and Ang-(1-7) levels in the SHR artery. It was also observed a significant reduction of the AngII/Ang-(1-7) ratio, which occurred simultaneously with a significant fall in BP (5%). The authors concluded that physical training reduces the levels of the two major RAS mediators in Wistar Kyoto (WKY) rats, maintaining the balance between both axesat a lower level[43].

The BP control and vasodilation are important capacities for chronic heart failure (CHF) patients. Gomes-Santos et al (2014)[44] showed that running training in rats with CHFinduced by left coronary artery ligation reduced serum ACE activity and plasma levels of Ang II. On the other hand, exercise increased serum ACE2 activity and plasma Ang-(1–7)/Ang II ratio in these animals[44]. Training also reduced plasma levels of Ang II in sham-operated animals. Furthermore, these authors showed a reduction of Ang II concentration in soleus and plantaris muscles, a reduction of the expression of AT_1 receptor in the soleus muscle and an increase of Ang-(1–7) in the plantaris muscle of CHF rats[44]. Although we did

not investigate RAS components in skeletal muscles, previous studies show that Ang-(1-7) decreases atrophy, reduces insulin resistance, induce AKT phosphorylation, increases skeletal muscle glucose uptake, restores muscle strength in dystrophic muscle, improves locomotor phenotypes in muscular dystrophy, inhibits TGF-β signaling and decreases p38 MAPK phosphorylation[34].

The present study revealed that aerobic physical exercise acutely increases the activity of the RAS counter-regulatory pathway, mostly the MICE protocol. These findings contribute to our understanding on the role of exercise in the modulation of RAS components. It also suggests that the stimulation of the counter-regulatory RAS axis may take part in the beneficial effects of physical exercise. Future studies focusing on the evaluation of the chronic effect of different exercise protocols in RAS molecules both in ill patients and in healthy individuals are necessary. It would provide valuable information about the adaptability and responsiveness of RAS after several training sessions, as well as the clinical relevance of these protocols in the treatment of these patients.

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8. CONSIDERAÇÕES FINAIS

O exercício físico aeróbico alterou as concentrações plasmáticas e urinárias de parte dos biomarcadores investigados. Durante o protocolo de exercício de alta intensidade (HIIE), os indivíduos que realizaram maior trabalho no cicloergômetro, tiveram maiores níveis de TNF circulante no período pós-exercício. Esta modulação sugere um estímulo inflamatório dependente do esforço, induzido pelo exercício de alta intensidade e, provavelmente, relacionado à proteólise muscular. No entanto, é necessário a investigação de moléculas relacionadas à lesão tecidual, como, por exemplo, a creatina fosfoquinase, para melhor elucidar este desfecho.

O protocolo HIIE promoveu um aumento nas concentrações de adiponectina, sugerindo uma provável influência da intensidade do exercício no perfil metabólico.

O eixo contra regulatório do sistema renina-angiotensina também foi estimulado pelo exercício aeróbico. Embora os dois protocolos tenham aumentado os níveis de ECA2 e de Ang (1-7), o protocolo MICE promoveu uma resposta de maior magnitude comparado ao HIIE. Apesar de serem necessários estudos sobre os efeitos crônicos do exercício, nossos resultados podem contribuir para o entendimento dos mecanismos envolvidos nas adaptações cardiovasculares e renais promovidas pelo exercício aeróbico. Além disso, faz-se necessária a investigação dos efeitos do exercício físico em indivíduos com doenças relacionadas ao comprometimento do eixo ECA2/Ang(1-7)/Mas para identificar se há potencial terapêutico do exercício aeróbico para esta população.

ANEXOS

ANEXO 1 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Nome do voluntário participante:

_____

Efeito agudo do exercício aeróbico sobre biomarcadores plasmáticos e urinários em indivíduos saudáveis.

