Universidade Estadual de Londrina

FÁBIO DO NASCIMENTO BASTOS

EFEITO DE DIFERENTES TÉCNICAS RECUPERATIVAS SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO E MARCADORES INFLAMATÓRIOS APÓS EXERCÍCIO DE ALTA INTENSIDADE DE ESFORÇO

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Orientador: Prof. Dr. Rubens Cecchini.

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Londrina, 17 de agosto de 2015.

“Que mistério, a Natureza!
E como ainda está atrasada
a ciência dos homens!”

Dona Benta
(O poço do Visconde, 1937).

Dedicatória

Aos meus familiares: Maria Antonia (mãe), Arivaldo (pai), Ligia (irmã) e Pedro (irmão),
A minha esposa: Lilian,
Ao meu grande amigo: Carlos Marcelo Pastre

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BASTOS, Fábio do Nascimento. **EFEITO DE DIFERENTES TÉCNICAS RECUPERATIVAS SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO E MARCADORES INFLAMATÓRIOS APÓS EXERCÍCIO DE ALTA INTENSIDADE DE ESFORÇO.** 2015, 95f. Tese (Doutorado em Patologia Experimental) – Universidade Estadual de Londrina, Londrina, 2015.

RESUMO

O objetivo do presente estudo foi investigar o perfil de marcadores de estresse oxidativo, inflamatórios e de dano muscular após exercício de alta intensidade de esforço em três diferentes processos de recuperação [imersão em água fria (IAF), recuperação ativa (RA) e recuperação passiva (RP)], considerando-os como efeitos deletérios ou adaptativos do exercício e da técnica. A casuística foi composta por 24 indivíduos do sexo masculino. Na primeira visita, foi realizado um teste incremental para determinar o consumo máximo de oxigênio e a velocidade máxima associada (MAS). Após 72h foram iniciadas as três visitas restantes para a realização dos testes exaustivos na MAS e os diferentes métodos de recuperação, todos por 6 min. Os três testes foram separados por intervalos de 7 dias (randomizados: IAF, RA ou RP). As variáveis sanguíneas foram analisadas nos momentos basal, 90 min, 24, 48 e 72 h após os testes máximos. Cada participante realizou todos as técnicas recuperativas em um período de 25 dias no total. A análise estatística foi realizada baseada nas inferências da mínima mudança detectável. Para a proteína carbonílica (PC) as diferenças entre RP e RA e entre RP e IAF não foram estatisticamente significantes em todos os momentos analisados. As diferenças entre os grupos mostraram que o malondialdeído (MDA) foi superior em 90 min e 24 h, além disso, também foi superior em 48h e 72h após o exercício no grupo RP em relação à RA. MDA foi maior em todos os momentos analisados para o grupo RP em relação à IAF. Para capacidade antioxidante total (TRAP) as diferenças entre RP e RA, e entre RA e IAF não foram conclusivas. No entanto, a TRAP foi maior no grupo RP nos momentos 24h e 48h após o exercício em relação à IAF. Em relação ao perfil das citocinas não houveram diferenças entre as técnicas. Menores valores de CKmb e CKmm foram observados no grupo IAF e RA quando comparado à RP a partir de 90 min. A lipoperoxidação esteve mais pronunciada no grupo PAS quando comparado aos grupos IAF e RA. É possível concluir, que IAF e a RA apresentam menos danos à ultraestrutura da célula e menos efeitos deletérios em comparação à RP.

Palavras-chave: recuperação de função fisiológica, estresse oxidativo, crioterapia.

BASTOS, Fábio do Nascimento. **EFEITO DE DIFERENTES TÉCNICAS RECUPERATIVAS SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO E MARCADORES INFLAMATÓRIOS APÓS EXERCÍCIO DE ALTA INTENSIDADE DE ESFORÇO.** 2015, 95 pages. Thesis (Doctoral Degree Thesis) – State University of Londrina. Londrina, 2015.

ABSTRACT

The aim of this study was to investigate the profile of markers of oxidative stress, inflammation and muscle damage after high intensity exercise in three different recovery processes [cold water immersion (CWI), active recovery (ACT) and passive recovery (PAS)], considering them as deleterious or adaptive effects of exercise and technique. The sample consisted of 24 male subjects. On the first visit, an incremental test was performed to determine the maximal oxygen uptake and the associated maximum speed (MAS). After 72 h started the remaining three visits to the achievement of exhaustive testing in the MAS and the different recovery methods, all for 6 min. The three tests were separated by intervals of 7 days (randomized: CWI, ACT or PAS). The blood variables were assessed at baseline, 90 min, 24, 48 and 72 h after the maximum tests. Each participant performed all the recuperative techniques over a period of 25 days in total. Statistical analysis was performed based on inferences of the minimum detectable change. For carbonylated proteins (CP) differences between PAS and ACT and between PAS and CWI were not statistically significant in all analyzed times. Differences between groups showed that the malondialdehyde (MDA) was higher at 90 min and 24 h, moreover, was also higher in 48 h and 72 h after exercise in the PAS group in relation to the ACT. MDA was higher at all times analyzed for the PAS group in relation to the CWI. For total antioxidant capacity (TRAP) differences between PAS and ACT and between ACT and CWI were not conclusive. However, TRAP was higher in the PAS group in moments 24 and 48 h after exercise compared to the CWI. Regarding the profile of cytokines there were no differences between the techniques. Lower values of CKmb and CKmm were observed in the CWI and ACT group when compared to the PAS group from 90 min. Lipid peroxidation was more pronounced in the PAS group compared to the CWI and ACT groups. It is possible to conclude that CWI and the ACT have less damage to the ultrastructure of the cell and less deleterious effects compared to the PAS.

Key words: Post-exercise recovery; oxidative stress; cryotherapy.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

CK	Creatina quinase
CO_2	Dióxido de carbono
CP	Carbonilação de proteínas
DNA	Ácido desoxirribonucléico
ERO	Espécies reativas de oxigênio
GH	Hormônio de crescimento
GPx	Glutationa peroxidase
GR	Glutationa redutase
H_2O_2	Peróxido de hidrogênio
HO•	Radical hidroxila
HPLC	Cromatografia líquida de alta performance
IAF	Imersão em água fria
IL-1	Interleucina-1
IL-10	Interleucina-10
IL-1ra	Interleucina-1 receptor antagonista
IL-1β	Interleucina-1beta
IL-6	Interleucina-6
MAS	Velocidade máxima associada ao consumo máximo de oxigênio
MDA	Malondialdeído
^oC	Graus Celsius
PC	Proteína carbonílica
PCR	Proteína C-reativa
pH	Potencial hidrogeniônico
RA	Recuperação ativa
RP	Recuperação passiva
SOD	Superóxido dismutase
TBARS	Ácido tiobarbitúrico
TNF-α	Fator de necrose tumoral-alfa
TRAP	Capacidade antioxidante total
VFC	Variabilidade de frequência cardíaca

SUMÁRIO

1 INTRODUÇÃO

Vários praticantes de diversas modalidades esportivas têm utilizado exercícios de alta intensidade de esforço pensando no treinamento, recuperação e desempenho. O exercício de alta intensidade é caracterizado por intensas contrações musculares, microtraumatismos teciduais e altos níveis de impacto, além de envolver alto gasto energético, tal condição é identificada como estressor fisiológico e metabólico principalmente em sujeitos fisicamente ativos (BASTOS et al., 2012). Os principais sintomas após a prática de um exercício exaustivo é a redução de força muscular, dor muscular tardia, diminuição da amplitude de movimento. Além do exposto, o exercício, em diferentes intensidades, pode levar ao aumento de marcadores inflamatórios e gerar espécies reativas de oxigênio (ERO) que pode ser consequência de possíveis danos musculares (FINAUD; LAC; FILAIRE, 2006; POWERS; NELSON; HUDSON, 2011).

Nesse sentido a recuperação, faz parte de todo programa de condicionamento físico, pois a partir dela que o praticante pode restaurar as condições fisiológicas basais. Entretanto, muitos praticantes treinam de forma extremamente intensa, sem respeito ao tempo necessário para restauração adequada de substratos utilizados durante o esforço, antes de submeterem-se a um novo estímulo, caracterizando uma condição inadequada (FINAUD; LAC; FILAIRE, 2006; WILCOCK et al., 2006; BARNETT, 2006; SELLWOOD et al., 2007). Ações de campo são investigadas para constatar a efetividade da recuperação, e cada vez mais novas ferramentas são propostas na quantificação dos efeitos do estresse causado pelo exercício e o potencial de recuperação dos sistemas orgânicos (PASTRE et al., 2009). Por outro lado, a literatura é escassa na procura de parâmetros que possam investigar possíveis efeitos deletérios por meio de intervenções recuperativas específicas.

A imersão em água fria (IAF) tem sido utilizada como método de recuperação pós esforço em populações diversas como atletas de alto rendimento de diferentes modalidades (DE PAUW et al., 2014; VERSEY et al., 2013) e sujeitos fisicamente ativos (ROBERTS et al., 2014; BASTOS et al., 2012) nos mais variados tipos de estresse físico. Há evidências de sua resposta sobre aspectos clínicos (BLEAKLEY et al., 2012), funcionais (ROBERTS et al., 2014; HOWATSON et al., 2009), autonômicos e fisiológicos (BASTOS et al., 2012), sendo expostas lacunas

considerando a relação dose-resposta, e principalmente a possível ocorrência de efeitos deletérios (BLEAKLEY; DAVISON, 2010). A cronicidade na realização do exercício físico, promove uma cascata de eventos que podem ter princípio no dano da ultraestrutura celular e repercutir em cenários diversos, sejam fisiológicos, metabólicos e até funcionais (VOLLAARD et al., 2005; FINAUD; LAC; FILAIRE, 2006; POWERS; NELSON; HUDSON, 2011).

O conjunto de informações levantado a partir de pesquisas relacionadas ao tema mostra, em sua maioria, efeitos benéficos para a IAF. No entanto, alguns efeitos são questionados, sobretudo pela incongruência nas formas que são utilizados os protocolos de exercício e recuperação. Há uma diversidade de estudos que abordam a técnica buscando somente seus desfechos favoráveis.

Uma das técnicas de recuperação mais antigas é a recuperação ativa (RA), que consiste na realização de exercícios ativos aeróbios e de baixa intensidade de esforço. Pastre et al. (2009), em estudo de revisão, sugerem intensidade do esforço entre 20 e 50% do VO_{2max}. Embora a RA seja amplamente discutida no âmbito científico, os pesquisadores discutem respostas que envolvam aspectos isolados, como a remoção do lactato sanguíneo, e níveis de potencial hidrogeniônico (pH).

Sabendo que o exercício, dependendo de sua intensidade, pode proporcionar efeitos positivos e negativos no que tange a formação de ERO, e levando em consideração que a utilização da RA é utilizada após exercícios de alta intensidade de esforço, torna-se interessante investigar se a RA pode provocar efeitos nocivos a partir da análise de variáveis que envolvem o perfil do estresse oxidativo.

1.1 Exercício físico e recuperação pós esforço

Atualmente, estudos têm analisado o processo de recuperação pós esforço como estratégia para integrar ações de campo no treinamento esportivo. Para tanto, diferentes técnicas são empregadas para análise de desfechos normalmente relacionados ao desempenho, sinais clínicos, marcadores de lesão e funções fisiológicas (BARNETT, 2006). Entretanto, cada qual pode oferecer desfechos particulares e, portanto, merecem análise.

