

Carolina Cabral Santos

**RESPOSTA METABÓLICA E INFLAMATÓRIA NO EXERCÍCIO
AERÓBIO MODERADO CONTÍNUO E INTERMITENTE DE ALTA
INTENSIDADE COM VOLUME EQUALIZADO**

Presidente Prudente

2015

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MODERADO CONTÍNUO E INTERMITENTE DE ALTA INTENSIDADE COM
VOLUME EQUALIZADO**

Dissertação apresentada à Faculdade de Ciências e Tecnologia - FCT/UNESP, campus de Presidente Prudente, para obtenção do título de Mestre no Programa de Pós-Graduação em Fisioterapia.

Orientador: Profº Dr Fábio Santos de Lira

Presidente Prudente

2015

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE CAROLINA CABRAL SANTOS, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM FISIOTERAPIA, DO(A) FACULDADE DE CIÊNCIAS E TECNOLOGIA DE PRESIDENTE PRUDENTE.

Aos 30 dias do mês de outubro do ano de 2015, às 14:00 horas, no(a) Anfiteatro, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Prof. Dr. FABIO SANTOS DE LIRA do(a) Departamento de Educação Física / Faculdade de Ciências e Tecnologia de Presidente Prudente, Prof. Dr. EDUARDO ZAPATERRA CAMPOS do(a) Faculdade de Ciências e Tecnologia de Presidente Prudente, Prof. Dr. GUSTAVO DUARTE PIMENTEL do(a) Universidade Federal de Goiás, sob a presidência do primeiro, a fim de proceder a arguição pública da DISSERTAÇÃO DE MESTRADO de CAROLINA CABRAL SANTOS, intitulada "RESPOSTA METABÓLICA E INFLAMATÓRIA EM EXERCÍCIO AERÓBIO MODERADO CONTÍNUO E INTERMITENTE DE ALTA INTENSIDADE COM VOLUME EQUALIZADO". Após a exposição, a discente foi arguida oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final: **Aprovada**. Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.


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APRESENTAÇÃO

Esta dissertação é composta de uma introdução, dois artigos científicos originados de pesquisas realizadas no Laboratório de Fisiologia Celular do Exercício (LAFICE), do Departamento de Educação Física da FCT/UNESP – Presidente Prudente, conclusão a partir de ambas pesquisas, referência bibliográfica utilizadas na introdução e anexo as normas dos periódicos. Em consonância com as regras do Programa de Pós-Graduação em Fisioterapia, os artigos foram redigidos de acordo com as normas das revistas que foram submetidos, exceto pelas figuras, que foram inseridas no corpo do texto.

Artigo 1

“Physiological acute response to 5-km running performance in high-intensity intermittent and moderate-intensity continuous”

Carolina C. Santos, José Gerosa-Neto, Daniela S. Inoue, Fabrício E. Rossi, Jason M. Cholewa, Eduardo Z. Campos, Fábio S. Lira.

Submetido à apreciação no “International Journal of Sports Medicine”

Artigo 2

“Similar anti-inflammatory acute response of moderate-intensity continuous and high-intensity intermittent exercise”

Carolina Cabral Santos, José Gerosa-Neto, Daniela Sayuri Inoue, Valéria Leme Gonçalves Panissa, Luís Alberto Gobbo, Alessandro Moura Zagatto, Eduardo Zapaterro Campos, Fábio Santos Lira.

Publicado no “Journal of Sports Science and Medicine”

RESUMO

Com o objetivo de investigar as respostas fisiológicas do exercício moderado contínuo (MICE) versus exercício intermitente de alta intensidade (HIIE) com volume equalizado, 12 sujeitos fisicamente ativos completaram dois protocolos experimentais em ordem randomizadas: 5km de corrida em esteira de modo contínuo (MICE: 70% vVO_{2max}) ou de modo intermitente (HIIE: 1×1 a 100% vVO_{2max}). Amostras sanguíneas, consumo de oxigênio (VO_2), concentração de lactato, percepção subjetiva de esforço (PSE), frequência cardíaca (FC) e gasto energético pós-exercício (EPOC) foram coletados em repouso, durante e 60 minutos após cada sessão de exercício. As concentrações de lactato exibiram maiores valores imediatamente no momento pós-exercício quando comparado com o repouso (HIIE: 1.43 ± 0.25 para 7.36 ± 2.78 ; MICE: 1.64 ± 1.01 para 4.05 ± 1.52 mmol•L⁻¹, $p=0.0004$), porém HIIE promoveu maior elevação ($p=0.001$). Houve diferença no tempo de consumo de O₂ em todos os momentos analisados em ambos os grupos ($p<0.001$). Ambos protocolos promoveram aumento do EPOC (HIIE: 6.61 ± 1.85 L; MICE: 5.32 ± 2.39 L, $p<0.005$), verificou-se maiores valores no HIIE, porém o tempo total do exercício pode ter contribuído para este achado. O HIIE foi mais efetivo em modificar a FC e PES RPE (HIIE: 183 ± 12.54 e 19; MICE 172 ± 8.5 e 16, respectivamente). Em resumo, ambos protocolos apresentaram diferenças em FC, PSE e concentração de lactato quando a distância percorrida foi equalizada. Como esperado, a contribuição metabólica também apresentou diferenças, uma vez que o HIIE induziu um gasto energético maior, porém, a duração total do exercício deve ser levada em consideração. Além disso, quando utilizado o protocolo de exercício moderado contínuo, a porcentagem do sVO_{2pico} e o do limiar anaeróbio podem influenciar o exercício e a resposta fisiológica do treinamento.

Palavras-chaves: Exercício intermitente de alta intensidade; exercício moderado contínuo; Respostas fisiológicas; Gasto energético.

ABSTRACT

To investigate the physiological responses to moderate-intensity continuous, and high-intensity intermittent exercise (MICE and HIIE), 12 physically active male subjects completed two experimental sessions in randomized order; a 5-km run on a treadmill continuously (70% sVO₂peak) or intermittently (1:1 min at sVO₂max). Oxygen uptake, EPOC, lactate concentration, rating perceived exertion (RPE) and heart rate (HR) data were recorded during and after each session. The lactate levels exhibited higher values immediately post-exercise than at rest (HIIE: 1.43 ± 0.25 to 7.36 ± 2.78 ; MICE: 1.64 ± 1.01 to 4.05 ± 1.52 mmol•L⁻¹, $p=0.0004$), but HIIE promotes higher values ($p=0.001$, $\eta^2=0.69$). There was difference across time on VO₂ consumption of all moments tested in both groups ($p<0.001$, $\eta^2=0.98$), both exercise conditions promote increased in EPOC (HIIE: 6.61 ± 1.85 L; MICE: 5.32 ± 2.39 L, $p<0.005$), with higher values in HIIE, however exercise time may have contributed to this differences. HIIE was more effective in modify HR and RPE (HIIE: 183 ± 12.54 and 19; MICE 172 ± 8.5 and 16, respectively). In conclusion, same distance MICE and HIIE exhibits different HR, lactate concentration and RPE. As expected the metabolic contribution also differed, and HIIE induced higher energy expenditure. However, total session duration may be taken into account, moreover, when using moderate-intensity training, the percentage of sVO₂peak, and anaerobic threshold might influence exercise and training responses.

Keywords: Physiologic Responses, Energy Expenditure, Lactate Concentration, High Intensity Intermittent Exercise, Acute Exercise.

INTRODUÇÃO

A prática regular de exercícios físicos impõe uma série de desafios as vias bioenergéticas e a musculatura esquelética em atividade, resultado em adaptações metabólicas e funcionais específicas a fim de otimizar os diferentes sistemas recrutados durante a atividade contrátil. Porém, essas adaptações são dependentes do tipo de exercício e suas características, como volume e intensidade do esforço.

Sabe-se que a magnitude e a utilização das vias metabólicas tem uma relação linear com a intensidade do exercício, ou seja, à medida que a intensidade do exercício aumenta, ocorre aumento progressivo do metabolismo de carboidratos e diminuição do metabolismo de gordura. Isso se deve principalmente ao maior recrutamento de fibras rápidas (ricas em enzimas glicolíticas e poucas enzimas lipolíticas e mitocôndrias) (Ball, 2015) e concentrações sanguíneas crescentes de adrenalina (aumentando a atividade de fosforilase e aumento na quebra de glicogênio muscular) (Lapin, 2007).

O exercício físico promove a elevação da lipólise do tecido adiposo pela ação de hormônios lipolíticos (adrenalina, noradrenalina, glicocorticoides e hormônio do crescimento e glucagon) liberados principalmente durante os exercícios de longa duração (Lapin, 2007). Porém exercícios de alta intensidade e curta duração utilizam primariamente as vias metabólicas anaeróbias para ressintetizar a molécula de adenosina trifosfato (ATP) necessária para a contração muscular. Durante esse tipo de esforço, a ATP é ressintetizada, predominantemente, pela degradação da fosfocreatina e do glicogênio muscular com consequente formação de lactato (Medbo, 2006;

Caputo, 2009). Ainda, estímulos anaeróbios constantes podem favorecer o maior gasto energético pós-exercício por manter a taxa metabólica basal em concentrações elevada, utilizando a gordura proveniente do tecido adiposo como principal fonte de substratos e proporcionando um desbalanço entre lipólise e lipogênese (Caputo, 2009).

As concentrações de lactato têm sido amplamente utilizadas para se estimar a contribuição do metabolismo glicolítico durante o exercício físico (Bertuzzi, 2015), onde observa-se concentrações sanguíneas estáveis e baixas (~ 1 mmol/L) durante o exercício moderado e contínuo uma vez que a sua remoção é mais rápida. Porém, no exercício de alta intensidade e pouco volume, a produção aumentada de lactato inibe o metabolismo de gordura e diminui sua disponibilidade como substrato energético, determinando assim o uso dos carboidratos como fonte primária de energia (Ball, 2015).