Introdução:Você está sendo convidado a participar desta pesquisa. É muito importante que você leia e compreenda a seguinte explicação sobre os procedimentos propostos antes de aceitar participar da mesma. Esta declaração descreve o objetivo, procedimentos, benefícios e riscos do estudo, e o seu direito de sair do estudo a qualquer momento. Nenhuma garantia ou promessa pode ser feita sobre o resultado do estudo. Estas informações estão sendo dadas para esclarecer quaisquer dúvidas sobre a pesquisa, antes de obter o seu consentimento. Se ainda assim persistirem dúvidas a respeito do estudo, você pode perguntar, em qualquer momento, aos pesquisadores responsáveis.

Objetivo:O objetivo deste estudo é avaliar a quantidade de determinadas substâncias (adipocinas/ miocinas/ citocinas/ componentes do sistema renina-angiotensina) que podem ser medidas no sangue e urina das pessoas antes e após realizar exercício físico. Esse estudo tenta saber se a quantidade dessas substâncias muda depois de fazer exercício aeróbico. Essa medida da quantidade das substâncias antes e depois do exercício poderá ajudar a entender os benefícios do exercício aeróbico à saúde.

Resumo: A falta de exercícios físicos regularmente pode gerar muitas complicações à saúde como obesidade, diabetes, hipertensão arterial, infarto do coração, derrame cerebral e encefálicos e depressão. Quando a pessoa tem algumas dessas doenças ou complicações, podem aparecer no sangue e na urina algumas substâncias (adipocinas/ miocinas/ citocinas/ componentes do sistema renina-angiotensina). Pode ser que o fato de a pessoa se exercitar modifique a quantidade dessas substâncias. No entanto, a falta de tempo para se dedicar à prática de exercício físico é uma justificativa muito utilizada pelas pessoas. Com o objetivo de sugerir alternativas a essas pessoas, pretendemos estudar os efeitos para a saúde do exercício aeróbico de curta duração e alta intensidade comparado com os efeitos do exercício de

duração e intensidades moderadas. O entendimento dos efeitos de diferentes tipos de exercício aeróbico sobre essas substâncias pode contribuir para a indicação adequada do exercício como forma de prevenção e tratamento de doenças associadas ao sedentarismo.

Procedimentos: Este estudo inclui avaliação clínica padronizada realizada por profissional capacitado. No primeiro encontro com o voluntário, este profissional aplicará alguns questionários e testes para saber sobre as condições de saúde dos indivíduos e sua prontidão para o exercício físico. A aplicação desses testes e questionários não traz riscos ao voluntário e o único incômodo refere-se ao tempo de duração da entrevista, que pode durar de 20 a 40 minutos. Posteriormente, será realizado um teste (teste de Wingate) de aproximadamente 10 minutos para avaliar a capacidade anaeróbica do voluntário. No segundo encontro será realizada uma avaliação antropométrica com duração de aproximadamente 30 minutos, seguida de teste (teste adaptado de Balke) para avaliar a capacidade aeróbica do voluntário. Este teste terá a duração de aproximadamente 20 minutos. No terceiro e no quarto encontros, cinco minutos antes e cinco minutos após os diferentes de exercícios, serão coletados 10 mL de sangue e um frasco de urina com material descartável apropriado. Esse sangue será encaminhado para o estudo laboratorial. A coleta de sangue venoso implica em risco mínimo de acidente de punção, caracterizado por extravasamento sanguíneo para o tecido debaixo da pele, provocando uma pequena "mancha roxa" no local. Para minimizar este risco, a coleta de sangue será realizada por profissional treinado, com capacidade técnica e experiência que estará atento e tomará todas as providências necessárias. Em todos os encontros, serão realizados protocolos de exercício aeróbico/anaeróbico no cicloergômetro. O exercício físico oferece poucos riscos para esta população, dentre eles, existe risco, ainda que muito pequeno, de alteração na pressão arterial, desmaio e alteração do ritmo cardíaco. No entanto, para minimizar estes riscos, serão realizadas avaliações prévias para conhecer o estado de saúde, histórico de atividade física e posteriormente estratificar o risco para a realização do exercício físico. No caso de alguma informação negativa em relação à prontidão para o exercício físico, o voluntário não realizará os protocolos propostos. Além disso, estarão disponíveis equipamentos emergenciais e equipe treinada para lidar com situações incomuns que possam surgir.