Considerando o exposto, no âmbito do exercício e recuperação,

pode-se refletir sobre alguns achados. Dentre as técnicas mais utilizadas nos processos de recuperação pós esforço destacam-se a recuperação passiva (RP), que utiliza o repouso, sem qualquer tipo de intervenção, para retomada da homeostase; a recuperação ativa (RA), que consiste na realização de exercícios de baixa intensidade de esforço por curtos períodos de tempo (SPENCER et al, 2006); e a IAF – comumente relacionada ao gerenciamento de lesões musculoesqueléticas, mas que tem como objetivo resfriar segmentos do corpo em água com temperatura igual ou inferior a 15ºC (BLEAKLEY et al., 2012). Cada uma das técnicas supracitadas pode ser realizada em diferentes condições, havendo variação quanto ao tempo, temperatura e frequência de exposição à técnica.

Com base na literatura científica, tais técnicas são discutidas nos âmbitos clínicos, funcionais, metabólicos e autonômicos. Há opções clássicas utilizadas para inferir sobre a recuperação após exercícios, como o desempenho (DE PAUW et al., 2014; WHITE et al., 2014; ROBERTS et al., 2014), índices lactacidêmicos (BASTOS et al., 2012; BROPHY-WILLIAMS et al., 2011; HEYMAN et al., 2009; CROWE et al., 2007), percepção de recuperação pós esforço (HALSON et al., 2008; STANLEY et al., 2012; SELLWOOD et al., 2007), dor (HALSON et al., 2008; SELLWOOD et al., 2007), frequência cardíaca e sua variabilidade (BASTOS et al., 2012; STANLEY et al., 2012; Al Haddad et al., 2012; PAROUTY et al., 2010; BUCHHEIT et al., 2009), e hormonais (SILVA et al., 2013; WAHL et al., 2013; ABDERRAHMANE et al., 2013).

Considerando os resultados presentes na literatura frente ao efeito da IAF sobre o desempenho, deve-se considerar que a comparação dos resultados não é feita entre estudos de mesma natureza. Dessa forma, antes de afirmar quaisquer condições que remetem ao efeito da IAF, é preciso cautela. Assim como a comparação com outras técnicas recuperativas.

Bastos et al. (2012), em estudo com 20 sujeitos fisicamente ativos, utilizaram após exercício de alta intensidade de esforço a IAF, RA e RP. Os autores observaram que do ponto de vista da análise de marcadores clássicos de atividade metabólica, não houve diferença significativa entre a IAF e a RA, pois ambas foram capazes de acelerar a remoção de lactato quando comparada a RP. No entanto, somente a IAF foi capaz de promover reativação parassimpática e proporcionar melhores valores de variabilidade de frequência cardíaca (VFC). Resultados semelhantes no que diz respeito a VFC, foram observados em vários ensaios

(STANLEY et al., 2012; Al Haddad et al., 2011; PAROUTY et al., 2010; BUCHHEIT et al., 2009).

Sobre os desfechos clínicos como por exemplo a dor muscular tardia, a literatura também apresenta resultados significantes a favor da IAF. A maioria das comparações se remetem a RP (BUCHHEIT et al., 2009; INGRAM et al., 2009; KING; DUFFIELD, 2009; HALSON et al., 2008), mas também são encontradas comparações com a RA (KING; DUFFIELD, 2009). Duas recentes meta-análises (BLEAKLEY et al., 2012; LEEDER et al., 2012) encontraram evidências de baixa qualidade, relatando que IAF é uma estratégia eficaz para reduzir a dor muscular tardia após uma variedade de tipos de exercícios, além disso, as revisões (BLEAKLEY et al., 2012; LEEDER et al., 2012) apontam para efeitos inconclusivos sobre a função muscular. Diante da quantidade de evidências inconclusivas, é possível inferir que talvez sejam necessários marcadores mais sensíveis, na comparação entre protocolos iguais.

1.2 Exercício físico, estresse oxidativo, inflamação e dano muscular

A prática de atividade física dependendo da intensidade pode promover vários efeitos benéficos para a saúde do praticante. Apesar disto, vários estudos têm mostrado que o exercício físico pode favorecer o aumento de radicais livres além de provocar danos teciduais, estimulando o sistema de defesa antioxidante por diferentes vias, o que caracteriza o estresse oxidativo (POWERS; NELSON; HUDSON, 2011; BLEAKLEY; DAVISON, 2010; BLOOMER; GOLDFARD, 2004).

Os primeiros estudos que mostraram que o exercício físico pode levar ao estresse oxidativo são datados de mais de 30 anos atrás. A partir desta data o termo "estresse oxidativo" foi definido por alguns autores (SIES, 1985; JONES, 2006; SIES; JONES, 2007) como um desequilíbrio entre oxidantes e antioxidantes, em favor dos oxidantes, conduzindo a uma ruptura da sinalização redox e do controle de danos moleculares.

Há evidências de que o estresse oxidativo resulte do excesso de produção de ERO (POWERS; NELSON; HUDSON, 2011). O aumento inicial de ERO durante o exercício, bem como após no período de recuperação, pode levar ao aumento de substâncias pró-oxidantes por meio do *burst* respiratório, perda da

homeostase do cálcio e/ou a destruição de proteínas que contém ferro (FISHER-WELLMAN; BLOOMER, 2009) (Figura 1). Este excesso pode danificar lipídios, proteínas e ácidos nucléicos, e a investigação sugere que o estresse oxidativo possa contribuir para o envelhecimento e desenvolvimento de várias doenças, incluindo a demência, aterosclerose, diabetes, doenças neurológicas, o câncer, entre outras (BLOOMER; GOLDFARB, 2004; COVAS et al., 2002). Portanto, parece paradoxal que, embora o exercício possa promover o estresse oxidativo, uma rotina de exercício físico regular esteja associada à inúmeros benefícios à saúde, incluindo um menor risco de mortalidade.

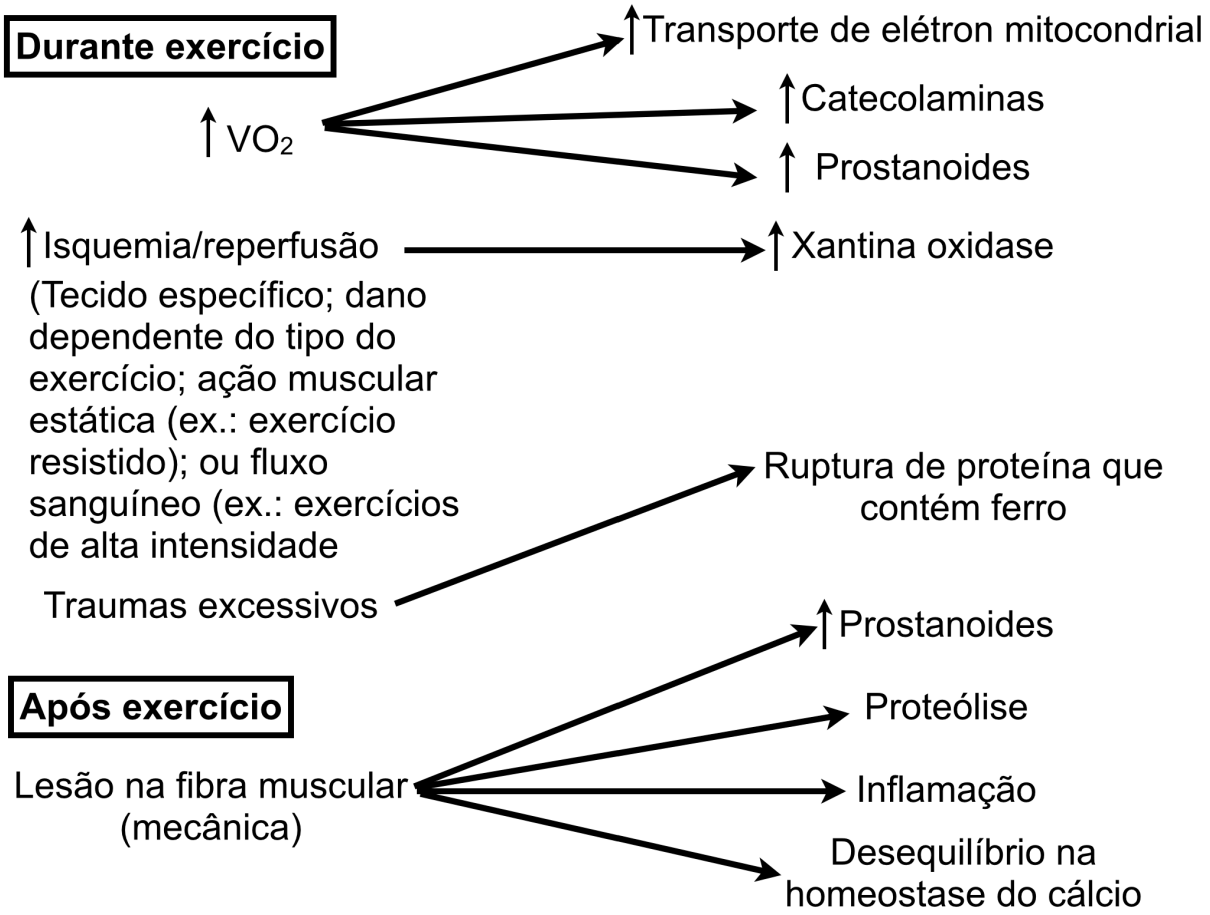

Figura 1. Potencial mecanismo para o aumento das espécies reativas de oxigênio e nitrogênio relacionado ao exercício agudo. **Fonte:** Adaptado de Bloomer; Goldfarb, 2004.

Segundo Powers, Nelson e Hudson (2011), apesar de níveis elevados de produção de ERO danifiquem componentes celulares, níveis baixos a moderados de oxidantes celulares desempenhem papéis importantes no que tange

a adaptação frente ao exercício físico.

"Espécies reativas" é um termo genérico que engloba a produção de moléculas quimicamente reativas, classificadas tanto como radicais como radical hidroxila ($HO^{\bullet}$), como não-radicais, como por exemplo peróxido de oxigênio (H_2O_2). Estes radicais livres incluem ânion superóxido, H_2O_2, $HO^{\bullet}$, ácido hipocloroso, e peroxinitritos (COVAS et al., 2002). Os efeitos biológicos proporcionados pelos radicais livres podem acometer lipídeos, ácido nucleico (DNA) e proteínas (FISHER-WELLMAN; BLOOMER, 2009).

As proteínas são os principais alvos para as ERO devido à sua elevada abundância nos sistemas biológicos. Segundo Fisher-Wellman e Bloomer (2009), as proteínas podem sequestrar a maioria (50-75%) das ERO geradas no metabolismo. O dano oxidativo a proteínas pode ocorrer diretamente por interação da proteína com as ERO ou indiretamente; por interação da proteína com um produto secundário que pode ser resultante da interação de radical com lípidio ou uma molécula de carboidrato (DALLE-DONNE et al., 2006). A formação e acúmulo de carbonilação de proteínas (CP) tem sido utilizados para avaliar a oxidação total de proteínas em relação ao exercício (CECI et al., 2014; FATOUROS et al., 2010; ZEMBRON-LACNY; SLOWINSKA-LISOWSKA; ZIEMBRA, 2009; FALONE et al., 2009). Ressalta-se que a oxidação de proteínas é mais frequentemente representada pela formação de derivados carbonil e pode levar à perda de função catalítica ou estrutural, o que torna estas proteínas susceptíveis a degradação proteolítica (LEVINE; STADTMAN, 2001).