Classicamente, exercícios aeróbio realizados de maneira contínua e prolongada, são preconizados para potencializar melhora da capacidade cardiorrespiratória e redução dos depósitos de gordura corporal. No entanto, mais recentemente, têm demonstrado que exercícios aeróbios realizados de maneira intermitente e em alta intensidade, nomeados como HIIE ou HIIT (do inglês para *high intensity intermitten exercise* ou *training*), também direcionam para melhora das mesmas variáveis que o exercício contínuo (Gibala, 2006). Têm sido averiguado nesse modelo de treinamento uma elevação na oxidação da glicose, elevação dos estoques de glicogênio, maior capacidade de transporte de lactato da fibra muscular para a circulação sanguínea nos músculos exercitados. Entretanto, os protocolos utilizados nos diferentes estudos não levam em consideração a carga interna e externa (volume

exercício) quando comparam exercícios aeróbios contínuos e intermitentes (Skelly et al, 2014).

Além das alterações metabólicas, o exercício físico agudo e/ou exaustivo, também promove aumento dos marcadores inflamatórios que agem como mediadores entre os diferentes sistemas atuantes do organismo. Várias citocinas (pequenos polipeptídios com papel imunoregulatório) facilitam o influxo de linfócitos, neutrófilos, monócitos e demais células para o foco da inflamação tecidual e/ou sistêmica (Pedersen, 2009). De acordo com a resposta inflamatória desencadeada, as citocinas são divididas em dois grandes subgrupos de caráter pró-inflamatórias (como IL-1ra e IL-10) e citocinas anti-inflamatórias (como o TNF- α).

Pontualmente, a IL-6 possui efeitos imunomodulatórios peculiar e atua de forma pleiotrópica no organismo. A IL-6 age via receptor na célula alvo e associa-se a proteína transmembrana gp130, permitindo a transdução de sinal, entretanto, pode também se ligar a receptores solúveis presentes no sangue (Wunderlich, 2013), assim pode-se inferir que a IL-6 atue em todas as células do organismo.

Recentemente, descobriu-se que a ativação da enzima calcineurina dentro do miócito, provocada pela necessidade do aumento de influxo de Ca^{2+} , para possibilitar a contração muscular, é uma etapa essencial para estimular a produção de mRNA e proteína IL-6 (Banzet et al, 2007).

Uma vez que o aumento na concentração de IL-6 é dependente da intensidade, volume, duração do exercício físico e da aptidão física do sujeito (Pedersen, 2009), o principal responsável pela sua produção e liberação são as fibras musculares e sua expressão é modulada pelo exercício físico agudo.

Estudos demonstraram a relação do aumento da IL-6 sobre diferentes parâmetros metabólicos relacionados com a manutenção da homeostase energética durante a sessão de exercício físico. A IL-6 circulante estimula a glicogenólise e a gliconeogênese hepática fornecendo uma quantidade maior de glicose na circulação para ser captada pelo músculo esquelético. Sua elevação propicia aumento na captação de glicose devido à uma maior translocação do transportador do citoplasma para membrana plasmática, aumentando também a oxidação deste substrato e disponibilizando energia para a contração muscular (Pal et al, 2014). Em incubação de músculo esquelético humano, na presença de IL-6 recombinante humana, em concentrações semelhantes às encontradas no exercício, detectou-se a fosforilação de diversas enzimas associadas ao aumento da captação e oxidação intramuscular de glicose e ácidos graxos, via PI3K e AMPK (Al-Khalili et al, 2006).

No entanto, em casos inflamação crônica de baixo grau, como observado na obesidade e na síndrome metabólica, a IL-6 pode aumento de ácidos graxos livres circulantes nesses pacientes (Pedersen, 2009). Adicionalmente, a IL-6 modula processos anti-inflamatórios, já que esta citocina é capaz de aumentar a produção de IL-10, IL-1ra e inibir a produção de TNF- α em humanos (Neto, 2009). Sendo assim, a IL-6 é considerada uma citocina chave para a melhor compreensão da interação entre os diferentes tecidos.

Em contrapartida, na família das consideradas pró-inflamatórias, destaca-se o fator de necrose tumoral alfa (TNF- α). Produzido principalmente por monócitos e macrófagos ativados, além de outras células, como linfócitos, fibroblastos, neutrófilos, músculo liso e mastócitos, esta citocina pode atuar

sobre quase todo os tipos de células nucleadas, através de dois tipos de receptores de membrana, o tipo I (RTNF-I, p55) e o tipo II (RTNF-II, p75) ou ainda, como molécula solúvel. O aumento da transcrição gênica do TNF- α é mediado pela via fator de transcrição nuclear kappa B (NF- κ B). Quando não estimulado, NF- κ B encontra-se no citoplasma ligado a uma proteína inibitória: o I κ B que impede a translocação do NF- κ B para o núcleo.

O aumento da expressão gênica e proteica de TNF- α no músculo esquelético é uma importante resposta inicial, em decorrência de microlesões ocasionadas pelo exercício físico, visto que o aumento desta citocina promove o acúmulo de neutrófilos e macrófagos a fim de auxiliar no remodelamento da fibra muscular (Neto, 2009). No entanto, se o TNF- α permanecer elevado por um período prolongado pode prejudicar a ativação dos mecanismos de regeneração (Pedersen, 2012).

Adicionalmente, o TNF- α é um importante regulador dos processos metabólicos durante o exercício, porém sua elevação crônica, induz a fosforilação de IRS-1 e IRS-2 em resíduos de serina e treonina, causando prejuízo na sinalização da insulina, tanto em adipócitos como em miócitos (Hotamisligil, 1996). Além disso, o TNF- α é capaz de atuar sobre o metabolismo lipídico, aumentando a disponibilidade de ácidos graxos livres na circulação através do processo de lipólise, via mecanismo dependente da HSL (lipase hormônio sensível) e modificar o padrão de perilipinas no tecido adiposo (Cawthorn et al, 2008). Ainda, diminui a expressão da LPL (enzima lipase lipoproteica) neste tecido e reduzindo a hidrólise dos triacilglicerol das lipoproteínas (quilomícrons e VLDL) e a captação e acúmulo de ácidos graxos (Yang et al, 2011). Com base nesse fato, podemos deduzir que a elevação do

TNF- α no tecido adiposo e na musculatura esquelética, durante e/ou após a sessão de exercício, parece contribuir no desenvolvimento muscular e na manutenção da homeostase metabólica.

A Interleucina 10 (IL-10), de características anti-inflamatórias, é produzida por uma série de diferentes tipos celulares, em especial por células inflamatórias como macrófagos e linfócitos T, para as quais é o principal inibidor da síntese de citocinas e da atividade funcional de macrófagos. Sua atividade biológica é mediada através de seu receptor de membrana (IL-10R) e a ação da IL-10 reflete sua ação pela ativação da via JAK-STAT, especificamente sobre a JAK1 e STAT3 em macrófagos, e essa ativação é dependente da SOCS3 (Murray, 2007).

O mecanismo proposto para explicar o efeito anti-inflamatório da IL-10 no exercício agudo se deve a sua propriedade de inibir a produção de várias citocinas pró-inflamatórias, como a TNF- α , ao aumentar a liberação dos receptores solúveis do TNF- α (TNFR), os quais podem antagonizar os efeitos do TNF- α dificultando sua ligação nos receptores. Essa linha de raciocínio sugere que a IL-10 atuaria como um mecanismo de retroalimentação negativa, contrabalanceando o excesso de citocinas pró-inflamatórias.

Hoje em dia, utiliza-se a razão TNF- α /IL-10 como marcador do estado inflamatório, pois se considera essa razão mais importante na avaliação do quadro inflamatório do que a concentração isolada de cada uma dessas citocinas. Redução nessa razão é correlacionada com pior prognóstico e diminuição na expectativa de vida de pessoas que possuem diferentes morbidades (Neto, 2009).

As doenças metabólicas frequentemente observadas na sociedade moderna têm um elo comum, dentre as diferentes condições, que é um persistente quadro inflamatório crônico de baixo grau. Elevações sutis nas concentrações de mediadores inflamatórios podem deflagrar condições de quadro de resistência à ação da insulina, dislipidemia, proteólise acentuada, entre outras disfunções (Pedersen, 2009). Em contrapartida, o envolvimento com diferentes programas de treinamento físico, principalmente de caráter aeróbio, favorece em parte a melhora do quadro metabólico e inflamatório.

OBJETIVO

- a) Averiguar as respostas metabólicas e fisiológicas no exercício aeróbio moderado contínuo comparado ao exercício intermitente de alta intensidade com o volume equalizado;
- b) Averiguar a resposta imunometabólica frente ao exercício intermitente de alta intensidade.

ARTIGO 1

Physiological acute response to 5-km running performance in high-intensity intermittent and moderate-intensity continuous: implications for training prescription

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Abstract

The aim of this study was to investigate the physiological responses to moderate-intensity continuous and high-intensity intermittent exercise. We recruit 12 physically active male subjects completed a 5-km run on a treadmill in two experimental sessions in randomized order: continuously (70% VO₂max) or intermittently (1:1 min at VO₂max). Oxygen uptake, excess post-exercise oxygen consumption, lactate concentration, heart rate and rating perceived exertion data were recorded during and after each session. The lactate levels exhibited higher values immediately post-exercise than at rest (High-Intensity: 1.43 ± 0.25 to 7.36 ± 2.78 ; Moderate-Intensity: 1.64 ± 1.01 to 4.05 ± 1.52 mmol•L⁻¹, $p=0.0004$), but High-Intensity promotes higher values ($p=0.001$, $\eta^2=0.69$) than Moderate-Intensity. There was difference across time on oxygen uptake of all moments tested in both groups ($p<0.001$, $\eta^2=0.98$). Both exercise conditions promote increased in excess post-exercise oxygen consumption (High-Intensity: 6.61 ± 1.85 L; Moderate-Intensity: 5.32 ± 2.39 L, $p<0.005$), but higher values observed in High-Intensity. High-Intensity was more effective in modify heart rate and rating perceived exertion (High-Intensity: 183 ± 12.54 and 19; Moderate-Intensity: 172 ± 8.5 and 16, respectively). In conclusion, in same distance, Moderate-Intensity and High-Intensity exhibits different lactate concentration, heart rate and rating perceived exertion. As expected the metabolic contribution also differed, and High-Intensity induced higher energy expenditure, however, total duration of session may be take into account. Moreover, when using moderate-intensity training, the percentage of sVO₂max, and anaerobic threshold might influence exercise and training responses.