Critérios de inclusão: Adultos jovens que não apresentem nenhuma contra-indicação ao exercício físico de intensidade moderada e alta.

Critérios de exclusão: Indivíduos com contra-indicação à prática de exercício moderado-intenso, como problemas ortopédicos, metabólicos, renais, pulmonares e cardiovasculares. Pessoas que tenham história de qualquer doença crônica e que usem diariamente qualquer tipo de medicamento. Serão também excluídas temporariamente pessoas que estejam com alguma infecção, inflamação e/ ou alergia no momento de participar da pesquisa. Também serão excluídos indivíduos treinados (tecnicamente) em ciclismo além de indivíduos que estiveram sob o uso de anti-inflamatórios e antibióticos nas ultimas quatro semanas.

Benefícios: Não haverá compensação financeira pela sua participação, nem remuneração financeira do pesquisador, cujo interesse é apenas científico. O participante não terá nenhum benefício direto além de estar contribuindo para o desenvolvimento científico e a melhor compreensão dos efeitos do exercício aeróbico à saúde. A participação no estudo também não implicará em ônus financeiro (despesas) para você.

Confidencialidade: Os registros de sua participação neste estudo serão mantidos confidencialmente até onde é permitido por lei e todas as informações estarão com a equipe responsável pelo projeto. No entanto, o pesquisador e, sob certas circunstâncias, o Comitê de Ética em Pesquisa/UFOP, poderão verificar e ter acesso aos dados confidenciais que o identificam pelo nome. Qualquer publicação dos dados não o identificará. Ao assinar este formulário de consentimento, você autoriza o pesquisador a fornecer seus registros médicos para o Comitê de Ética em Pesquisa/UFOP, além da divulgação dos dados desta para o meio científico desde que não haja quebra de confidencialidade.

Desligamento: A sua participação neste estudo é voluntária e sua recusa em participar ou seu desligamento do estudo não terá penalidades ou perda de benefícios aos quais você tem direito. Você poderá cessar sua participação a qualquer momento.

Emergência / contato com a Comissão de Ética: É garantido seu direito a tirar dúvidas que surgirem durante o estudo. Assim, se você tiver qualquer dúvida ou apresentar qualquer problema médico, contate a Profa. Ana Cristina Simões e Silva pelo telefone 3409-8073, Daniel Massote Magalhães, pelo telefone 3409-8073 ou o Comitê de Ética em Pesquisa (CEP/UFOP) no telefone *(31)3559-1368*. O CEP/UFOP localiza-se no *Morro do Cruzeiro-ICEB II, Sala 29 -PROPP/UFOP*, Campus Universitário, Ouro Preto, CEP *35.400-000*.

Consentimento: Li e entendi as informações precedentes. Tive a oportunidade de fazer perguntas e todas as minhas dúvidas foram respondidas a contento. Este formulário está sendo

assinado voluntariamente por mim, indicando o meu consentimento para participar do estudo, até que eu decida o contrário.

TERMO DE CONSENTIMENTO

Declaro que, após convenientemente esclarecido (a) pelo pesquisador e ter entendido o que me foi explicado, autorizo a coleta de 10 mL de sangue e um frasco de urina para ser utilizado na pesquisa descrita acima.

Data: ____/____/____

Assinatura do voluntário participante:
_____

Nome pesquisador responsável:
_____

Data: ____/____/_____

Assinatura do pesquisador responsável:

_____

ANEXO 2 – PARECER CEP/UFOP

UNIVERSIDADE FEDERAL DE OURO PRETO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos de diferentes protocolos de exercício físico, agudos e crônicos no comportamento, qualidade de vida, marcadores imunológicos, inflamatórios, de estresse oxidativo e do sistema renina angiotensina.