No âmbito do exercício físico, algumas investigações têm apontado para o comportamento da CP. Ceci et al. (2014), objetivaram em seu estudo quantificar o impacto de um treinamento resistido sobre a CP. Os autores puderam concluir que há um aumento gradativo dos valores de CP imediatamente após o esforço com aumento gradativo até 24h. O que chamou atenção dos pesquisadores foi que após treinamento com aumento gradual de carga, houve diferença significativa entre o grupo treinamento e o grupo controle ($p < 0,05$), mostrando que o parâmetro parece ser um bom marcador de estresse oxidativo.

Além da avaliação clássica da CP para indicar o dano oxidativo, outros métodos são bastante utilizados, como a avaliação da peroxidação lipídica. A quantficação do malondialdeído (MDA), produto da peroxidação lipídica, representa o ensaio mais utilizado. O MDA é um aldeído de baixo peso molecular com três

cadeias de carbono, produzido durante a decomposição de um hidroperóxido lipídico. Spirlandeli et al. (2014), apontam para as incongruências encontradas na literatura a partir da avaliação do MDA. Os autores questionam as metodologias utilizadas nos ensaios clínicos. Para tanto, avaliaram o MDA por meio de três métodos, cromatografia líquida de alta performance (termo em inglês HPLC); reação com o TBARS, e ensaio do MDA com 1-metil-2-fenilindol. Os resultados mostram que as técnicas mais sensíveis para identificar a lipoperoxidação através do MDA após exercícios agudos são o método de cromatografia líquida e TBARS, embora este último possa superestimar os valores de MDA. Os autores (SPIRLANDELI et al., 2014) ainda relatam que o HPLC é o método padrão ouro e encorajam este método de análise para utilização nas análises laboratoriais e de campo.

Estudos têm mostrado aumento dos níveis de MDA de zero a quatro horas dependendo da intensidade do esforço (SPIRLANDELI et al., 2014; FISHER-WELLMAN; BLOOMER 2009; MICHAILIDIS et al., 2007). No entanto, há evidências de que haja aumento do MDA nas primeiras 24h seguido de decréscimo conforme estudos de Silva et al., (2013) e Fatouros et al., (2010).

Para conter e balancear as respostas promovidas pelos radiais livres levando ao estresse oxidativo, o organismo busca respostas do sistema antioxidante. O termo antioxidante pode ser definido como qualquer mecanismo, estrutura e/ou substância que retarde, impeça ou retire modificações oxidativas de uma molécula alvo (HALLIWELL; GUTTERIDGE, 2007; PAMPLONA; CONSTANTINI, 2011). Assim, o sistema de defesa antioxidante objetiva proteger as células contra o excesso de produção das ERO. Os antioxidantes podem ser moléculas complexas, tais quais a superóxido dismutase (SOD) e peroxirredoxinas, ou mais simples, tais quais o ácido úrico e glutationa (GUTTERIDGE; HALLIWELL, 2010). Eles podem ser classificados de acordo com a sua função em: i) eliminadores de radicais livres, ii) sequestradores de radicais livres e iii) agentes que inibem a geração de espécies reativas.

É sabido que a geração de espécies reativas, como o oxigênio, radical superóxido e radical hidroxila ocorre como consequência do metabolismo celular normal (HALLIWELL; GUTTERIDGE, 2007). Há evidências de que as fibras musculares alteram o ambiente fisiológico em consequência de uma intensa ou prolongada atividade contrátil. Com isso pode haver predisposição das fibras para maior produção das ERO (POWERS; NELSON; HUDSON, 2011). Outros fatores

que podem aumentar a produção das ERO frente ao exercício são alterações na temperatura, aumento da concentração de dióxido de carbono (CO_2) e diminuição do potencial hidrogeniônico (pH). Essas mudanças induzidas pela contração muscular podem estimular a produção de íons superóxido (POWERS; NELSON; HUDSON, 2011).

O radical superóxido pode fazer com que haja quebra nas cadeias do DNA e modificações de base simples, oxidação de cadeias laterais de aminoácidos e de fragmentação de polipeptídeos, e a degradação dos ácidos graxos poli-insaturados, além dos fosfolípidos por peroxidação lipídica (SPIRLANDELI et al., 2014). O processamento do radical superóxido é realizado pelo sistema endógeno de defesa antioxidante, que inclui a atividade enzimática do SOD, glutationa-peroxidase (GPx) glutationa redutase (GR), bilirrubina, ácido úrico, etc; e em conjunto com antioxidantes exógenos consumidos por meio da dieta através das vitaminas E, C, carotenóides e compostos fenólicos (COVAS et al., 2002).

Alguns desfechos são encontrados na literatura científica no que diz respeito à capacidade antioxidante frente ao exercício físico e ao treinamento. O aumento no consumo de oxigênio durante o exercício aeróbio é acompanhado por uma elevação das ERO (POWERS; NELSON; HUDSON, 2011). Exercício aeróbio agudo gera ERO, criando perturbação no transporte de elétrons, o que leva ao escape excessivo de radicais superóxido. No entanto, o treinamento de resistência de longa duração reduz efetivamente o dano associado ao aumento do consumo de oxigênio, melhorando as defesas antioxidantes do organismo. Tem sido demonstrado que o sistema de defesa antioxidante melhora em resposta ao treinamento de resistência (CECI et al., 2014; MARTINOVIC et al., 2010; ZEMBRON-LACNY; SLOWINSKA-LISOWSKA; ZIEMBRA, 2009).

Falone et al. (2009), encontraram aumento significativo da TRAP após exercício aeróbio. Ceci et al. (2014), observaram através da glutationa e glutationa oxidada uma melhor capacidade antioxidante em 12 semanas de treinamento resistido gradual. Além da cascata de respostas que as ERO podem induzir frente ao exercício, dependendo da intensidade de esforço, pode haver microtraumatismos e consequentemente levar a liberação de marcadores inflamatórios, como as citocinas, e de dano muscular como a creatina quinase (CK) (FINAUD; LAC; FILAIRE, 2006; POWERS; NELSON; HUDSON, 2011).

Embora os efeitos do exercício tenham sido estudados há muitos

anos, recentes estudos ainda se preocupam em traçar perfis fisiológicos frente a estímulos específicos. Autores observam diferenças significativas de CK após 24 e 48h do término de uma atividade, sendo que os valores de pico acontecem em 24h (Silva et al., 2013). Vale ressaltar também que os valores de MDA e de TRAP também estavam aumentados 24 e 48h após partida de futebol. Para Fatouros et al., (2010) o pico de dor muscular tardia também aconteceu em 24h após partida de futebol, enquando que os picos de CK, CP e glutationa oxidada aconteceram em 48h pós esforço.

Lesões estruturais, como apontado nos estudos supracitados, podem promover uma intensa cascata de desfechos celulares, incluindo respostas inflamatórias. Estas muitas vezes induzem a produção de citocinas como interleucina-1 (IL-1) e fator de necrose tumoral-α (TNF-α), estimulando assim a proliferação de interleucina-6 (IL-6) que por sua vez, induz o fígado a produzir substancias da fase aguda, como a proteína C-reativa (PCR) (CZARKOWSKA-PACZEK, 2005).

Consepcion-Huertas et al., (2013) acompanharam durante um ano atletas de handebol em seus treinamentos e competições. Observaram que do ponto de vista de estresse oxidativo, os atletas adquirem adaptação e reduzem os possíveis efeitos adversos ao longo da temporada, como dores musculares. Por outro lado, do ponto de vista de marcadores inflamatórios, como IL-1, IL-6 e TNF-α os autores encontraram que os atletas não conseguiram adquirir adaptação frente ao exercício, pois os valores se mantiveram praticamente inalterados. Semelhante resultado Zembron-Lacny, Slowinska-Lisowska e Ziemba (2010) tiveram quando investigaram por 6 meses jogadores profissionais de basquetebol. Estudos sobre os efeitos de uma sessão, ou da diferença entre modalidades, ainda são obscuros.

1.3 Estresse oxidativo, inflamação, dano muscular e recuperação pós esforço

Os padrões de recuperação usualmente utilizados são passivos –, caracterizados por repouso sem quaisquer tipos de modalidades de intervenção (BASTOS et al., 2012), e ativos, utilizando exercícios de curta duração e baixa intensidade de esforço visando uma melhor resposta metabólica relacionada ao fluxo sanguíneo (DE PAUW et al., 2014) e, além dessas, a utilização de recursos

diversos incluindo a IAF (BASTOS et al., 2012).

Sugere-se como efeitos da IAF uma resposta do sistema de termorregulação resultando em redirecionamento de fluxo (BLEAKLEY et al., 2012; VAILE et al., 2011), a redução de impulso elétrico promovendo analgesia (BLEAKLEY; MCDONOUGH; MACAULEY, 2004) e a potencialização do retorno venoso e linfático considerando os efeitos da pressão hidrostática (WILCOCK et al., 2006). Por outro lado, reduções de temperatura podem promover respostas nocivas em âmbito metabólico, funcional e estrutural (BLEAKLEY; DAVISON, 2010; CROWE et al., 2007; PEIFFER et al., 2009). Danos podem ser observados considerando reduções importantes de temperatura, mas pouco se relata sobre práticas de IAF e a ocorrência de efeitos deletérios.

Atualmente a IAF talvez seja o método mais popular no que tange a recuperação pós exercício (BIEUZEN et al., 2013). Há evidências de que a IAF possa auxiliar na recuperação da dor muscular tardia (TAKEDA et al., 2014), e no desempenho (ROWSELL et al., 2014), pois pode alterar a temperatura tecidual e o fluxo sanguíneo. Além disso, o efeito da pressão hidrostática promovido pela imersão em água pode criar um deslocamento de fluidos a partir da periferia para as regiões centrais do corpo. Esta pressão resulta em várias alterações fisiológicas, incluindo um aumento no transporte de substrato e do débito cardíaco, bem como uma diminuição da resistência periférica e do volume do fluido extracelular por meio de gradientes osmóticos intracelulares e intravasculares (WILCOCK et al., 2006). A IAF induz uma série de respostas fisiológicas e bioquímicas, tais como vasoconstrição, estimulação do retorno venoso, remoção de metabólitos após o exercício, redução de edema e dor muscular (LEEDER et al., 2012).

Estudos têm avaliado os desfechos das técnicas recuperativas após exercício de alta intensidade de esforço. Bailey et al. (2007), utilizaram em seu estudo 20 sujeitos fisicamente ativos e avaliaram o efeito da IAF (10 min a 10^{o}C) após exercício de alta intensidade. Os autores observaram que houve pico de dor muscular tardia e CK 24h pós esforço. Porém, concluíram que os achados não foram influenciados pela realização da técnica recuperativa, ou seja, a IAF não influenciou os níveis de CK. Corroborando os achados de Ingram et al. (2009), o que diferiu foi a população estudada, no caso atletas, o tempo de IAF (2 x 5 min a 10^{o}C), e o protocolo de estresse (simulação de partida de futebol – *sprints* repetidos).

Também utilizando o mesmo tempo de imersão e a mesma

temperatura Jakeman et al. (2009), induziram a lesão muscular realizando 10 séries com 10 repetições de saltos verticais e logo após, os indivíduos foram submetidos a IAF (10 min a 10^{o}C) e obtiveram como resultados que a técnica não foi suficiente para reduzir a concentração de CK após lesão muscular induzida pelo exercício. Por outro lado, Ascensão et al. (2011), avaliaram o efeito da IAF (10 min a 10^{o}C) em jogadores de futebol após a partida, obtendo redução das concentrações de CK.