Keywords: Physiologic Responses, Energy Expenditure, Lactate Concentration, High Intensity Intermittent Exercise.

INTRODUCTION

The implementation of low to moderate intensity and long duration continuous efforts has been classically prescribed for the maintenance or improvement of aerobic capacity and health promotion in different population (Haskell et al., 2007; Nelson et al., 2007). Several meta-analyses showed benefits of moderate-intensity continuous exercise (MICE) on body composition, metabolic risk factors and improve maximum oxygen uptake (VO₂max). MICE promotes metabolic health via anti-inflammatory effects, increasing the activity of aerobic enzymes, intramuscular glycogen, mitochondrial and capillary densities in the muscles, oxidation of lipids in skeletal muscle and liver and improvement aerobic capacity (Kelley et al., 2006; Kelley and Kelley, 2008; Thorogood et al., 2011; Ismail et al., 2012). As such, the American College of Sports Medicine and the American Heart Association both recommend 30 min or more of MICE (64-76% of maximal heart rate or 46-63% of VO₂max) preferably all days of the week for protection against chronic diseases and in at least 60–90 min of moderate-intensity activity daily to sustain weight loss in adults who have lost substantial body weight.

The high-intensity interval exercise (HIIE) has been used as an interesting method for improve health markers, VO₂max, oxidative capacity, since it induces similar or higher effect during low volume high intensity exercise (i.e. ≤10 minutes of intense exercise) (Gibala et al., 2006; Burgomaster et al., 2008) when compared to the traditional MICE (vigorous intensity: 77-95% of maximal heart rate or 64-90% of VO₂max) (Garber et al., 2011). Both protocols 4-6 x 30-s Wingate with 4 minutes of recovery, such as 10 x 60-s at 90% of maximal heart rate interspersed with 60-s of recovery have been used for HIIT to improve glucose control, metabolic and vascular risk factors in overweight/obese sedentary men and patients with type 2 diabetes. (Whyte et al., 2010; Little et al., 2011). The 10 x 60-s protocol is considered more feasible than all-out efforts for different types of population, due to its effectiveness, safety reports and easy adherence.

When physically active subjects perform MICE, they may run for approximately 5 km (mean of 70% of maximal aerobic speed), while the HIIE volume is significantly lower than the performed during MICE (Gibala et al., 2006; Burgomaster et al., 2008), which induces reduction on energy expenditure during training (352 ± 34 versus 547 ± 65 kJ respectively; $p < 0.001$), without difference on excess post-exercise oxygen consumption (EPOC) (Skelly et al., 2014). However Skelly et al. (2014) have observed relatively lower intensity during the HIIE (i.e. $77 \pm 3\%$ of peak power output) than suggested.

In this sense, aiming to induce higher aerobic adaptation to training, no study has investigated whether HIIE training performed with higher intensity (i.e. 100% of maximal aerobic speed) and same volume as MICE results in different physiological responses and metabolic adaptations during and after training, respectively. Thus, the objective of this study was to compare the effects of 5 km MICE and HIIE on the physiological response in young adults.

MATERIALS AND METHODS

Subjects

Twelve physically active male subjects [age 23.22 ± 5.47 years, height 1.73 ± 0.06 m, weight 74.60 ± 6.61 kg, body mass index 24.63 ± 1.97 kg•m²-1 and peak oxygen uptake 58.58 ± 5.60 ml•kg•min⁻¹] volunteered to participate in this study. They presented a health and neuromuscular status which demonstrated their ability to complete the study protocol. All procedures performed in the study were in accordance with the ethical standards of the University Research Ethics Committee for studies involving human participants. Written informed consent was obtained from all subjects after they had been informed about the purpose and risks of the study.

Procedures

Subjects completed three experimental trials at the laboratory. The first visit aimed to determine peak oxygen uptake ($\text{VO}_{2\text{Peak}}$), and the speed associated with $\text{VO}_{2\text{Peak}}$ ($\text{sVO}_{2\text{Peak}}$). During the remaining two visits, all subjects were submitted a two protocols of 5km running on treadmill in randomized sequence: high-intensity exercise (HIE), or moderate-intensity exercise (MIE), separated by at least 72h. All tests took place at the same time of the day, between 1:00 p.m. and 6:00 p.m., at a average temperature of between 20°C and 24°C. The subjects were instructed to abstain from strenuous exercise for at least 24 hours prior to each exercise session and were encouraged to maintain their usual nutritional and hydration routines. Moreover, they were also request not to ingest stimulants (tea, coffee, soda, chocolate, chocolate powder) or alcoholic beverages during this period.

Maximal endurance running test

The subjects were submitted to an incremental test on at treadmill (Inbramed MASTER CI, Inbrasport®, Porto Alegre, Brazil). The initial speed was set at 8 $\text{km}\cdot\text{h}^{-1}$, increasing by 1 $\text{km}\cdot\text{h}^{-1}$ every 2-min until volitional exhaustion. Strong verbal encouragement was given during the test. The oxygen uptake was measured (Quark PFT, Cosmed®, Rome, Italy) throughout the test and the average of the last 30 s defined as $\text{VO}_{2\text{Peak}}$. The $\text{sVO}_{2\text{Peak}}$ was assumed as the final incremental test speed. When the subject was unable to complete at stage, the speed was expressed according to the time in the final stage, determined as follow: $\text{sVO}_{2\text{Peak}} = \text{speed of final complete stage} + [(\text{time, in seconds, remaining at the final incomplete stage} / 120\text{s}) * 1 \text{ km}\cdot\text{h}^{-1}]$ (Kuipers et al., 1985). Heart rate was also continuously recorded throughout the tests (Polar Vantage NV, Electro Oy, Finlândia). The 6–20 Borg scale (Borg, 1982) was used to measure the rating of perceived exertion during the test.

High-intensity intermittent and moderate-intensity continuous exercise

For both exercise trials, the subjects performed a warm-up consisting of a running at 50% of sVO_2Peak for five minutes at 1% inclination. The HIE was performed intermittently with subjects running on a treadmill for one minute at 100% of sVO_2Peak (Little et al., 2011), interspersed by one minute of passive recovery (without exercise) until they had complete 5 km. The MIE consisted of a continuous 5 km run on the treadmill at 70% of sVO_2Peak .

Energy expenditure

To estimate the energy expenditure of all exercises the sum of the contribution of the three energy systems (aerobic, anaerobic lactic and alactic) was used. Aerobic metabolism was estimated using the oxygen uptake integral during the exercise (Whyte et al., 2010), anaerobic alactic was assessed using the fast phase of excess of oxygen uptake as presented by Bertuzzi et al. (2010), and the lactic anaerobic contribution using net blood lactate accumulation as proposed by Di Prampero and Ferretti (1999).

Oxygen uptake was measured continuously and for 60 min after exercise protocols. At 1, 3, 5 and 7 min after the end of each test, blood samples were collected by venipuncture to measure lactate concentration. The highest lactate value ($[\text{La-}]$) measured was considered the peak lactate concentration ($[\text{La-}]_{\text{peak}}$). The difference between the $[\text{La-}]_{\text{peak}}$ and pre exercise lactate concentration ($[\text{La-}]_{\text{rest}}$) was expressed as a net lactate accumulation ($\Delta[\text{La-}]$). A metabolic equivalent of $3 \text{ mL O}_2 \cdot \text{kg}^{-1}$ for each $1 \text{ mmol} \cdot \text{L}^{-1}$ of $\Delta[\text{La-}]$ was considered the anaerobic lactic contribution (Di Prampero and Ferretti, 1999).

For the anaerobic alactic contribution, the fast component of excess post-exercise oxygen consumption was determined using a modified bi-exponential decay equation. The anaerobic alactic contribution corresponded to the product of bi-exponential fast component amplitude and tau (Bertuzzi et al., 2007; Zagatto et al., 2011). The aerobic metabolism was estimated by subtracting rest oxygen consumption from exercise oxygen consumption. To estimate the total energy expenditure and

oxygen consumption during each protocol, the energy expenditure were summed and converted to kJ, assuming that 1 L of oxygen consumed was equivalent to 20.9 kJ (Gastin, 2001).

Statistical Analysis

The differences during the tests was analyzed by repeated measurements analyses and the comparison between MICE and HIIE was performed by two-way repeated measure of ANOVA (group x time). When a significant difference in group or interaction was observed, a Tukey post hoc test was conducted. For all measured variables, the estimated sphericity was verified according to Mauchly's W test, and the Greenhouse-Geisser correction was used when necessary. The effect size (eta-squared; η^2) of each test was calculated for all analyses. Statistical significance was set at $p < 0.05$. The data were analyzed using the Biostat (version 5.0).

RESULTS

Table 1 presents the mean values of age, body weight, height, $\text{VO}_{2\text{peak}}$, and $\text{sVO}_{2\text{peak}}$ of all subjects at the baseline of this study.

Table 1. Subjects characteristics.