Pesquisador: Albená Nunes da Silva

Área Temática:

Versão: 4

CAAE: 60064216.5.0000.5150

Instituição Proponente: Universidade Federal de Ouro Preto

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.881.170

Apresentação do Projeto:

"Pesquisa interinstitucional (UFMG, UFOP, UNIBH) cujo objetivo será avaliar o efeito, em biomarcadores no sangue, tanto de uma sessão de exercício físico quanto do treinamento crônico. Prevê 80 participantes voluntários (estudantes do centro universitário UNIBH Belo Horizonte) serão submetidos á uma sessão de treino e as amostras sanguíneas serão coletadas imediatamente antes e após uma sessão de treino. Os participantes também serão submetidos a um período de 8 semanas de treinamento e as amostras serão coletadas, antes, no meio (semana 4) e ao final do treinamento crônico de 8 semanas. A coleta será realizada no Laboratório de biomecânica e a sessão de treinamento acontecerá no laboratório de musculação, ambos localizados no Centro Desportivo da UFOP(CEDUFOP) sob a supervisão de um ou mais profissionais de educação física devidamente treinados. Em seguida, as amostras serão conduzidas para o Laboratório de Imunobiologia da Inflamação (LABIIN, UFMG) e para o Laboratório Piloto de Análises Clínicas da Farmácia(LAPAC/UFOP).

As análises das mesmas moléculas serão realizadas antes e após este período de treinamento."

Objetivo da Pesquisa:

"Objetivo Primário:

Avaliar e analisar o efeito de diferentes protocolos de exercício físico, agudos e crônicos no

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UF: MG Município: OURO PRETO
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comportamento, qualidade de vida, marcadores imunológicos, inflamatórios, de estresse oxidativo e do sistema renina angiotensina.

Objetivo Secundário:

Objetivo 1- Quantificar as células do sistema imunológico (neutrófilo, basófilo, neutrófilo e monócito) em voluntários submetidos a um protocolo de treinamento de força na musculação com duração de 08 semanas.Objetivo 2- Quantificar a produção de biomarcadores plasmáticos e urinários (CCL2, IL-8, CCL5, IL-1, IL-2, IL-4, IL-6, IL-10, IL-17 e TNF) em voluntários submetidos a um protocolo de treinamento de força na musculação com duração de 08 semanas.Objetivo 3- Quantificar marcadores de lesão muscular (CK, LDH e escala subjetiva de dor) em um protocolo de treinamento de força na musculação com duração de 08 semanas.Objetivo 4- Avaliar o equilíbrio oxidante redutor através da análise das substâncias reativas do ácido tiobarbitúrico (TBARS), relação nitrito/nitrato, glutationa reduzida (GSH) capacidade antioxidante do plasma (CAP) e produtos de oxidação proteica (AOPP).Objetivo 5- Avaliar a hipertrofia muscular e o aumento da capacidade física força após um período de 08 semanas de treinamento na musculação.Objetivo 6- Avaliar os níveis plasmáticos de Angiotensina II, Angiotensina-(1-7), enzima conversora de angiotensina (ECA) e ECA2 após um período de 08 semanas de treinamento de hipertrofia na musculação.Objetivo 7- Avaliar comportamento humano e qualidade de vida em praticantes de atividade física."