Em recente estudo, Leeder et al. (2015) investigaram os efeitos da IAF (14^{o}C por 14 min) na posição sentada e na posição ortostática, de forma que pudessem avaliar a diferença da pressão hidrostática. Para isso, utilizaram 24 atletas de alto rendimento e após exercício de alta intensidade de esforço, avaliaram aspectos funcionais, CK, PCR, IL-6 e percepção de dor. Os autores concluiram que não há diferenças entre os tipos de IAF, bem como entre o grupo controle, questionando as reais potencialidades da técnica.

Rowsell et al. (2014), avaliaram o efeito da IAF (10^{o}C por 5 x 1 min com 1 min de repouso entre as sessões) após exercício de alta intensidade em triatletas a partir de variáveis fisiológicas, psicológicas e marcadores bioquímicos. Os autores puderam concluir que a IAF foi eficaz em melhorar o desempenho dos atletas quando comparado a imersão em água (34^{o}C). Por outro lado, os resultados mostraram-se inconclusivos em relação a CK, lactato desidrogenase, mioglobina, IL-6, exceto para a interleucina-10 (IL-10) o qual mostrou-se benéfica para a IAF. Tal condição pode sugerir um efeito anti-inflamatório para a IAF.

Takeda et al. (2014), avaliaram o efeito da IAF (15^{o}C por 10min) em atletas após partida de rugby. Utilizaram como variáveis potência muscular e marcadores bioquímicos de dano muscular. Os autores encontraram melhor percepção de recuperação e de função para o grupo IAF. Por outro lado, não houve quaisquer diferenças significativas entre a IAF e o grupo controle para os marcadores aspartato transaminase, creatinina, CK, lactato desidrogenase e lactato, pós intervenção e 24h após partida de rugby.

Dugué et al., (2005) avaliaram os efeitos a curto e longo prazo (12 semanas) do resfriamento de corpo inteiro (-110^{o}C por 2 min) e IAF em nadadores a partir da TRAP. Os autores concluiram que embora houvesse aumento da TRAP após o estresse térmico, a longo prazo não foi possível identificar quaisquer alterações quando comparado aos valores basais.

White, Rhing e Wells (2014), investigaram os efeitos de diferentes

protocolos em IAF a partir de respostas inflamatórias e funcionais após exercício de alta intensidade de esforço. Com uma amostra de 8 sujeitos fisicamente ativos, completaram 5 ensaios de *sprints* repetidos. Os protocolos de IAF (IAF-10min a 20^{o}C, IAF-30 min a 20^{o}C, IAF-10 min a 10^{o}C, ou IAF-30 min a 10^{o}C) foram comparados com a RP. Os autores concluiram que a IAF parece facilitar a recuperação do músculo esquelético, mas não para atividades que exigem exercícios de potência para contração concêntrica. Além disso, essas mudanças parecem não estar relacionadas aos níveis de citocinas inflamatórias. Por outro lado, embora discretamente, os autores relatam que o protocolo de 30 min de IAF independente da temperatura, pode causar o aumento de citocinas inflamatórias circulantes.

Pournot et al. (2011), ao estudar atletas de alto rendimento, observaram os efeitos da IAF a partir de marcadores inflamatórios, avaliação de força e potência. Os resultados apontam para eficácia da IAF no que tange a remoção da CK, lactato desidrogenase e desempenho, quando comparada ao grupo controle. Para os demais marcadores inflamatórios não houve qualquer diferença entre os grupos.

Nemet et al., (2009) avaliaram o efeito da crioterapia (pacote de gelo) em atletas de handebol entre o intervalo de um treinamento de alta intensidade de esforço. Para tanto, utilizaram variáveis para observar os efeitos anabólicos, catabólicos, pró-inflamatórios e anti-inflamatório. O exercício proporcionou aumento do IL-6, hormônio de crescimento (GH) e testosterona. A aplicação da crioterapia esteve associada à diminuição da IL-1β, IL-1 durante a recuperação. Os autores concluiram que a aplicação da crioterapia imediatamente após treinamento de alta intensidade está associada a diminuição de marcadores pró e anti-inflamatórios no período de recuperação.

Autores (ASCENSÃO et al., 2011; POURNOT et al., 2011) descrevem que no período pós-exercício, a IAF auxilia na redução da liberação de proteínas intramusculares para o sistema linfático e na redução do dano tecidual. Afirmam ainda que a restauração do dano muscular pode estar associada com a redução da permeabilidade celular e, com isso, a IAF pode atenuar as respostas inflamatórias neste período. Assim, com base nos estudos levantados pela literatura científica, antes de inferir sobre benefícios e malefícios de respostas imediatas a partir da IAF deve-se considerar a relação dose resposta para a referida técnica, ou

seja, tempo de exposição e temperatura de aplicação.

Em revisão sistemática e meta-análise, Torres et al., (2012) observaram que há muitos estudos objetivando amenizar os efeitos da dor muscular tardia. Dentre as técnicas mais utilizadas, destacam-se a crioterapia e a RA. No entanto, os autores concluem que há falta de evidências que comprovam os reais efeitos dessas técnicas.

Em relação a RA, há escassez de estudos na literatura que avaliam marcadores inflamatórios e estresse oxidativo a partir desta intervenção. Devido a técnica objetivar efeitos no que dizem respeito ao aumento de fluxo sanguíneo na tentativa de acelerar a remoção de catabólitos produzidos durante e após o esforço, a maioria dos estudos se atentam à variáveis metabólicas como o lactato sanguíneo ou testes de desempenho.

De Pauw et al. (2014), avaliaram o efeito da RA, RP e IAF após a realização de vários exercícios. Todas as técnicas foram aplicadas por 15 min. Após os primeiros testes não houve diferenças significativas entre os métodos, em relação à potência, desempenho, concentração de lactato sanguíneo. No entanto, após o segundo ensaio, foram observados melhores efeitos para o grupo que realizou a IAF.

Webb et al., (2013) utilizaram 21 atletas de alto rendimento e investigaram a eficácia das técnicas RA, banho de contraste e IAF após partida de rugby. Em relação às variáveis estudadas, CK, percepção de dor muscular e desempenho, os autores observaram melhores resultados para o banho de contraste e IAF quando comparados a RA, 42h após o esforço.

O primeiro estudo a avaliar os efeitos da RA a partir dos níveis de estresse oxidativo e capacidade antioxidante após partida de futebol foi realizado por Andersson et al., (2010b). Os autores utilizaram atletas de futebol feminino de alto rendimento e após duas partidas investigaram os efeitos da RA e RP. De acordo com os achados, os pesquisadores concluiram que a RA traz efeitos benéficos para o praticante, pois pode prevenir a lipoperoxidação após partida de futebol. No entanto, os autores utilizaram simultaneamente dieta rica em antioxidantes. Assim, não parece adequado demonstrar as reais potencialidades da técnica, pois a mesma não foi realizada de forma isolada.

Andersson et al., (2010a) utilizaram o mesmo protocolo supracitado para avaliar os efeitos das citocinas circulantes. Os autores concluiram que

respostas evidentes de marcadores pró e anti-inflamatórios aparecem após a primeira partida de futebol, não aparecendo após a segunda partida (72h após). Os autores ainda concluem que não houve diferenças entre a RA e a RP em relação aos marcadores inflamatórios. Nesse sentido, visto o limitado referencial bibliográfico, deve-se atentar para possíveis efeitos deletérios provocados pela RA, além da observação do comportamento de parâmetros de estresse oxidativo.

1.4 Justificativa

Tanto efeitos favoráveis quanto aspectos deletérios das diversas técnicas recuperativas já foram abordados de formas e modelos diversos, mas a estratégia de apontar possíveis efeitos deletérios induzidos pelo exercício e pela técnica não parece ter sido considerado ainda em âmbito mais profundo e acurado.

Portanto, considerando a abrangência do uso da técnica em campo devido a sua popularização e facilidade de execução e as lacunas referentes aos possíveis efeitos deletérios ao corpo, entende-se como pertinente a investigação do tema em questão, ao comparar diferentes técnicas recuperativas com os modelos mais utilizados para este fim. Assim, basear-se em marcadores metabólicos, de danos da ultraestrutura, marcadores inflamatórios, e na sua capacidade antioxidante pode apontar não só uma resposta do perfil do estresse do exercício físico, mas também a influência de diferentes técnicas durante o período de recuperação após exercício. O estresse oxidativo pode assim constituir-se como um importante marcador de estresse metabólico e adaptativo, além de apontar importantes mecanismos dentro dos processos e métodos de recuperação pós-esforço.

2 OBJETIVOS

2.1 Objetivo geral

- Investigar o perfil de marcadores de estresse oxidativo, inflamatórios e de dano muscular após exercício de alta intensidade de esforço em três diferentes processos de recuperação (imersão em água fria, recuperação ativa e recuperação passiva), considerando-os como efeitos deletérios ou adaptativos do exercício e da técnica.

2.1 Objetivos específicos

- Verificar o perfil de marcadores de estresse oxidativo, inflamatórios e de dano muscular a partir de um exercício de alta intensidade de esforço;
- Investigar se o aumento ou diminuição do estresse oxidativo pós exercício tem relação com as diferentes técnicas aplicadas;
- Investigar se o aumento ou diminuição dos marcadores inflamatórios pós exercício tem relação com as diferentes técnicas aplicadas;
- Investigar se o aumento ou diminuição dos marcadores de dano muscular pós exercício tem relação com as diferentes técnicas aplicadas;
- Verificar se existe diferença entre os métodos recuperativos aplicados e se algum destes pode proporcionar efeitos nocivos ao praticante.

3 CONSIDERAÇÕES

O presente trabalho, juntamente com a metodologia empregada originaram dois artigos científicos que estão incluídos como anexo. Este trabalho foi realizado em colaboração com o Laboratório de Fisioterapia Desportiva (LAFIDE) da Universidade Estadual Paulista (FCT/UNESP – Campus Presidente Prudente), e permitiu a elaboração das seguintes conclusões:

- Os parâmetros de estresse oxidativo se comportam de forma distinta no que diz respeito às diferentes técnicas recuperativas. Respostas semelhantes acontecem entre a imersão em água fria e recuperação ativa.
- Os parâmetros de citocinas estabelecem padrões parecidos independente da técnica aplicada.
- Os parâmetros de CK se comportam de forma distinta no que diz respeito às diferentes técnicas recuperativas principalmente quando comparada à recuperação passiva.

- As técnicas de imersão em água fria, recuperação ativa e recuperação passiva, parecem não proporcionar efeitos deletérios ao praticante após exercício de alta intensidade de esforço.
- O perfil de comportamento das variáveis analisadas parecem favorecer para as técnicas de imersão em água fria e recuperação ativa.

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ANEXO A

ARTIGO 1: EVOLUTION OF OXIDATIVE STRESS PARAMETERS AS METABOLIC MARKERS IN DIFFERENT PROTOCOLS OF POST EXERCISE RECOVERY: THE IMPORTANCE OF MAGNITUDE-BASED ANALYSIS

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Dear Dr.
Cecchini,

Thank you for the submission of your manuscript entitled "OXIDATIVE STRESS PARAMETERS AS METABOLIC MARKERS ON DIFFERENT PROTOCOLS OF POST EXERCISE RECOVERY" to the International Journal of Sports Medicine. Your manuscript will now go into the reviewing process.