Variable	Subjects (n=12)
Age (years)	23.22 ± 5.47
Body Mass (kg)	74.60 ± 6.61
Height (m)	1.73 ± 0.06
BMI (kg·m ²)	24.63 ± 1.97
VO ₂ peak (ml·kg·min ⁻¹)	58.58 ± 5.6
sVO ₂ max (km·h ⁻¹)	14.23 ± 1.20

Values are mean ± standard deviation. BMI= Body Mass Index; VO₂peak=peak oxygen uptake; sVO₂max= speed associated with maximal oxygen uptake.

A summary of both exercise protocols are shown in Table 2, and significant differences were found for HIIE compared to MICE (p=0.01) for total speed and exercise session duration.

Table 2. Summary of exercise descriptors for high-intensity exercise (HIIE) and moderate-intensity continuous exercise (MICE) protocols (n=12).

Variable	MICE	HIIE
Protocol	run at 70% sVO ₂ max	1:1 minute at 100% sVO ₂ max
Speed (km·h⁻¹)	10.14 ± 0.84	14.23 ± 1.20*
Exercise session (min)	29.77 ± 2.46	21.07 ± 1.78*
Total exercise duration (min)	29.77 ± 2.46	41.14 ± 3.56*

Values are mean ± standard deviation. * = significant differences from MICE (p<0.05).

The figure 1 presents the difference on $[La^-]$ between MICE and HIIE. There was statistically significant difference across time, mainly in HIIE, as well as, 30, 45 and 60 minutes after exercise session ($p < 0.001$, $\eta^2 = 0.69$). There was statistically significant difference between MICE and HIIE groups ($p = 0.001$, $\eta^2 = 0.66$) and interaction was observed (time x group: $p < 0.001$, $\eta^2 = 0.38$).

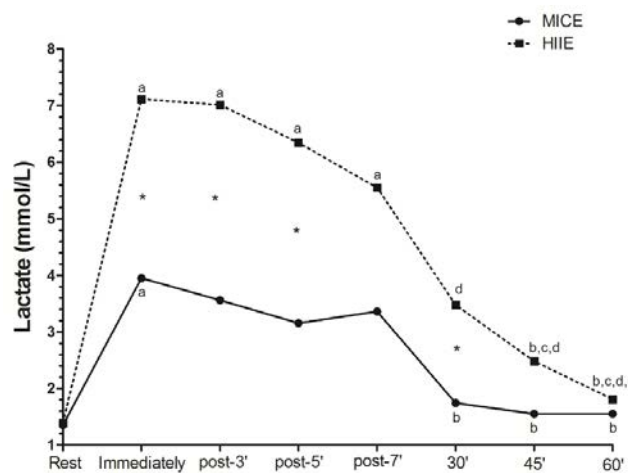


Figure 1: Difference on $[La^-]$ between MICE and HIIE. a = Tukey's post-hoc test with p-value < 0.05 compared to rest; b= Tukey's post-hoc test with p-value < 0.05 compared to immediately moment; c= Tukey's post-hoc test with p-value < 0.05 compared to post-3 minutes; d= Tukey's post-hoc test with p-value < 0.05 compared to post-5 minutes; e= Tukey's post-hoc test with p-value < 0.05 compared to post-7 minutes; *= statistically significantly difference between MICE and HIIE.

The figure 2 shows the differences in VO_2 consumption during 30 minutes of exercise and 30, 45 and 60 minutes after the exercise interruption in both conditions.

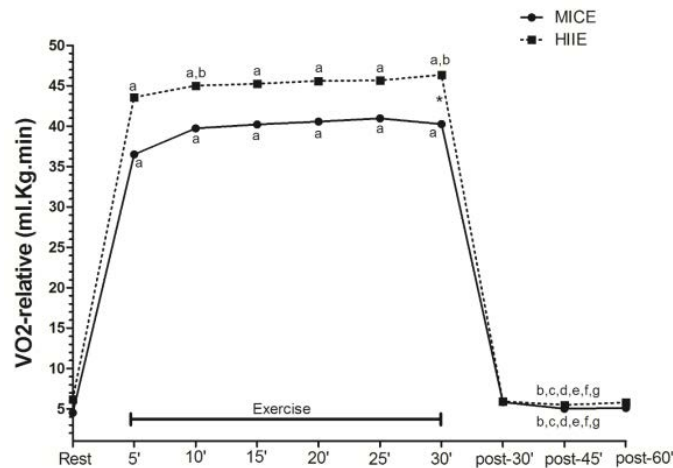


Figure 2: Difference on oxygen uptake (VO₂relative) between MICE and HIIE.

a = Tukey's post-hoc test with p-value < 0.05 compared to rest; b= Tukey's post-hoc test with p-value < 0.05 compared to five minutes of exercise; c= Tukey's post-hoc test with p-value < 0.05 compared to 10 minutes of exercise; d= Tukey's post-hoc test with p-value < 0.05 compared to 15 minutes of exercise; e= Tukey's post-hoc test with p-value < 0.05 compared to 20 minutes of exercise; f= Tukey's post-hoc test with p-value < 0.05 compared to 25 minutes of exercise; g= Tukey's post-hoc test with p-value < 0.05 compared to 30 minutes of exercise; *= statistically significantly difference between MICE and HIIE.

There was statistically significant difference across time on VO₂ ($p < 0.001$, $\eta^2 = 0.98$). Post hoc analysis revealed that in 10 and 30 min of exercise, there was difference on HIIE compared to rest and five minutes. All groups presented difference in relation to rest. After 30, 45 and 60 minutes of exercise cessation, the VO₂ was different of all time during exercise in both groups. There was significant differences between group in 30 minutes of exercise ($p = 0.044$, $\eta^2 = 0.59$) and interaction (time x group: $p = 0.002$, $\eta^2 = 0.41$).

When analyzed HR and RPE (figure 3) there were significant difference across time (HR: $p < 0.001$, $\eta^2 = 0.98$; RPE: $p = 0.001$, $\eta^2 = 0.80$), between group (HR: $p = 0.038$; $\eta^2 = 0.92$; RPE: $p = 0.002$; $\eta^2 = 0.66$) and interaction (time x group, HR: $p = 0.003$, $\eta^2 = 0.75$; RPE: $p < 0.001$, $\eta^2 = 0.92$).

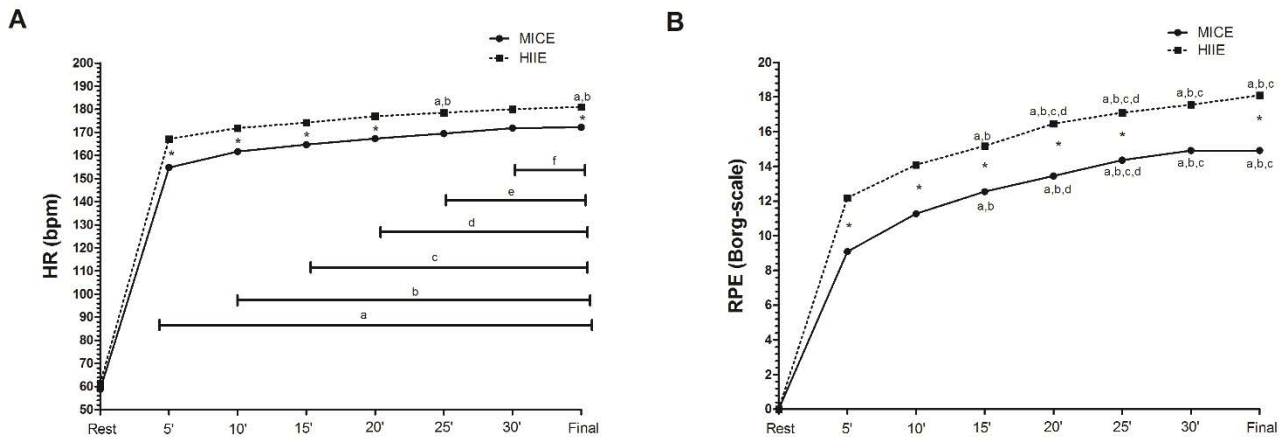


Figure 3: Difference on rating of perceived exertion (RPE) and heart rate (HR) between MICE and HIIE. **For RPE:** a = Tukey's post-hoc test with p-value < 0.05 compared to five minutes of exercise; b= Tukey's post-hoc test with p-value < 0.05 compared to 10 minutes of exercise; c= Tukey's post-hoc test with p-value < 0.05 compared to 15 minutes of exercise; d= Tukey's post-hoc test with p-value < 0.05 compared to 20 minutes of exercise. **For HR:** a = Tukey's post-hoc test with p-value < 0.05 compared to rest; b= Tukey's post-hoc test with p-value < 0.05 compared to five minutes of exercise; c= Tukey's post-hoc test with p-value < 0.05 compared to 10 minutes of exercise; d= Tukey's post-hoc test with p-value < 0.05 compared to 15 minutes of exercise; e= Tukey's post-hoc test with p-value < 0.05 compared to 20 minutes of exercise; f= Tukey's post-hoc test with p-value < 0.05 compared to 25 minutes of exercise;

g= Tukey's post-hoc test with p-value < 0.05 compared to 30 minutes of exercise; *= statistically significantly difference between MICE and HIIE.

Both aerobic and lactic anaerobic contribution were higher on HIIE than on MICE, while alactic anaerobic contribution only tended to be different ($p = 0.08$) (Table 3). Furthermore, HIIE presented higher total energy expenditure and EPOC.

Table 3. Mean $\pm$ standard deviation of aerobic, anaerobic lactic and alactic contribution, total energy expenditure, and excess post oxygen consumption during moderate intensity continuous exercise, and high intensity interval exercise ($n = 12$).