Avaliação dos Riscos e Benefícios:

"Riscos:

Este estudo respeitará todas as normas estabelecidas pelo Conselho Nacional em Saúde envolvendo pesquisas com seres humanos (Resolução 466/2012) e somente terá início após aprovação pelo Comitê de Ética em Pesquisa em Seres Humanos (UFOP). Antes de iniciarem qualquer atividade neste projeto, os voluntários receberão todas as informações quanto aos objetivos, ao processo metodológico, bem como os possíveis riscos e benefícios de participação no estudo. Caso aceitem participar, os voluntários assinarão um Termo de Consentimento Livre e Esclarecido (TCLE) no qual tomarão ciência de que a qualquer momento poderão deixar de participar da pesquisa. Serão tomadas precauções no intuito de preservar a privacidade dos voluntários, sendo que a saúde e o bem-estar estarão sempre acima de qualquer outro interesse. Todos os procedimentos adotados neste estudo estão de acordo com as "Diretrizes e Normas Regulamentadoras das Pesquisas Envolvendo Seres Humanos" do Conselho Nacional da Saúde (Res. 196/96) envolvendo pesquisas com seres humanos. A coleta será realizada no Laboratório de biomecânica e a sessão de treinamento acontecerá no laboratório de musculação, ambos

localizados no Centro Desportivo da UFOP(CEDUFOP) sob a supervisãode um ou mais profissionais de educação física devidamente treinados. Em seguida, as amostras serãoconduzidas, em condições de transporte de amostras biológicas padrão, para o Laboratório de Imunobiologia da Inflamação (LABIIN) e para o Laboratório Piloto de Análises Clínicas da Farmácia(LAPAC). A realização deste estudo envolve os riscos gerais relacionados à prática de exercícios físicos,como lesões músculoesqueléticas e traumatismo. Porém, a freqüência com que esses eventos ocorrem em condições laboratoriais é mínima.

Benefícios:

Com os dados gerados por este estudo, será possível um melhor entendimento do efeito de uma sessão de treinamento de força na musculação em biomarcadores inflamatórios e de estresse oxidativo, o que permitirá um melhor entendimento da carga de treinamento e suas consequências. Com estes dados será possível analisar a melhor maneira de aplicar uma sessão de treino, bem como no tempo necessário para recuperação. Ainda será possível fazer inferências sobre os níveis de citocinas e as possíveis respostas adaptativas geradas por estes estímulos."

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante.

Considerações sobre os Termos de apresentação obrigatória:

Termos apresentados e adequados.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_773635.pdf	14/12/2016 07:40:38		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_UFOP.docx	14/12/2016 07:39:32	Albená Nunes da Silva	Aceito
Outros	CARTA_CEP1.doc	14/12/2016 07:39:16	Albená Nunes da Silva	Aceito

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Outros	APOIO_LAMOV.jpg	12/12/2016 23:36:25	Albená Nunes da Silva	Aceito
Outros	APOIO_LAPAC.jpg	26/10/2016 15:07:21	Albená Nunes da Silva	Aceito
Outros	APOIO_IRMAZIO.pdf	26/10/2016 13:23:08	Albená Nunes da Silva	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DETALHADO.docx	24/10/2016 13:05:26	Albená Nunes da Silva	Aceito
Outros	APOIO_UNI.pdf	23/10/2016 17:25:39	Albená Nunes da Silva	Aceito
Outros	Gasto.doc	23/10/2016 16:41:12	Albená Nunes da Silva	Aceito
Declaração de Pesquisadores	APOIO_LABFE.pdf	14/09/2016 12:52:43	Albená Nunes da Silva	Aceito
Declaração de Pesquisadores	APOIO_LIIM.pdf	14/09/2016 12:52:16	Albená Nunes da Silva	Aceito
Folha de Rosto	Folha_Rosto.pdf	14/09/2016 10:25:38	Albená Nunes da Silva	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

OURO PRETO, 23 de Dezembro de 2016

Assinado por:
Núncio Antônio Araújo Sól
(Coordenador)