Manuscript authors:
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ARTIGO 1

EVOLUTION OF OXIDATIVE STRESS PARAMETERS AS METABOLIC MARKERS IN DIFFERENT PROTOCOLS OF POST EXERCISE RECOVERY: THE IMPORTANCE OF MAGNITUDE-BASED ANALYSIS

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ABSTRACT

The aim of the present study was to observe the evolution of the total antioxidant capacity behavior, lipid peroxidation and protein carbonylation after high-intensity exercises in three different recovery procedures: cold water immersion (CWI), active recovery (ACT) and passive recovery (PAS). Consideration was given to both the harmful effects decurring from the exercise and the implications of the employed techniques. 24 male subjects were recruited. On the first visit, an incremental test was performed to determine maximal oxygen consumption and the associated speed (MAS). The remaining 3 visits for the performance of constant velocity exhaustive tests at MAS and different recovery methods (6 min) were separated by 7-day intervals (randomized: CWI, ACT or PAS). For carbonyls protein (CP) the differences between PAS and ACT group and between PAS and CWI group were unclear in all moments analyzed. The differences between group showed that malondialdehyde (MDA) almost certain was higher 90 min and 24h, very likely was higher 48h and 72h following exercise in PAS group than in ACT group. MDA very likely was higher all moments analyzed in PAS group than in CWI group. For total radical-trapping antioxidant parameter (TRAP) the differences between PAS and ACT group and between ACT and CWI group were unclear. However, TRAP was higher in PAS group 24h and 48h following of exercise than in CWI group. It is possible concluding, that CWI and the ACT present less harmful effects in comparison to the PAS.

Key words: Post-exercise recovery; oxidative stress; cryotherapy; active recovery

INTRODUCTION

Cold water immersion (CWI) has been used as a post-exercise recovery method in various populations, such as high performance athletes from several sports [1,12,34] and physically active individuals [3,30], under a number of different physical stress types. There are evidence of its response regarding clinical [5], functional [20,30], autonomic and physiological aspects [3]. Gaps are exposed when one considers the dose-response relation, mainly the possible occurrence of harmful effects. The dynamics of practicing physical exercises itself leads to a chain of events that might begin with damages caused to the ultrastructure and that reflect on different scenarios, whether physiological, metabolic and even functional [14,27,35].

The often employed recovery patterns are passive – characterized by rest periods, without any type of intervention modality [3] - and active; they use low-intensity effort exercises to achieve better metabolic response with regard to blood flow [12]. Despite the aforementioned, they also use several sources that include CWI [3].

The thermoregulation system response resulting in flow redirection [5,32], reduction in electric impulses that provide analgesia [7] and the enhancement of venous and lymphatic return - taking under consideration the hydrostatic pressure effects [38] - are assumed as CWI effects. On the other hand, temperature decreases may lead to harmful responses in the metabolic, functional and structural extent [11,25]. Damage can be observed when important temperature decreases are considered, although little is reported about CWI practices and the occurrence of harmful effects.

CWI and recovery methods can increase intra-muscle pH post-exercise, suggesting a positive effect on the muscle buffering system [39]. In addition, some evidence suggest that the reduction on oxidative demand caused by post-exercise recovery methods can attenuate reactive oxygen species (ROS) generation and/or tissue interaction with its end products,

declining the consequent muscle damage mediated by ROS [28]. One of the sources of oxidative stress during physical exercise are the inflammatory response mediated by neutrophils or enhanced activity of respiratory chain during increased metabolic activity [24].

When dealing with athletic performance, where in some cases a minimal difference is meaningful, some prominent researchers have proposed inspecting the magnitudes covered by the confidence interval of a difference to make statements about how big or small the true difference could be [4]. The effect size represents the magnitude of the treatment effect of a given variable, while the magnitude-based inference tests the likelihood chances that the true value (or difference) lies below the lower limit or above the upper limit of the 90% confidence interval. Some researchers prefer to use and find it easier to interpret the effect size, while others find it more meaningful to present the quantitative/qualitative analysis proposed by Hopkins et al. [19].

Investigating the scope of the technique's use in this area is understood as pertinent - when one compares it to the most used models applied to the same purpose -, due to its popular and easy execution and the gaps linked to its possible harmful effects on the body. Therefore, making conclusions based on oxidative markers and on its antioxidant capacity may lead not only to post exercise effort responses, but also to the influence of the technique during the post-exercise recovery period.

Thus, the aim of the current study was to observe the evolution of the total antioxidant capacity behavior, lipid peroxidation and protein carbonylation after high-intensity exercises in three different recovery procedures: cold water immersion (CWI), active recovery (ACT) and passive recovery (PAS). Consideration was given to both the harmful effects decurring from the exercise and the implications of the employed techniques

aims to observe the total antioxidant capacity behavior, lipid peroxidation and protein carbonylation after high-intensity exercises in three different recovery procedures (cold water immersion, active recovery and passive recovery), by considering them enlightening effects from the exercise and the technique. There is the assumption of different behaviors among groups and their possible harmful effects on the CWI group.

METHODS

Participants

Twenty-four male physical education students (age: 21 ± 2 years; height: 175 ± 8 cm; body mass: 72 ± 11 kg; body mass index: 23.5 ± 2.1 $kg \cdot m^{-2}$; VO_{2max}: 47.1 ± 3.1 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in this study. All participants were classified as physically active, as they were regularly engaged in various intermittent activities (i.e., basketball, handball and soccer 3 times per week), with each session lasting one to two hours. None of the participants were taking any medication that would affect the results of study. After receiving verbal and written explanations regarding the testing and recovery procedures, all subjects signed a term of informed consent agreeing to participate in the study. The experimental protocol was approved by the Research Ethics Committee of the associated institution and was performed in accordance with the ethical standards of the International Journal of Sports Medicine [18].

Study Design

All tests were performed between 2:00 and 6:00 pm. Each participant came to the laboratory four times at exactly the same hour. On the first visit, an incremental test was performed to determine maximal oxygen consumption (VO_{2max}) and maximal aerobic speed (MAS). The remaining three visits used for the performance of constant velocity exhaustive tests and different recovery methods were separated by seven-day intervals. The subjects were

asked to refrain from any exercise and not to consume alcoholic or caffeinated beverages 24 h prior to each test. The subjects were also asked to have their last meal (similar carbohydrate, proteins and lipids intake) and fluid consumption at least 2 hours prior to each test. The adherence to the instructions was checked before testing and all of the subjects complied with the requirements. All tests were performed in a quiet laboratory under standardized conditions (temperature: 21 to 23^{o} C; relative humidity: 40-60%) and were preceded by 5 min of jogging at 7 km·h^{-1} and 5 min of passive recovery (standard warm-up).

Maximal Incremental Test

Maximal oxygen consumption (VO_{2max}) and MAS were determined on a treadmill (Inbramed Super ATL, Inbrasport, Rio Grande do Sul, Brazil) using an incremental protocol. The incremental protocol consisted of running at a starting velocity of 10 km·h^{-1} for 1 min, with increments of 1 km·h^{-1} per minute until exhaustion. The inclination was kept constant at 1% [17].

Gas exchange was continuously measured and averaged every 30 s (VO_{2000}, MedGraphics, Minnesota, USA). Expired O_2 and CO_2 concentrations were determined using a galvanic fuel cell for the oxygen and a non-dispersive infrared analyzer for the carbon dioxide. Flow was determined using a bi-directional differential pressure pneumotach (preVent™). Heart rate (HR) was recorded with a Polar S810i monitor in the RR mode, but averaging HR at 10 s intervals (Polar Electro, Kempele, Finland) [33].

At least two of the following criteria were required to ensure that VO_{2max} had been attained: 1) the occurrence of a plateau (i.e., < 150 mL·min^{-1} increase between two successive stages); 2) a respiratory exchange ratio above 1.10; and/or 3) HR in excess of 90% of age-predicted maximum. The MAS was recorded as the speed corresponding to the last complete stage on the incremental test [23].

Constant Velocity Exhaustive Test

After arriving to the laboratory, subjects had performed the aforementioned standardized warm-up, the treadmill velocity was set at each individual's MAS and the participant ran until volitional exhaustion. Strong verbal encouragement was provided throughout the test. Exhaustion was determined by the inability to maintain the exercise intensity or by clinical signs, such as dizziness, paleness and nausea, accompanied by the attainment of maximal HR obtained during the maximal incremental test.

Recovery Interventions

After the completion of the constant velocity exhaustive test at MAS, the subjects were submitted to three different recovery methods (separated by seven-day intervals) on different occasions in a random order (raffle card): passive recovery (PAS), ACT and CWI. The participants were allowed 1 min immediately following the completion of the running test to perform the transition to the recovery mode (e.g., removing shoes for CWI). The duration of each intervention was 6 min [3].

During PAS, the subjects stood in the orthostatic position without any attempt to accelerate recovery; this condition was considered the control. Within the first minute post-exercise, subjects could incline their trunk and support their weight on the handrails of the treadmill. No participants passed out or experienced any ill sensations in the remaining six minutes of PAS. Thus, the participants tolerated PAS and the other recovery modes relatively well.

During the ACT, the subjects walked/ran on the treadmill at a low intensity (30% of VO_{2max}) [15]. During the CWI intervention, the subjects were submerged in the orthostatic position in cold water at a level that covered the anterior superior iliac spine [3]. For this study water temperature was maintained constant at 11 ± 2^{o} C, which is within the 10-15^{o} C

range suggested in the review of Bleakley et al. [5] and previously used in several investigations [3,26,30,32,37]. According to Wilcock et al. [38] and Halson [16], very cold water (< 5^{o} C) temperature is not suitable for post-exercise recovery of performance, but for treatment of acute injuries and reduce inflammation and oedema. Participants were instructed to stand in the bath, with as little movement as possible.

After each of the recovery methods, the subjects laid supine for an additional 83 min. Recovery was performed at 90 min following the post-exercise testing session, and at 24, 48, and 72 h post-exercise. Blood samples were collected at baseline, 90min, 24, 48 and 72 h post exhaustion.

Collection and preparation of blood samples for biochemical analysis

All blood samples were analyzed in duplicate. To avoid interassay variation, all samples were analyzed in a single batch at the end of the study, with the exception of blood parameters, which were performed on the day of the collection. Venous blood samples were collected pre-exercise (Baseline) and at each of the four post-exercise time-points at 90 min, 24, 48 and 72 h. Each blood sample (5 mL) was collected from a superficial forearm vein using standard venipuncture techniques. All samples were collected directly into serum separator collection tubes and serum separated by centrifugation at 3,000 x g for 10 min at 4°C. 500 μL (serum samples) aliquots were immediately removed and stored frozen at -80°C until analysis.

Content of protein carbonyls in plasma

For detecting protein carbonyl, the colorimetric method proposed by Buss et al. [8] was used by the reaction with dinitrophenylhydrazine (DNPH), forming hydrazones. Diluted plasma samples (1:5 in monobasic potassium phosphate buffer 50mM, 1mM EDTA, pH 7,4)

were submitted to reaction with DNFH. Subsequently, protein precipitations with TCA 20 and 10% was performed, followed by treatment with ethyl acetate-ethanol (1:1 – v/v) to remove the excess of DNFH. Final pellets were re-suspended in Guanidine 6M and an absorbent peak was obtained from the reading between 355-390 nm, in spectrophotometer. Results were expressed in carbonil/mg nanomols of total protein in 1mL of plasma.