Variable	MICE	HIIE
Aerobic contribution		
L	83.66 $\pm$ 11.28	96.59 $\pm$ 8.23*
Kj	1748.47 $\pm$ 235.73	2018.66 $\pm$ 172.04*
Anaerobic alactic contribution		
L	3.23 $\pm$ 0.77	2.38 $\pm$ 1.10
kJ	67.48 $\pm$ 15.99	49.82 $\pm$ 22.96
Anaerobic lactic contribution		
L	1.27 $\pm$ 0.76	0.64 $\pm$ 0.67*
kJ	26.59 $\pm$ 15.78	13.33 $\pm$ 13.93*
Total energy expenditure		

L	87.15 ± 11.77	101.83 ± 8.91*
kJ	1821.37 ± 246.07	2128.17 ± 186.19*
EPOC		
L	5.32 ± 2.39	6.61 ± 1.85*
kJ	111.19 ± 49.97	138.21 ± 38.69*

MICE: moderate intensity continuous exercise; HIIE: high intensity interval exercise; EPOC: excess post-exercise oxygen consumption. * significantly different of MICE

Discussion

The main findings were that the same volume HIIE induced higher RPE, HR, post [La-] than MICE, while exercise VO₂ was higher only at the end of exercise. In addition, aerobic and lactic anaerobic contribution, total energy expenditure, and EPOC were higher on HIIE than MICE.

High intensity interval training and all-out maximum effort or capacity have been used to promote weight loss, glycemic control, and increase on aerobic fitness (Burgomaster et al., 2008; Little et al., 2011; Gibala et al., 2012). However, all-out exercise needs specific equipment (cycloergometer, i.e. Wingate equipment), and may be unusual for some population, limiting its feasibility. Thus the high intensity interval training seems to be interesting to overcome this limitation (Skelly et al., 2014).

As expected, HR and RPE were higher on HIIE than MICE, since linear relation exist between HR and RPE with exercise intensity (Karvonen and Vuorimaa, 1988). As well the [La-]peak was higher after the HIIE than MICE, however, even MICE presented mean [La-] values immediately after the exercise close to anaerobic threshold (4.74 ± 2.43 mmol•L⁻¹), while HIIE presented 8.25 ± 2.95 mmol•L⁻¹. This result indicates the higher contribution of anaerobic metabolism during the HIIE in comparison with MICE. Bucchheit and Laursen (2013) have stated that aerobic interval

training could be prescribed by the HR during training. However, the present study revealed that even though HR is higher during HIIE, the VO₂ response is not (Figure 2). However, taking into account the VO₂ area during the exercise, HIIE presented greater amount of consumed oxygen which may have occurred due to longer exercise time (Table 2). Thus, since VO₂ consumption during exercise training is one important index for indicate aerobic zone (Zagatto et al., 2011), HIIE was more effective in stimulating aerobic metabolism compared with MICE. Nevertheless the exercise time must be considered. When the HIIE exercise time is matched for the MICE, the VO₂ integral of HIIE is significantly lower than MICE (68.07 ± 6.19 L and 83.66 ± 11.28 L, respectively; $p=0.0001$), evidencing that for iso-time exercise, continuous 5 km at 70% of sVO₂max is better than HIIE (with same time exercise session time), however, we cannot assume that after exercise energy expenditure (i.e. EPOC) would or not be different.

Different studies have compared high and moderate intensity training on physical fitness, however, diverse intensities are used to compare training exercises (Trapp et al., 2008; Gillen et al., 2013; Williams et al., 2013; Skelly et al., 2014). In relation to moderate training, high intensity training has been proposed to presents higher or same physical fitness adaptations (Gibala et al., 2006; Wisløff et al., 2007; Buchheit and Laursen, 2013), however, intensity used in MICE may also influence the results. Aiming to compare HIIE and MICE, Skelly et al. (2014) have used 77% and 33% of peak power output, respectively, which is considerably lower than the intensity used in the present study (100% and 70% of sVO₂max). While for our subjects, 70% of sVO₂ax could be at or slightly above anaerobic threshold, and mean exercise HR was $86.20 \pm 2.76\%$ of maximal HR, much higher than others moderate intensity training (Burgomaster et al., 2008; Sperlich et al., 2011; Gibala et al., 2012; Williams et al., 2013). Thus, the assumption that training at HIIE is better than MICE to improve physiological responses must be done with caution since MICE intensity is usually too low to induce high adaptation. Other studies may want to verify whether at or above

anaerobic threshold moderate intensity training induces similar adaptation to high intensity interval training.

The analysis of recovery period has been also proposed to be important on the effects of different types of training on physical fitness (Williams et al., 2013; Skelly et al., 2014). Skelly et al. (2014) did not show any difference on EPOC after HIIE and MICE in physically active subjects, however, MICE intensity was too low to induce higher EPOC, and HIIE presented low volume, hampering the comparison with the present study. Williams et al. (2013) compared sprint interval exercise with MICE (HIEE: four 30-s Wingates separated by 4.5 min of active rest; MICE: 60% at peak power output) and did not verify differences on EPOC in both sessions (HIEE: 33.5 ± 16.3 kcal; MICE: 41.5 ± 13.8 kcal). In our study, HIIE EPOC was significantly higher than MICE, however, as presented before, the HIIE total session duration may have influenced our results. The EPOC of HIIE could be similar than MICE in the present study if the exercise time was the same, however this is yet to be determined.

In addition to exercise volume (external load), and the intensity (i.e. RPE, [La-], or HR; (internal load), and their product (i.e. training impulse –TRIMP) must also be taken into account (Borresen and Lambert, 2008a; Borresen and Lambert, 2008b; Manzi et al., 2009; Foster et al., 2011; Minganti et al., 2011). Although some studies have equalized exercise energy expenditure (Gibala et al., 2012), training impulse (product between external and internal load) is not considered when comparing exercise training. Calculating training impulse from [La-], HIIE presented higher value (293 ± 123.98 a.u.) than MICE (120.44 ± 53.0 a.u.), nevertheless, when the rest period of HIIE is not taken into account (Minganti et al., 2011), no difference was found between them (150.53 ± 62.73 a.u. and 120.44 ± 53.0 a.u., respectively; $p=0.13$). Thus, it is still important to sought (i) whether physiological differences exist between HIIE and MICE when training impulse is equalized, and (ii) whether passive resting period may have been taken into account when calculating training impulse.

In conclusion, HIIE was more effective in modify HR, and RPE, however, not for oxygen uptake, even though aerobic area were higher on HIIE, likely due longer exercise duration. Furthermore, when the exercise time was equalized, no difference existed in aerobic contribution between HIIE and MICE. EPOC was higher after HIIE, however exercise time may have contributed to this differences. Thus, equalizing training impulse or time exercise could induce different results. Finally, depending on subjects training status, and/or exercise intensity, HIIE may be induced benefits as good as MICE. The characteristics provided by this research can be used to help physical training programs for all populations, due to the easily benefit from proper implementation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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ARTIGO 2

Similar anti-inflammatory acute response of moderate-intensity continuous and high-intensity intermittent exercise

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Abstract

The purpose of this study was to compare the effect of high-intensity intermittent exercise (HIIE) versus volume matched steady state exercise (SSE) on inflammatory and metabolic responses. Eight physically active male subjects completed two experimental sessions, a 5-km run on a treadmill either continuously (70% $\dot{V}O_{2\max}$) or intermittently (1:1 min at $\dot{V}O_{2\max}$). Blood samples were collected at rest, immediately, 30 and 60 minutes after the exercise session. Blood was analyzed for glucose, non-ester fatty acid (NEFA), uric acid, lactate, cortisol, and cytokines (IL-6, IL-10 and TNF- α) levels. The lactate levels exhibited higher values immediately post-exercise than at rest (HIIE 1.34 ± 0.24 to 7.11 ± 2.85 , and SSE 1.35 ± 0.14 to 4.06 ± 1.60 mmol·L⁻¹, $p < 0.05$), but HIIE promoted higher values than SSE ($p < 0.05$); the NEFA levels were higher immediately post-exercise than at rest only in the SSE condition (0.71 ± 0.04 to 0.82 ± 0.09 mEq/L, respectively, $p < 0.05$), yet, SSE promoted higher values than HIIE immediately after exercise (HIIE 0.72 ± 0.03 vs SSE 0.82 ± 0.09 mEq·L⁻¹, $p < 0.05$). Glucose and uric acid levels did not show changes under the different conditions ($p > 0.05$). Cortisol, IL-6, IL-10 and TNF- α levels showed time-dependent changes under the different conditions ($p < 0.05$), however, the area under the curve of TNF- α in the SSE were higher than HIIE ($p < 0.05$), and the area under the curve of IL-6 in the HIIE showed higher values than SSE ($p < 0.05$). In addition, both exercise conditions promote increased IL-10 levels and IL-10/TNF- α ratio ($p < 0.05$). In conclusion, our results demonstrated that both exercise protocols, when volume is matched, promote similar inflammatory responses, leading to an anti-inflammatory status; however, the metabolic responses are different.

Keywords: High intensity intermittent exercise, steady state exercise, metabolism, inflammation, energy expenditure, cytokines.

Introduction

Metabolic diseases are frequently observed in modern society, primarily as persistent, chronic low-grade inflammation conditions. These disorders are caused pre-dominantly by physical inactivity and food intake imbalance (Pedersen, 2009). There is evidence that a single session of exercise promotes a lower risk of chronic disease, which is associated with morbidity, compared to sedentary individuals, and contributes to improvements in health (Bassuk and Manson, 2005).

It is well established that, in long-term training, physical exercise mediates and promotes improved metabolic processes (such as reduced total cholesterol, triglycerides and low density lipoprotein, and enhances high density lipoprotein) and may act as a trigger for reduction in body fat, principally through increased energy expenditure and adaptations of oxidative metabolism, especially in skeletal muscle (Gillen et al., 2013). In addition, this training protocol is powerful in inducing the inflammatory response (hence skeletal muscle is the major source of the increase in the release of interleukin-6 (IL-6), interleukin-10 (IL-10), it is an interleukin 1 receptor antagonist (IL-1ra), and it reduces tumor necrosis factor alpha (TNF- α) and interleukins (1 β , IL-2) (Neto et al., 2011; Pedersen and Fabbraio, 2009).