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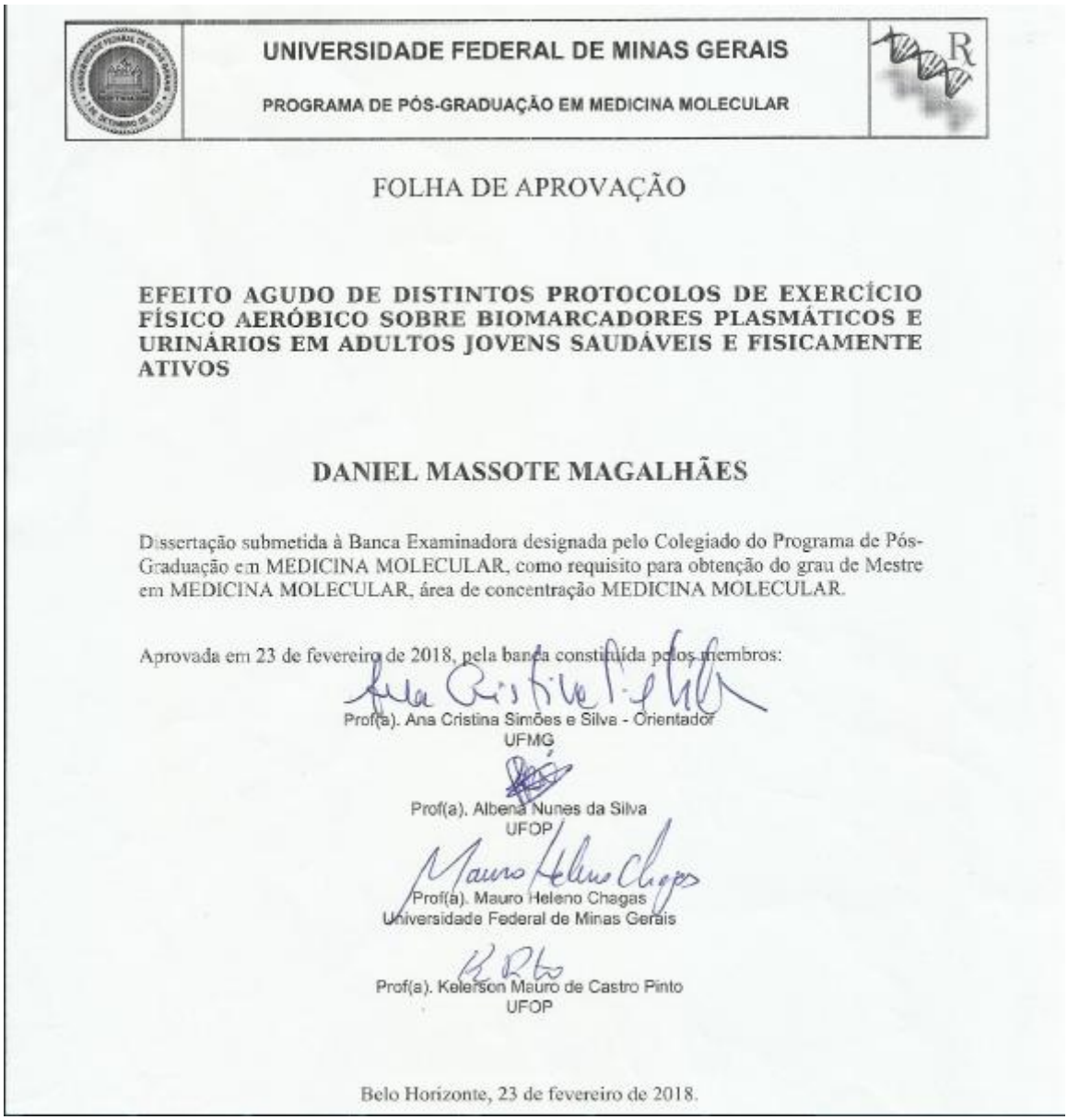
ANEXO 3 – DECLARAÇÃO DE APROVAÇÃO DA DEFESA



UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA MOLECULAR

FOLHA DE APROVAÇÃO

EFEITO AGUDO DE DISTINTOS PROTOCOLOS DE EXERCÍCIO FÍSICO AERÓBICO SOBRE BIOMARCADORES PLASMÁTICOS E URINÁRIOS EM ADULTOS JOVENS SAUDÁVEIS E FISICAMENTE ATIVOS

DANIEL MASSOTE MAGALHÃES

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em MEDICINA MOLECULAR, como requisito para obtenção do grau de Mestre em MEDICINA MOLECULAR, área de concentração MEDICINA MOLECULAR.