Lipid peroxidation – determination of MDA for HPLC methods

MDA-HPLC is the most suitable technique for accurate detection of MDA in sports and exercise area due to its sensitivity and accuracy. Thus, high-performance liquid chromatography (HPLC) determinations were made using an HPLC-20AT Shimadzu instrument equipped with an LC20AT pump and an SPDM20A UV absorbance detector and employing a C18 reverse-phase column. MDA levels were determined based on a standard curve. A standard solution of MDA was prepared by mixing 10 mL of 0.1 M HCl with 10 mL of 1,1,3,3-tetraethoxypropane, incubated for 5 min in a boiling water bath, and transferred to an ice bath [21].

A new method was adapted from Chirico [10] and Karatas et al. [21] in order to improve the purity of the chromatogram and conserve the HPLC column. In this method, 160 μL of plasma sample or standard solution was mixed with 100 μL of 0.5 M perchloric acid and incubated for 10 min on ice to precipitate the proteins. Samples were then centrifuged for 5 min at 5000×g/ 4°C. About 180 μL of supernatant was recovered and mixed with 100 μL of TBA. This reaction was incubated for 30 min in a boiling water bath and transferred to an ice bath to stop the reaction.

A 100 μL volume of 1 M NaH_2PO_4, pH 7, was added to each sample to stabilize the sample pH. The mobile phase consisted of 65% 50 mM KH_2PO_4 buffer and 35% HPLC-grade methanol. The supernatants were filtered and injected (20 μL) into the HPLC. The serial

dilutions of the standard solution of MDA were used as calibration points to obtain the linear regression equation and calculate the MDA concentration of the samples. Readings were obtained at 535 nm over 12 min at a flow rate of 0.8 mL/minute, and results were expressed as nanomolars of MDA.

Total radical-trapping antioxidant parameter

The total radical-trapping antioxidant parameter (TRAP) was measured as described by Repetto et al. [29]. This assay uses 2,2′-azo-bis (2-amidinopropane) (ABAP), a potent free radical generator that degrades itself to emit photons in a process that is amplified by the addition of luminol. This activity of ABAP is neutralized by antioxidants in the reaction mixture that inhibit its function. The photon emission profile of the ABAP solution was measured in a GloMax® luminometer 20/20 (Promega, Madison, USA) over 45 min at 5 readings/s.

ABAP emission in a reaction containing 900 μL of 0.1 M glycine buffer at pH 8.6, 50 μL of luminol, and 50 μL of ABAP was measured as a pro-emission standard. An antioxidant standard solution of 25 μM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-car- boxylic acid), an analog of hydrosoluble vitamin E, was added in order to neutralize ABAP autoxidation in a reaction containing 830 μL of 0.1 M glycine buffer pH 8.6, 70 μL of 25 μM Trolox, 50 μL of luminol, and 50 μL of ABAP that was used as a standard for antioxidant comparison.

Subsequently, TRAP was measured in plasma samples in reactions containing 830 μL of 0.1 M glycine buffer at pH 8.6, 70 μL of plasma sample, 50 μL of luminol, and 50 μL of ABAP. For TRAP calculation, the induction time of the sample (time for which the sample antioxidants can inhibit the ABAP action) was compared with that of the standard antioxidant (Trolox) using the following formula and the results were expressed as micromolars of

Trolox: TRAP (μM Trolox) = Sample induction time Trolox concentration in the reaction/Trolox induction time

Statistical Analysis

Data normality was tested with the Shapiro-Wilk's test. The MDA and TRAP presented skewed distributions and were log-transformed for analysis. The method of magnitude-based inferences was applied to detect the chances of an increase/inconclusive/decrease for both groups. For this purpose, the smallest worthwhile change (SWC – 0.2 multiplied by the between subject standard deviation) was obtained for each variable. The chance of the changes was greater than the SWC was obtained and compared between groups. The quantitative chances of a positive/trivial/negative effects were interpreted as follows: <1% almost certainly not; >1–5% very unlikely; >5–25% unlikely; >25–75% possible; > 75–95% likely; > 95–99 very likely; >99% almost certain. If the chances were higher than 5% for positive and negative effects, the true difference was interpreted as unclear [19]. The differences in MDA and TRAP were presented as percentage of means and coefficients of variation and the differences in CP was presented as mean and coefficients of variation. The confidence interval was 90%. The magnitude-based inferences were calculated with spreadsheets available at http://www.sportsci.org/.

RESULTS

Constant Velocity Exhaustive Test: Performance and Physiological Responses

No significant differences were detected in the duration of the constant velocity tests (15.1 ± 1.1 $km \cdot h^{-1}$) under the PAS (3.9 ± 0.8 min), ACT (3.9 ± 0.9 min) and CWI (3.9 ± 0.7 min) conditions ($p = 0.92$). All tests were terminated by voluntary exhaustion, without clinical signs of adverse health conditions.

Effect of Recovery Modes on Post-Exercise Protein Carbonyls

Data were described as mean and standard deviation in Table 1. In PAS group, CP likely increased 90 min following exercise. However, possible decreased 72 h following exercise. In ACT group, CP likely increase 90 min following exercise, possible increase 24h following exercise and possible decrease 72h following exercise. In CWI group, CP very likely increased 90 min following exercise, likely increased 24h following exercise and possible decreased 72 h following exercise. The differences between PAS and ACT group and between PAS and CWI group were unclear in all moments analyzed. However, CP was possibly higher in CWI group 90 min and 24h following exercise (Figure 1).

[Insert Table 1]

[Insert Figure 1]

Effect of Recovery Modes on Post-Exercise Lipid Peroxidation

In PAS group MDA almost certain increased in all moments analyzed following exercise. In ACT group, MDA very likely increased 90 min, 24h and 48h following exercise. In CWI group, MDA almost certain increased 90 min and 24h following exercise. Furthermore in this group MDA very likely increased 48h following exercise. The differences between group showed that MDA almost certain was higher 90 min and 24h, very likely was higher 48h and 72h following exercise in PAS group than in ACT group. MDA very likely was higher all moments analyzed in PAS group than in CWI group. In CWI group MDA was likely higher than PAS group 90 min following exercise (figure 2).

[Insert Figure 2]

Effect of Recovery Modes on Post-Exercise Total radical-trapping antioxidant parameter

In PAS group TRAP likely increased 24h and 48h following exercise. In ACT group, TRAP likely decreased 72h following exercise. In CWI group, TRAP possible decreased 48h and likely decreased 72h following exercise. The differences between PAS and ACT group and between ACT and CWI group were unclear. However, TRAP was higher in PAS group 24h and 48h following of exercise than in CWI group (Figure 3).

[Insert Figure 3]

DISCUSSION

This is the first study to use PC, MDA and TRAP markers as possible indicators of harmful effects caused by CWI after high-intensity exercises. The results point towards similar behavioral profile in most of the analyzed moments, in all used techniques. However, differently from the initial hypothesis, damage was not observe in CWI, fact that highlights the non-occurrence of harmful effects when comparing it to the PAS. It was also notice that both CWI and the ACT seem to minimize the harmful effects caused by exercises, despite not presenting any harm regarding tissue membrane damage. Besides, the cold-water immersion seems to provide higher antioxidant capacity in comparison to the passive recovery analyzed afterwards.

The use of CWI is controversial due to the scarcity of evidence related to its application. The different physical stress levels and the techniques' most different application models - by considering time and water temperature, among other variables - impair the interpretation of benefits and damages. Concerns about the harmful effects caused by oxidative stress appear to be irrelevant due to the findings. On the other hand, although the responses from the range of optimized antioxidant capacity in the group subjected to CWI

were better than those from the competitor groups, the extrapolation of this finding as being an effective benefit resulting from the technique is understood as premature.

As for the carbonylic protein variable, it is observed that the behavior throughout time was similar among recovery techniques. It is highlighted that there was carbonylic protein increase within 90 minutes and it resulted in reductions in the following moments. Our data are different from the findings by Ceci et al. [9] who verified increase in this variable up to 24 hours after the exercises. Such fact can be associated with the different exercise protocols, because the authors used incremental cycle ergometer tests in their experiment. The late behavior of carbonylic protein concentration is added to the literature about the proposed protocol - it demonstrated progressive reduction in the periods of 24, 48 and 72 hours after exercise. Such response profile points towards a behavior that predominantly results from the exercises rather than from the applied recovery technique.

Nevertheless, it is worth noticing that Bleakley et al. [6] in their systematic review study refer to aggression potential regarding oxidative stress increase due to the use of low temperatures in post-exercise recovery. Such responses were not observed in the present study and it can be explained by the different temperatures herein used (11 ± 2^{o} C) and in the consulted review studies ($< 4^{o}$C) [6]. Consequently, the dose-response relation must be considered in the referred technique, i.e., exposure time and temperature of application, before inferring about harms caused by immediate responses from cold-water immersion.

Another commonly used variable for oxidative stress response detection is MDA; HPLC is the other standard method to determine lipid peroxidation. All studied groups had MDA increase 90 minutes after the exercise and decrease 72 hours after it. It highlights that the exercise protocol used in the current experiment was able to cause structural lesion in the cell membrane. However, CWI and the active recovery had smaller MDA concentrations in all the assessed periods in comparison to the passive recovery.

It is known that lipid peroxidation results from increase in the number of free radicals; and it is the consequence of high-intensity exercises. Ascensão et al. [2], in a study with professional athletes, observed MDA increase up to 72 hours after a soccer match, such result was not verified in our findings. The condition described by them can be linked to effort intensity beyond the studied population.

The recovery period observed in the current study showed that either the CWI and the active recovery improved the efficiency of TBARS elimination mechanisms, which is the main MDA component. Similar results were observed by Sutkowy et al. [31] who have analyzed the effects of successive stimuli under low temperatures (-120 a -145 ^{o}C).

Two hypotheses may help understanding the results from the comparison done among techniques. The first one refers to the protective effect that both techniques could provide after high-intensity exercises. The other hypothesis refers to the hemodynamic response promoted by both low-intensity physical exercises and flow redirection by CWI. These hypotheses must be tested in further specific studies, since they have not been the focus of the present experiment.

The current study has shown that, in addition to the fact that there is no harm in TRAP due to the use of CWI, this technique has also proved to be better than the passive recovery 24 and 48 hours after the exercises. The antioxidant defense system can play an important role when it comes to the attenuation of oxidative modifications or to the promotion of accelerated post-exercise recovery [13]. According to Watson et al. [36], this system can be temporarily reduced in response to the production of oxygen reactive species, but it can increase during the recovery period due to the aggression suffered in the beginning of the exercise. TRAP increases in the passive recovery because of the increased MDA levels, fact that corroborates the findings by Marin et al. [22] and Ascensão et al. [2].

The same hypotheses used to explain the MDA behavior can be applied to TRAP. Additionally, according to what was described by Sutkowy et al. [31], it is possible inferring the CWI potential in stimulating the antioxidant system and in promoting heat stress.

The main limitation of the present study, although it has not been its central aim, was the non-execution of functional or performance analyses. The discussion about the findings could head towards the association between the behavior of the analyzed markers and the functional response from the participants in the research, due to the scarceness of studies with the same nature as the current one. Thus, the exploration of the cost-benefit relation with regard to the applied technique can be theme of interest for health and sports sciences. Finally, according to the situation herein presented and to the current findings, the use of CWI must be done when evidence highlight benefits such as those seen in cases of muscular pain that start later after the exercises and in autonomic balance recovery without undesirable effects, however, considering the cellular ultrasctructure damage caused by oxidative stress.