The metabolic and inflammatory changes from regular exercise training are dependent on duration, intensity and session volume, and these are crucial aspects of training (Lira et al., 2012; Neto et al., 2011). However, recently,

studies have suggested that aerobic exercise performed at a high intensity (typically ~90% VO₂max) and separated by recovery periods of lower intensity or complete rest, is a time-efficient strategy with a small total volume work and has the potential to promote similar health benefits compared to traditional aerobic exercises programs – such as improved maximal aerobic capacity functions, promotion of the reduction in body fat and serving to control body weight (Gibala 2012).

Study have indicate that high-intensity intermittent training (HIIT) (performed 8-12 HIIT sessions, with 60 x 75 second active rest, at 100% VO₂peak) increase the plasma concentrations levels of IL-10 during a following prolonged exercise in recreationally active males (Zwetsloot et al., 2014). In addition, the increase of IL-10 levels in athletes after HIIE (4 HIIT sessions of Wingate tests at 100% VO₂peak), implying that approaches designed to promote anti-inflammatory effects should be useful in attenuating the inflammatory milieu (Lira et al., 2015).

Especially worthy of note, the factor that probably has the greatest impact on inflammatory responses promoted by exercise session is workload, which is orchestrated by the duration and intensity (Pedersen, 2009). Most studies (Leggate et al., 2010; Skelly et al., 2014) have used protocols emphasizing exercise intensity, but these protocols have no equality of duration and volume of exercise session, which is a relevant aspect that must be considered in studies with the purpose of investigating the metabolic/immune responses during different exercise modes. The volume performed may not have been properly controlled and this is an important methodological issue that causes leads to mistakes in the interpretation of studies that compared the

effects of steady state and intermittent exercise on the magnitude of responses. Therefore, the aim of present study was to compare the effect of HIIE versus volume matched SSE on inflammatory and metabolic responses in young males.

Methods

Subjects

Eight physically active male subjects volunteered to participate in this study. Participants were free of health problems and/or neuromuscular disorders that could affect their ability to complete the study protocol. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Research Ethics Committee of UNESP – Presidente Prudente/SP and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all subjects after participants volunteered to participate in the study, after being informed about the purpose and risks of the study. Before conducting the study we checked the sample size needed ($n = 6$) using the G*Power 3.1 software (Düsseldorf, Germany) to guarantee an 80% power and a 5% significance level based on IL-10 using studies that measured differences between both protocols (Wadley et al., 2015) and using studies that measured the IL-6 pre and immediately post exercise as referenced by similar protocol (high intensity intermittent exercise) (Meckel et al., 2009; 2011; Legatte et al., 2010; Lira et al., 2015).

Procedures

Subjects completed three experimental sessions separated by at least 72 hours. During the first session, anthropo-metric, peak oxygen uptake ($\text{VO}_{2\text{peak}}$) and speed associated with $\text{VO}_{2\text{peak}}$ ($\text{sVO}_{2\text{peak}}$) measurements on a treadmill were performed. Two more experimental sessions were applied in randomized cross-over order: HIIE – a session in which participants performed a high-intensity intermittent aerobic exercise, and a steady state exercise (SSE) – a session in which participants performed a moderate continuous exercise. All tests took place at the same time of the day for each subject. The subjects were instructed to abstain from any strenuous exercise for at least 24 hours before each testing session and were encouraged to maintain their nutritional and hydration routines (Figure 1).

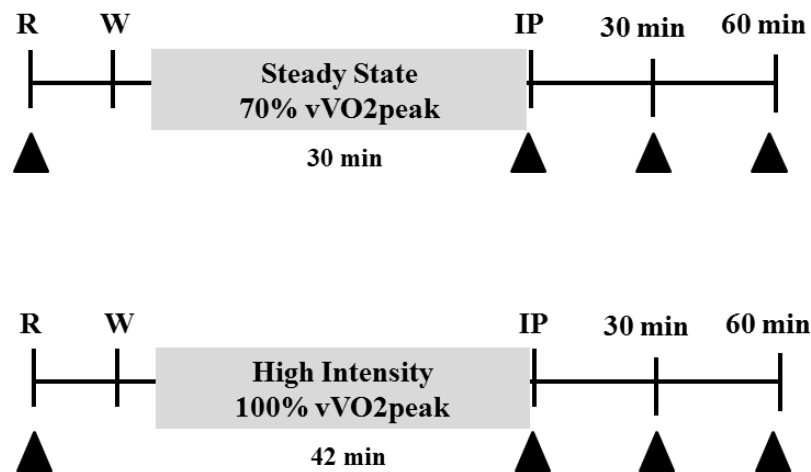


Figure 1: Schematic representation the study protocol. ▲ Blood samples (rest, immediately, 30 and 60 minutes after exercise); IP = immediately post-exercise; R= rest; W= warm up (n = 8).* = different from rest ($p < 0.05$)

Bioelectrical Impedance

Bioelectrical impedance in individuals was measured using the octopolar InBody 720 Composition Analyzer (Copyright®, 1996-2006, by Biospace Corporation, USA). The participant's age, gender and height were entered into the machine. The participants stood barefoot on the metal footplate and held the handles with their arms relaxed by their sides. Once impedance was measured, the results of Fat Mass (FM), Fat Free Mass (FFM) and %BF for five different body locations, each arm, each leg, and the trunk and one general overall set was printed. All anthropometric measurements were checked by the same person throughout the study to minimize interpersonal variations. Participants were asked to abstain from eating or drinking for two hours as well as to refrain from moderate or vigorous exercise for 24 hours before all testing. They were told to obtain a restful night's sleep, remain well hydrated, refrain from alcohol, and eat a regular meal in the morning before testing.

Maximal endurance running test

The subjects performed an incremental test to volitional exhaustion (Panissa et al., 2013). The initial treadmill (Inbramed, modelo MASTER CI, Brazil) speed was set at 8.0 km•h⁻¹ and it was increased by 1 km•h⁻¹ per 2-min stage until the participant could no longer continue. Strong verbal encouragement was given during the test. The oxygen uptake was measured (Quark PFT, Cosmed, Rome, Italy) throughout the test and the average of the last 30 s was defined as peak oxygen uptake (VO₂peak). When the subject was not able to finish the 2-min stage, the speed was expressed according to the

permanence time in the last stage, determined as the following: $sVO_{2peak} = \text{speed of last stage complete} + [(\text{time, in seconds, remained at the last stage incomplete} / \text{by } 120\text{s}) * 1 \text{ km.h}^{-1}]$ (Kuipers et al., 1985). Heart rate was also continuously recorded throughout the tests (Polar Vantage NV, Electro Oy, Finlândia). The 6–20 Borg scale (Borg, 1982) was used to measure the rating of perceived exertion during the test.

In order to establish whether subjects had given all-out effort, the verification procedure used for determination was three or more of the following criteria: (i) VO_2 plateau ($\leq 150 \text{ mL}\cdot\text{min}^{-1}$), (ii) attainment of the percent-age of the age-predicted maximal heart rate (HR_{max}) within ± 5 beats/min; (iii) the rating of perceived exertion (RPE) ≥ 18 ; and (iv) respiratory exchange ratio (RER) ≥ 1.10 (Howley 1995).

High-intensity intermittent exercise

Participants performed a warm-up at 50% at sVO_{2peak} for five minutes, and after a 1-min interval the exercise session was started. The exercise consisted of a 5-km run on treadmill performed in intermittently at 1-min at the sVO_{2peak} followed by 1-min of passive recovery. The subjects remained standing or sitting after each exercise bout (Table 2).

Steady state exercise

Participants performed a warm-up at 50% at VO_{2peak} for five minutes, and after a 1-min interval exercise was started. The endurance exercise consisted of 5-km run on treadmill continuous 70% at VO_{2peak} (Table 2).

Exercise energy expenditure

To estimate the energy expenditure of all exercises, the sum of the contribution of the three energy systems (aero-bic, anaerobic lactic and alactic) was used. Aerobic metabolism was estimated using the oxygen uptake during the exercise, anaerobic alactic using the fast phase of excess of oxygen uptake and the lactic using delta of blood lactate (Bertuzzi et al., 2007; Di Prampero and Ferretti, 1999; Zagatto et al., 2011).

Oxygen uptake was measured continuously and at 60 min after all the exercise sessions. At 1, 3, 5 and 7 min after the end of each test, blood was collected to measure lactate concentration.

The highest value measured was considered the peak lactate concentration ($[La-1]_{peak}$). The difference between the $[La-1]_{peak}$ and pre exercise lactate concentration ($[La-1]_{rest}$) was expressed as a delta value ($[La-1]_{\Delta}$). A value of $1 \text{ mmol} \cdot \text{L}^{-1}$ $[La-1]_{\Delta}$ was considered to be the equivalent to $3 \text{ mL O}_2 \cdot \text{kg}^{-1}$ body mass (Di Prampero and Ferretti, 1999). The fast component of excess post-exercise oxygen consumption was determined using a modified bi-exponential decay equation and the anaerobic alactic metabolism corresponded to the product of amplitude and tau (Bertuzzi et al., 2007; Zagatto et al., 2011). The aerobic metabolism was estimated by subtracting rest oxygen consumption from exercise oxygen consumption. To estimate the total energy expenditure and oxygen consumption during each protocol, the energy expenditure were summed and converted to kcal (Skelly et al., 2014).