Aprovada em 23 de fevereiro de 2018, pela banca constituída pelos membros:

Prof(a). Ana Cristina Simões e Silva - Orientador
UFMG

Prof(a). Albena Nunes da Silva
UFOP

Prof(a). Mauro Heleno Chagas
Universidade Federal de Minas Gerais

Prof(a). Kelerson Mauro de Castro Pinto
UFOP

Belo Horizonte, 23 de fevereiro de 2018.

APÊNDICE

I - Artigos Publicados:

- MANTOVANI, R. M. ROCHA, N. P., **MAGALHÃES, D. M**., BARBOSA, I. G., TEIXEIRA, A. L., & SIMÕES, A. C. Early changes in adipokines from overweight to obesity in children and adolescents. **Jornal de Pediatria (Versão em Português)**, v. 92, n. 6, p. 624-630, 2016.
- NUNES-SILVA, A.,ROCHA, G. C., **MAGALHAES, D. M**., VAZ, L. N., DE FARIA, S., Henrique, M., & SIMOES E SILVA, A. C.. Physical Exercise and ACE2-Angiotensin-(1-7)-Mas Receptor Axis of the Renin Angiotensin System. **Protein and peptide letters**, v. 24, n. 9, p. 809-816, 2017.

II – Apresentação dos resultados em eventos internacionais (pôsteres):

1 - 13TH INTERNATIONAL SOCIETY OF EXERCISE AND IMMUNOLOGY SYMPOSIUM - Coimbra, Portugal;

2 - III SIMPÓSIO INTERNACIONAL DE IMUNOLOGIA NO ESPORTE – São Paulo, Brasil.

- CHANGES IN INFLAMMATORY MOLECULES FOLLOWING MODERATE INTENSITY CONTINUOUS AND HIGH INTENSITY INTERMITTENT ACUTE EXERCISES IN YOUNG HEALTHY MEN

Daniel Massote Magalhães; Guilherme Carvalho Rocha; Lucas Neves Vaz; Marcelo Henrique Salviano de Faria; Albená Nunes-Silva; Natália Pessoa Rocha; Erica Leandro Marciano Vieira; Ana Cristina Simões e Silva

- PLASMA AND URINE LEVELS OF IRISIN IN RESPONSE TO MODERATE INTENSITY CONTINUOUS AND TO HIGH INTENSITY INTERMITTENT ACUTE EXERCISES

Daniel Massote Magalhães; Lucas Neves Vaz; Guilherme Carvalho Rocha; Erica Leandro Marciano Vieira; Marcelo Henrique Salviano de Faria; Natália Pessoa Rocha; AlbenáNunesSilva; Ana Cristina Simões e Silva

- ADIPOKINES LEVELS IN RESPONSE TODIFFERENT INTENSITY PHYSICAL EXERCISE PROTOCOLS IN YOUNG HEALTHY MEN

Daniel Massote Magalhães; Lucas Neves Vaz; Guilherme Carvalho Rocha; Natália Pessoa Rocha; Marcelo Henrique Salviano de Faria; Erica Leandro Marciano Vieira; AlbenáNunesSilva; Ana Cristina Simões e Silva

- PHYSICAL EXERCISE WITH DIFFERENT INTENSITIES ACUTELY MODULATES BOTH AXES OF THE RENIN-ANGIOTENSIN SYSTEM IN HEALTHY SUBJECTS

Daniel Massote Magalhães; Guilherme Carvalho Rocha; Lucas Neves Vaz; Albená NunesSilva; Marcelo Henrique Salviano de Faria; Erica Leandro Marciano Vieira; Natália Pessoa Rocha; Ana Cristina Simões e Silva