CONCLUSION

It is possible to conclude, that CWI and the active recovery present less harmful effects in comparison to the passive recovery. Differently from the previously established hypothesis, it was verified because of the smaller lipid peroxidation and the better overall antioxidant capability.

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Table 1. Mean and standard deviation of the blood concentration of TRAP, MDA and CP at different moments analyzed.

	Baseline	**90 min**	**24 h**	**48 h**	**72 h**
TRAP (µM Trolox)					
PAS	30.53 ± 19.11	30.45 ± 15.40	41.96 ± 22.10	39.42 ± 24.26	30.42 ± 17.78
ACT	28.32 ± 14.93	27.31 ± 12.54	34.37 ± 22.34	30.17 ± 16.07	20.88 ± 9.80
CWI	32.43 ± 20.65	34.50 ± 16.61	28.65 ± 15.33	26.84 ± 17.47	20.73 ± 9.74
MDA (nmol·L^{-1})					
PAS	0.04 ± 0.02	0.09 ± 0.04	0.11 ± 0.05	0.09 ± 0.04	0.07 ± 0.05
ACT	0.06 ± 0.03	0.07 ± 0.02	0.09 ± 0.04	0.08 ± 0.03	0.05 ± 0.03
CWI	0.05 ± 0.03	0.08 ± 0.04	0.09 ± 0.02	0.07 ± 0.02	0.05 ± 0.03
CP nmol·mg^{-1} total protein					
PAS	1.32 ± 0.48	1.54 ± 0.34	1.40 ± 0.35	1.36 ± 0.37	1.18 ± 0.36
ACT	1.37 ± 0.42	1.51 ± 0.41	1.46 ± 0.41	1.41 ± 0.42	1.32 ± 0.42
CWI	1.32 ± 0.38	1.53 ± 0.43	1.48 ± 0.44	1.32 ± 0.44	1.25 ± 0.42

Note: TRAP: Total radical-trapping antioxidant parameter; MDA: malondialdehyde; CP: Carbonylated proteins; PAS: passive recovery; ACT: active recovery; CWI: cold water immersion

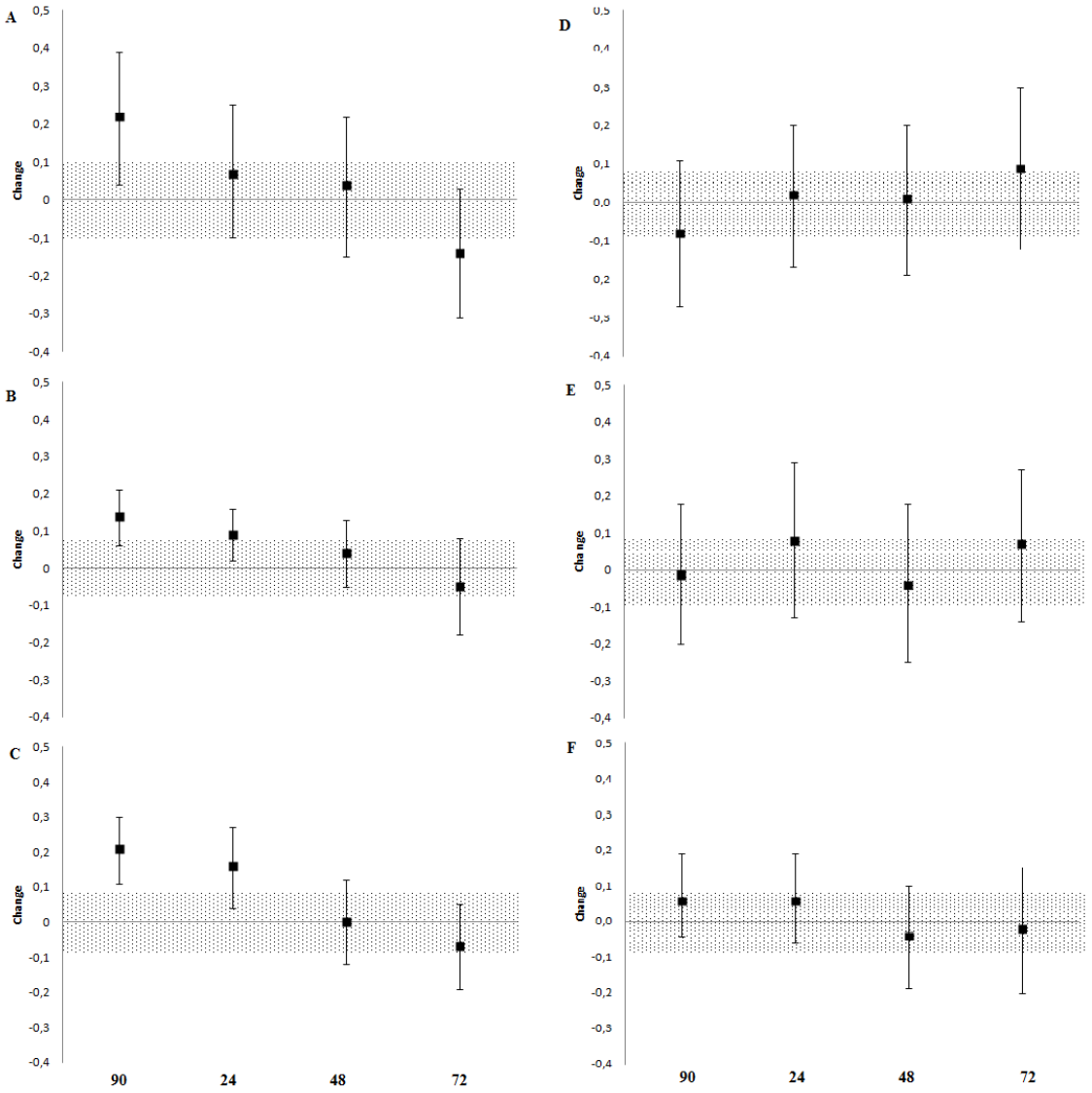

Figure 1. Differences in carbonylated protein following exercise compared with baseline. A) PAS group - 90 min (88/12/0), 24h (42/52/6), 48h(29/60/11), 72h (2/32/66); B) ACT group - 90 min (87/13/0), 24h (56/44/0), 48h(20/79/1), 72h (4/63/33); C) CWI group - 90 min (99/1/0), 24h (88/12/0), 48h(14/71/15), 72h (2/49/48). Comparing the differences between groups: D) ACT – PAS - 90 min (7/47/47), 24h (25/57/17), 48h(24/55/21), 72h (50/42/8); E) CWI – PAS - 90 min (20/54/26), 24h (48/43/9), 48h(17/48/35), 72h (44/45/11); F) CWI – ACT - 90 min (44/54/2), 24h (42/55/3), 48h(8/58/34), 72h (16/55/29).

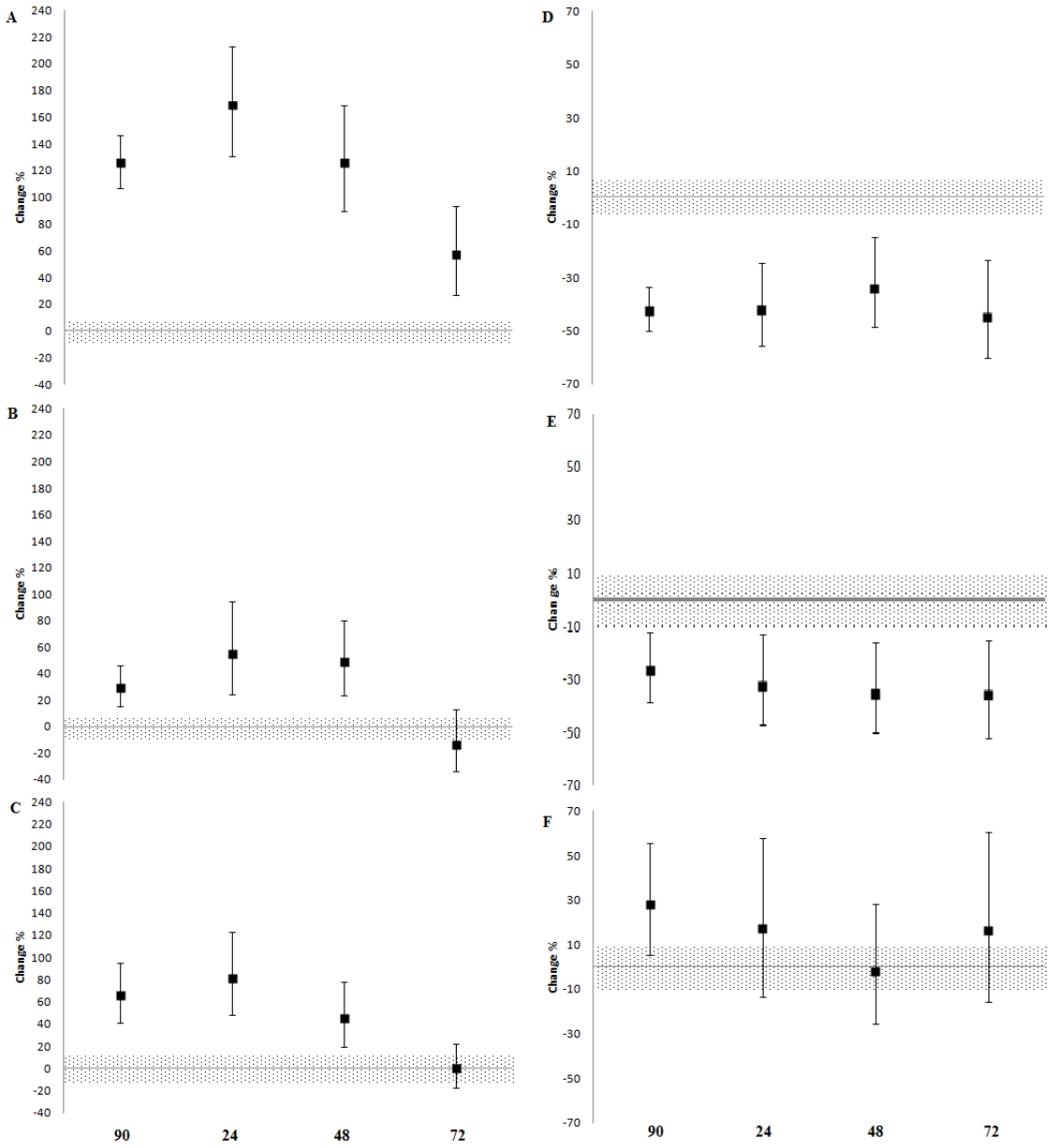

Figure 2. Differences in MDA following exercise compared with baseline A)PAS - 90 min (100/0/0), 24h (100/0/0), 48h(100/0/0), 72h (100/0/0); B)ACT - 90 min (99/1/0), 24h (99/1/0), 48h(99/1/0), 72h (7/29/64); C)CWI - 90 min (100/0/0), 24h (100/0/0), 48h(98/2/0), 72h (18/65/16). Comparing the differences between groups: D) ACT – PAS - 90 min (0/0/100), 24h (0/0/100), 48h(0/2/98), 72h (0/1/99); E) CWI – PAS - 90 min (0/3/97), 24h (0/3/97), 48h(0/2/98), 72h (0/2/98); C) CWI – ACT - 90 min (89/10/0), 24h (62/30/8), 48h(22/46/32), 72h (76/30/10).