Blood sampling and analyses

The blood samples were collected at rest, and immediately, 30, and 60 minutes after acute exercise sessions during HIIE and SSE. The blood samples (15 ml) were immediately allocated into two 5 mL vacutainer tubes (Becton Dickinson, BD, Juiz de Fora, MG, Brazil) containing EDTA for plasma separation and into one 5 mL dry vacutainer tube for serum separation. The tubes were centrifuged at 3.500 g for 15 minutes at 4°C, and plasma and serum samples were stored at -20°C until analysis. Cytokines IL6, IL-10 and TNF- α were assessed using ELISA commercial kits (R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, USA). Glucose, uric acid, and lactate were assessed using commercial kits (Labtest®, São Paulo, Brazil). Non-ester fatty acid (NEFA) was assessed by a colorimetric method with a commercial kit (Wako, 1-2, doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan). Serum cortisol was assessed using commercial kits (Cayman Chemical, Michigan, USA). Cortisol and glucose levels were assessed using serum, and NEFA levels were assessed using plasma.

Statistics

The data normality was verified using the Shapiro-Wilk test. For each variable, mean and standard deviations were calculated, and they were analyzed using the SAS statistical package (SAS version 9.3). Mixed models for repeated measures were used to examine differences in blood variables according to condition, time and interactions. The Tukey test was used post hoc when differences were found. The unpaired t test was used to examine differences in energy expenditure. The significance level was set at 5%.

Results

The subjects' characteristics, anthropometry measures and summary of incremental test are show in Table 1.

Table 1. Subjects characteristics.

Variable	Subjects (n=8)
Age (years)	24.56 ± 6.02
Body Mass (kg)	74.69 ± 7.48
Height (m)	1.75 ± 0.06
BMI (kg/m ²)	24.28 ± 1.74
Fat Mass (kg)	12.71 ± 4.18
%BF	16.85 ± 4.81
FFM (kg)	35.38 ± 3.38
VO ₂ peak (ml·kg·min ⁻¹)	59.93 ± 6.77

Values are mean ± standard deviation. BMI= Body Mass Index; %BF=

% Body Fat; FFM= Fat Free Mass; VO₂peak= peak oxygen uptake.

A summary of both exercise protocols are shown in Table 2, and significant differences are found in time commitment, since it is higher in HIIE than SSE ($p=0.0001$). For energy expenditure, heart rate and time commitment during exercises, there was a greater effect for condition ($p<0.01$) energy expenditure in HIIE than in SSE exercises.

Table 2. Summary of exercise descriptors for high-intensity exercise (HIE) and steady state exercise (SSE) protocols (n=8).

Variable	SSE	HIE
Protocol	run at 70% of $\dot{V}O_{2peak}$; 28-33 minutes	60 x 60 second rest; 20-23 bouts
Exercise session duration (min)	30.78 $\pm$ 2.09	42.09 $\pm$ 2.93*
Energy Expenditure (kcal)	454.36 $\pm$ 56.72	523.00 $\pm$ 40.06*
HR_{max} (beats·min⁻¹)	170.75 $\pm$ 8.35	181.63 $\pm$ 11.43*
[La]⁻rest (mmol·L⁻¹)	1.35 $\pm$ 0.14	1.34 $\pm$ 0.24
[La]⁻peak (mmol·L⁻¹)	4.06 $\pm$ 1.60	7.11 $\pm$ 2.85*
RPE_{final}	15 $\pm$ 2.81	20 $\pm$ 3.70

Values are mean $\pm$ standard deviation. HR_{max}= Maximal Heart Rate in exercise; [La]⁻= lactate concentration; RPE= Rate of Perceived exertion. * = different from SSE (p<0.05)

For [La⁻] there was a main effect of condition (p < 0.001), with higher values in HIE than SSE (p < 0.001), and in moment (p<0.001), with higher values immediately post exercise than at rest, 30 and 60 min-post exercise (p < 0.001 for all comparisons, Figure 2B). Moreover, there was a condition of interaction and moment (p < 0.001) in HIE where the values immediately post exercise were higher than at rest, 30 and 60 min-post exercise in the same condition (p < 0.001 for all comparisons); in SSE the values immediately post exercise were higher than at rest (p < 0.001), 30 (p = 0.003) and 60-min post exercise (p = 0.001) in the same condition.

For NEFA there was an interaction effect ($p = 0.044$), with higher values in SSE immediately post exercise than in HIIE at the same moment ($p < 0.050$); higher values in SSE immediately post exercise than at rest to the same condition ($p = 0.030$, Figure 2C). For glucose and uric acid there was no effect (Figure 2A and 2E).

For cortisol there was a main effect of moment ($p < 0.001$) with values at rest lower than immediately ($p < 0.001$), post-30 ($p = 0.003$), and post 60-min of exercise ($p = 0.024$, Figure 2D).

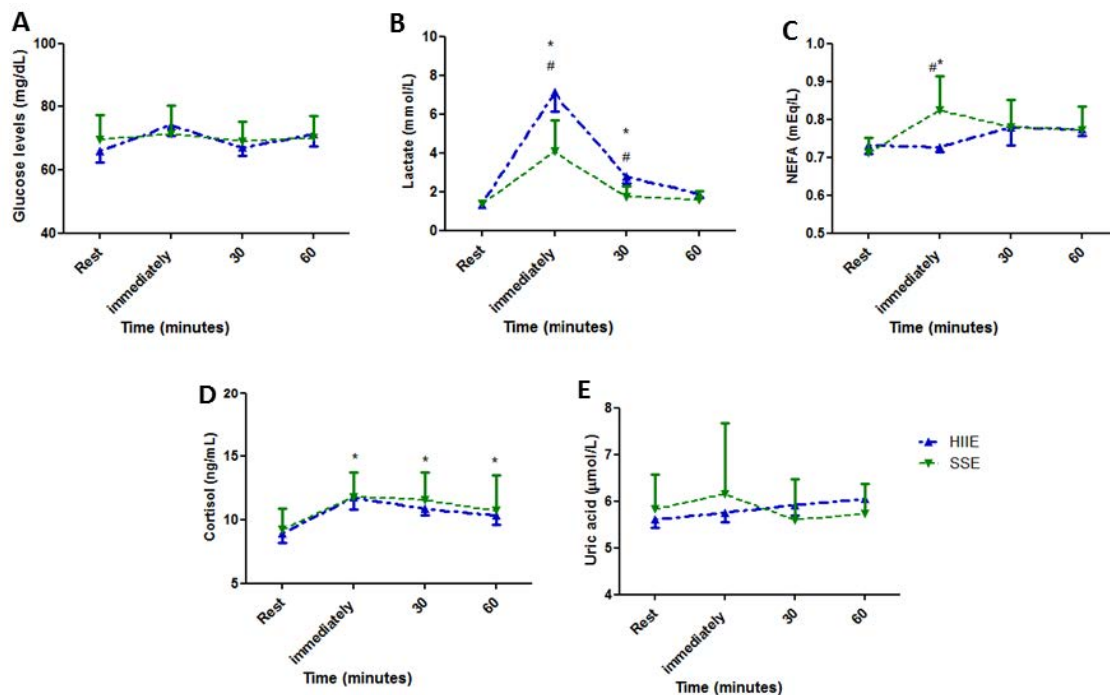


Figure 2: Metabolic parameters before and after single bout of SSE and HIIE exercise in males ($n = 8$; values are mean $\pm$ standard deviation). Figures: 2A (Glucose), 2B (Lactato), 2C (NEFA), 2D (Cortisol) and 2E (Acid Uric). * = different from rest ($p < 0.05$); ** = different SSE ($p < 0.05$); # = different from 30 minutes; \$ = different from 60 minutes.

As regards the cytokine levels (Figure 3), for TNF- α there was a main effect of condition ($p = 0.012$) with HIIE lower than SSE ($p = 0.012$), and for moment ($p = 0.050$) with values immediately post exercise higher than at rest ($p = 0.037$, Figure 3B). For IL-6 there was a main effect of condition ($p = 0.012$) with HIIE higher than SSE ($p = 0.012$), and moment ($p < 0.001$), with values at rest lower than immediately post-exercise ($p = 0.009$) and 30min-post exercise ($p = 0.039$); at 60min-post exercise lower than at immediately post-exercise ($p = 0.001$) and 30min-post exercise ($p = 0.007$, Figure 3A). For IL-10 there was a main effect of moment ($p = 0.002$), with values at rest lower than immediately ($p = 0.007$), 30min ($p = 0.047$) and 60 min-post exercise ($p = 0.001$, Figure 3C).

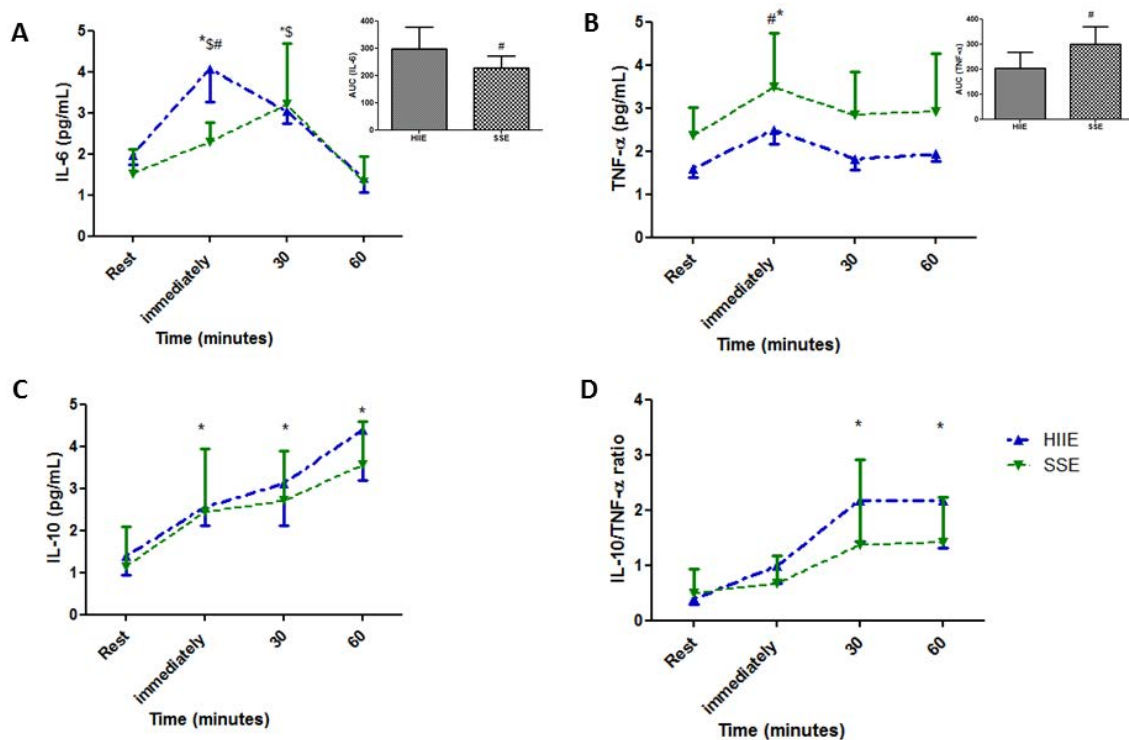


Figure 3: Cytokine levels before and after single bout SSE and HIIE exercise in males ($n = 8$; values are mean $\pm$ standard deviation). Figures: 3A (IL-6), 3B (TNF- α), 3C (IL-10), 3D (IL-10/ TNF- α). * = different from rest ($p < 0.05$); ** =

different SSE ($p < 0.05$); # = different from 30 minutes; \$ = different from 60 minutes.

For TNF/IL10 ratio there was a main effect of moment ($p=0.015$), with higher values immediately post exercise than at rest ($p=0.019$). There was also an interaction of condition and moment ($p=0.002$), where, in the HIIE condition, the values 30min-post exercise were higher than at rest ($p=0.011$), immediately ($p=0.006$) and 60min-post exercise ($p=0.042$) in the same condition; and in the SSE condition 30min-post exercise the values were higher than at rest ($p=0.005$) immediately ($p=0.004$) and 60min-post exercise ($p=0.020$, Figure 3D).

Discussion

The main finding of the present study was that HIIE elicited different total energy expenditure during exercise from SSE, despite matched volume. By design, energy expenditure was ~13% higher in the HIIE group (523 ± 40.06 versus 453 ± 56.72 kcal for SSE, $p < 0.02$) and session exercise time was 39% higher than the SSE group (30.78 ± 2.09 min versus 42.09 ± 2.93 min), whereas the session exercise time in the HIIE group that was spent in recovery between intense pause/session of run, thus actual exercise time was ~21 minutes compared to SSE. Recently, study have related similar energy expenditure (EE) in response to HIIE (10×60s at a workload that elicited 90% maximal heart rate with 60-s of active recovery at 50 W) and SSE (cycling at a workload that elicited 70% of maximal heart rate for 50 min) after 24h (Skelly et al., 2014), even despite the fact that the total energy expenditure during the exercise session was superior in SSE than HIIE (352 ± 34 versus

547 $\pm$ 65kcal, respectively; $p < 0.001$). This suggests that a session of HIIE may promote greater physiological stress than a bout of SSE, principally due to an increased hormonal response. Although our data has not exhibited significant differences between both protocol sessions, the energy expenditure following exercise (1h recovery) was 14% higher in HIIE than SSE. The difference in EE during exercise bouts (SSE vs HIIE) found in present study may be due to exercise protocols utilized, and here, we demonstrated that when the volume was equal between SSE and HIIE sessions, HIIE leads to more EE. In addition, the alterations found in our data after both exercise sessions are due to, at least in part, of hormonal changes, given that acute exercise promotes the enhancement of several hormones, principally the ones related to lipolysis in adipose tissue and glycogenolysis in skeletal muscle and the liver, promoting the availability of energetic substrates mainly by NEFA and glucose for muscle workload. Concomitant with the increased cortisol levels, our data demonstrate that HIIE promotes greater demands on the anaerobic metabolism (seen by peak lactate, Figure 2B) compared to SSE, while SSE promotes great demands on the aerobic metabolism (seen by peak NEFA, Figure 2C) compared with HIIE.

In our data, the HIIE did not promote accumulated NEFA levels immediately after exercise, while SSE did. During the steady state exercise, higher utilization of lipids in comparison with intermittent exercise is observed. Moreover, Jeppense and Kiens (2012) have reported that this response depends on acetyl CoA and CoA concentration ratios, carnitine availability, and hydrogen ion concentration. This last is likely higher during HIIE, due to

anaerobic metabolism. More studies are needed to better understand the mechanisms involved in this response.

The lack of accumulated NEFA levels immediately after HIIE can be, at least in part, a result of the higher fatty acid uptake by skeletal muscle during the repeated metabolic perturbations in the transitions from rest to exercise (pause/session cycles). We suggest that HIIE may be important in stimulate the lipolysis process, however an efficient clearance during pause favored by fatty acid uptake by skeletal muscle occurs, indicating that a supply for energy demand by aerobic metabolism also occurs. Studies have related that physiological adaptations resulting from brief sessions in Wingate-based HIIT over two weeks is a potential stimulus to enhance skeletal muscle oxidative capacity and induce adaptations that are apparent after several weeks, such as reduced rate of glycogen utilization and lactate production during exercise, and an increased capacity for whole-body and skeletal muscle lipid oxidation (Gibala et al., 2009; Burgomaster et al., 2008). Recently, our group has demonstrated that low-volume HIIE performance (4 sessions of Wingate-based HIIT, 30s x 3 minutes rest, ~2 minutes of exercise) promotes accumulated serum NEFA levels after the last session followed by rest (Lira et al., 2015). The higher serum NEFA levels can be a result of the lipolysis process; the need for an available substrate for maintenance of muscle contraction during exercise, but, as the exercise performed was short-duration, free fatty acid uptake by the skeletal muscle can be reduced, exposing the blood circulation to high concentrations of NEFA. Our data suggest that, HIIE performed in high-volume (5km) provides increased fatty acid uptake, principally by skeletal muscle.

On the other hand, a robust inflammatory response during the exercise session is observed. Skeletal muscle is a major source of some cytokines and the response is dependent on duration, intensity and session volume of exercise (Pedersen and Febbraio, 2009; Neto et al., 2011). The cytokines exert several functions, and have a crucial role in energy metabolism, such as IL-6 and TNF- α , that are important in the anti-inflammatory response and exert effects on glucose and lipid metabolism, stimulating increases in the lipolysis and glycogenolysis process in order to provide an energy supply for the skeletal muscle and other tissue after exercise.

In the present study, we observed that together with high cortisol levels in the SSE session, higher TNF- α levels, and the immune-endocrine profile can exert a potential effect on the lipolysis process, leading to accumulated NEFA levels after exercise. Rosa et al. (2009) have related that acute exhaustive exercise induces a pro-inflammatory response in the adipose tissue (observed by elevated IL-6 and TNF- α levels in adipose tissue) and this increase can contribute to lipolysis and the release of fatty acids as an energy supply for muscle and other tissues immediately after exercise. On the other hand, in the HIIE session higher IL-6 values were observed. Particularly, this immune-endocrine profile can favor the glucogenolysis process and the available glucose for skeletal muscle work. The results suggest that the alterations regarding cytokine kinetics during exercise are dependent on the exercise mode. However, more studies are needed for a better understanding of the mechanism involved.

In addition, increased IL-10 levels and IL-10/TNF- α ratio were observed in both exercise protocols, showing the anti-inflammatory role promoted by

exercise sessions. Classically, exercise leads to an anti-inflammatory status, and its condition is induced by an increase in IL-6 production in the skeletal muscle and, after exercise, higher IL-1ra and IL-10 levels are observed. The increased IL-10 levels can be related to higher IL-6 and TNF- α levels, and the principal role of these is to prevent the exacerbation of the pro-inflammatory status, blocking a possible persistent inflammatory status. Both HIIE and SSE were able to promote an anti-inflammatory status, as seen in an increased IL-10/TNF- α ratio. This suggests that both can be utilized as strategies for different populations, such as obesity, diabetes, dyslipidemia. More studies are necessary to better understand the mechanisms involved in HIIT in anti-inflammatory responses.

This study is limited mainly by the difference in total work performed. Even though the exercise volume was the same, HIIE likely induced higher internal loads. Future studies may want to verify whether work-matched HIIE and SSE exhibit different inflammatory and meta-bolic responses.

Conclusion

In conclusion, our results demonstrated that in both exercise protocols, when total volume is matched, the inflammatory response did not differ between group exercise modalities, leading to an anti-inflammatory status; however the metabolic response is different.

To the best of our knowledge, this is the first study comparing the metabolic and inflammatory responses to volume matched HIIE and SSE. Our initial hypothesis was that a more pronounced response would be found in the

HIIE, and would result in an increase in energetic substrates and cytokine levels. However this hypothesis was not confirmed.

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CONCLUSÃO

Em resumo, a respeito da resposta fisiológica e imunometabólica frente ao exercício intermitente de alta intensidade em jovens adultos, podemos concluir que:

- 1) HIIE foi mais eficiente em modular a FC, PSE e EPOC (apresentando valores mais elevados), mas não o VO_2 , durante a sessão de exercício quando comparado ao MICE;
- 2) HIIE exibiu uma resposta anti-inflamatória similar ao MICE quando a distância foi equalizada, entretanto, a resposta da IL-6, $\text{TNF-}\alpha$ e NEFA foram dissimilares.

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ANEXO 1

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Journal of Sports Science and Medicine

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