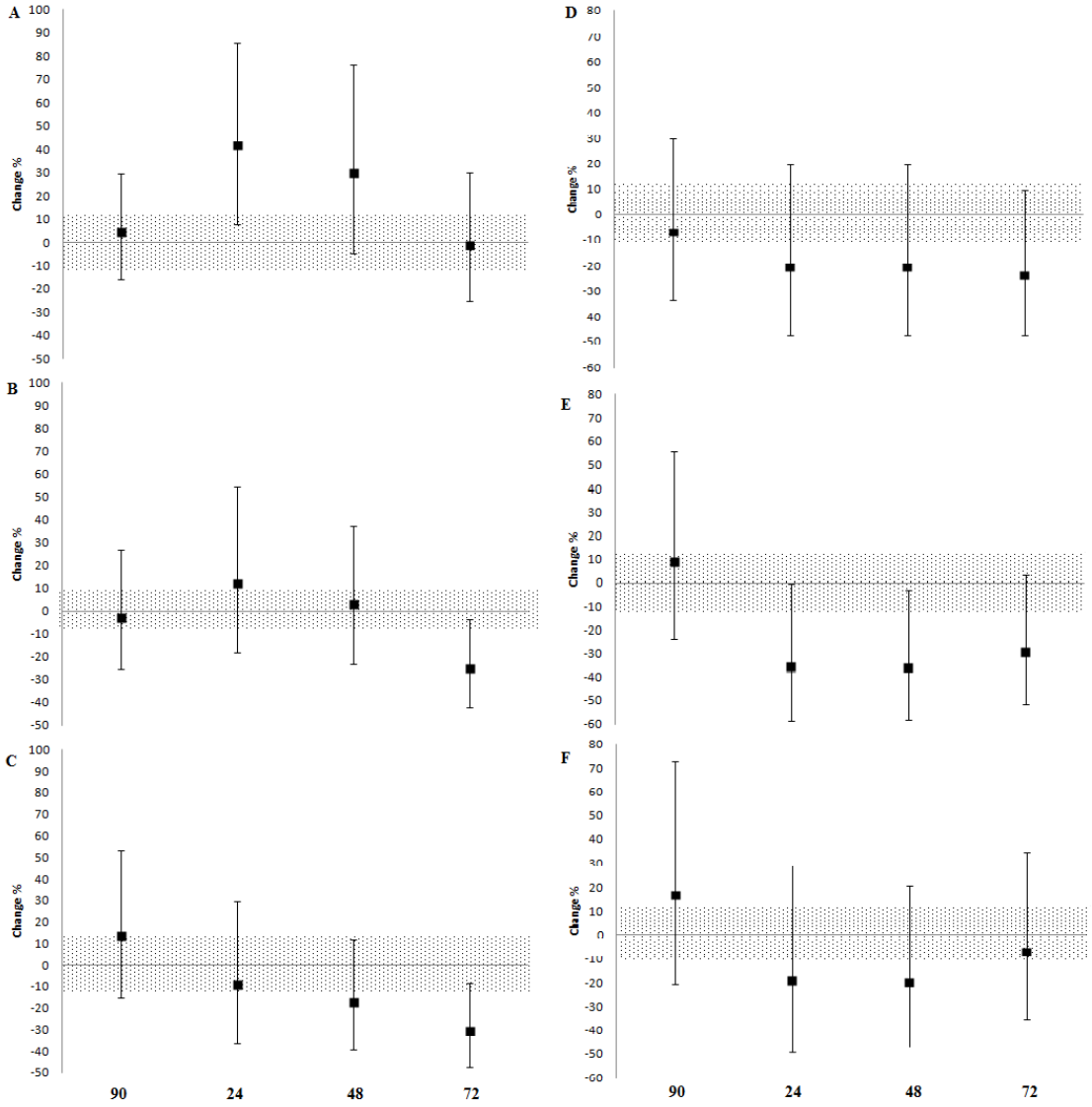

Figure 3. Differences in TRAP following exercise compared with baseline. A) PAS - 90 min (27/63/10), 24h (92/8/0), 48h(78/20/2), 72h (20/55/25); B) ACT - 90 min (22/44/34), 24h (55/32/14), 48h(35/41/24), 72h (1/9/90); C) CWI - 90 min (51/41/8), 24h (15/40/45), 48h(4/31/65), 72h (0/7/93). Comparing the differences between groups: D) ACT – PAS - 90 min (18/39/43), 24h (9/22/69), 48h(9/22/69), 72h (10/18/78); E) CWI – PAS - 90 min (43/40/17), 24h (2/9/89), 48h(1/8/91), 72h (6/14/85); F) CWI – ACT - 90 min (58/29/13), 24h (13/23/64), 48h(9/23/68), 72h (35/36/44).

ANEXO B

ARTIGO 2

THE EFFECTS OF DIFFERENT POST-EXERCISE RECOVERY METHODS ON SYSTEMIC CYTOKINES, CREATINE KINASE AND LIPID PEROXIDATION

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ABSTRACT

We investigated how and to what extent three different recovery strategies – passive recovery (PAS), active recovery (ACT), and cold water immersion (CWI) – affect oxidative stress biomarkers, injury and inflammatory markers after a high intensity exercise, by considering the harmful effects from the exercise and the technique. 24 male subjects were recruited. On the first visit, an incremental test was performed to determine maximal oxygen consumption and the associated speed (MAS). The remaining 3 visits for the performance of constant velocity exhaustive tests at MAS and different recovery methods (6 min) were separated by 7-day intervals (randomized: CWI, ACT or PAS). It was unclear if exist effect when the IL1-β blood concentration was compared between different groups. When the differences between groups in TNF-α were analyzed, it was found likely negative effect to ACT group compared with PAS group 90 min post exercise, likely negative effect to CWI group compared with PAS group 90 min post exercise, and possible positive effect to CWI group compared with ACT group 48 h post exercise. For CKmb, it was found likely, very likely and almost certain positive effect to ACT group compared with PAS group 24, 48 and 72h post exercise respectively, it was found likely, almost certain, likely and almost certain positive effect to CWI group compared with PAS group 90 min, 24, 48 and 72 h post exercise respectively. CLpeak blood concentration was found likely positive effect to ACT group compared with PAS group 90min, 24 and 48h post exercise, likely, likely and very likely positive effect to CWI group compared with PAS group. It is possible concluding, that CWI and the ACT present less harmful effects in comparison to the PAS.

Key words: Post-exercise recovery; oxidative stress; cryotherapy; active recovery

INTRODUCTION

Currently, several studies have analyzed the recovery process after exercise as a strategy to integrate fields of actions in training. Different techniques are used to analyze results, such as cold water immersion (CWI) and active recovery (ACT), usually related to performance (Roberts et al., 2014; White et al., 2014), clinical signs (Webb et al., 2013; Bleakley et al., 2012), injury markers (Leeder et al., 2015; Rowsell et al., 2014; Ascensão et al., 2011) and physiological functions (De Pauw et al., 2014; Bastos et al., 2012). However, the application of these techniques on molecular parameters has not been well explored.

High-intensity exercises can induce an acute state of oxidative stress, caused by the excess of reactive oxygen species (ROS) (Powers et al., 2011; Deminice et al., 2010). Oxidative stress is the condition in which cellular production of pro-oxidants exceeds the physiological capacity to stop the activity of the endogenous antioxidant system and the exogenous antioxidants, acquired through diet (Covas et al., 2002). ROS formation takes place during normal cell metabolism, but it may be increased under strenuous exercise conditions (Powers et al., 2011). Thus, ROS is not inherently harmful; however, the system can become unbalanced in response to chronic exposure to excessive and/or ectopic ROS production, potentially resulting in intracellular redox balance shifting towards a more oxidizing environment, instead of promoting oxidative damage, inflammation, ill-health, and disease (Dröge, 2002).

The recovery process directly influences the ability to return to normal training and performance levels. Sometimes, during training or competition season, the recovery time between two stimuli can be as short as 72 hours, and the return to training sessions may happen within 24 hours after the game. However, such short times might be insufficient for sports practitioners' physical performance normalization (Nédélec et al., 2012). Therefore,

recovery training has become increasingly important, and now it is recognized as a widespread key in the development of performance and injury prevention.

Numerous articles have reported that CWI may enhance performance recovery in different sports; water immersion at 10-15^{o} C for 5-15minutes is the most effective technique to accelerate performance recovery (Bleakley et al., 2012; Leeder et al., 2012 - see reviews). Although in recent years there has been an increase in the amount of researches focusing on CWI, findings remain unclear and have been assumed to be related to inconsistencies in exercise modalities. CWI protocols and dependent variables have been measured. Therefore, there is the demand of using similar protocols to better understand the physiological aspects and to compare studies on the same subjects.

Another commonly used recovery strategy is ACT. Although it is one of the oldest recovery techniques, their physiological effects are not well elucidated. Consequently, parameters as acute stress biomarker responses have not been investigated yet. Therefore, many recovery strategies have been used to minimize fatigue and accelerate post-exercise recovery from high intensity exercise (Leeder et al., 2015; Rowsell et al., 2014; Bastos et al., 2012). However, despite their widespread use, there is lack of guidelines and evidence considering the dose-response and especially the possible occurrence of harmful effects to support their implementation.

While studies have focused on the role of oxidative stress in regular training and recovery periods (Bessa et al., 2013), few studies have focused on recuperative techniques and on how they can influence practitioners' bodies. In order to add information about the current recovery methods, we investigated how and to what extent three different recovery strategies – passive recovery (PAS), ACT, and CWI – affect oxidative stress biomarkers, injury and inflammatory markers after a high intensity exercise. We hypothesized that there are different profiles among the groups, with possible deleterious effects on the CWI group.

METHODS

Participants

Twenty-four male subjects (age: 21 ± 2 years; height: 175 ± 8 cm; body mass: 72 ± 11 kg; body mass index: 23.5 ± 2.1 $kg \cdot m^{-2}$; VO_{2max}: 47.1 ± 3.1 $mL \cdot kg^{-1} \cdot min^{-1}$) were recruited via word of mouth and posters placed around the University State Paulista - UNESP. All were habitually active and typically engaged in intermittent exercise, such as soccer, handball and basketball, 3 to 4 times per week, with each session lasting one to two hours. This study had been approved by the University State Paulista Research Ethics Committee, Faculty Science and Technology. Prior to participation, the participants underwent a medical examination and were fully informed about the experimental procedures and a signed consent was obtained from the participants.

Study Design

Each participant attended the laboratory on four occasions separated by at least 7 days and all the laboratory tests for each participant were completed within four weeks. On the first visit, an incremental test was performed to determine maximal oxygen consumption (VO_{2max}) and MAS. The remaining three visits used for the performance of constant velocity exhaustive tests and different recovery methods were separated by seven-day intervals. The subjects were asked to refrain from any exercise and not to consume alcoholic or caffeinated beverages 24 h prior to each test. The subjects were also asked to have their last meal (similar high carbohydrate intake) and fluid consumption at least 2 hours prior to each test. The adherence to the instructions was checked before testing and all of the subjects complied with the requirements. All tests were performed in a quiet laboratory under standardized conditions (temperature: 21 to 23^{o} C; relative humidity: 40-60%) and were preceded by 5 min of jogging at 7 $km \cdot h^{-1}$ and 5 min of passive recovery (standard warm-up).

Maximal Incremental Test

Maximal oxygen consumption (VO_{2max}) and MAS were determined on a treadmill (Inbramed Super ATL, Inbrasport, Rio Grande do Sul, Brazil) using an incremental protocol. The incremental protocol consisted of running at a starting velocity of 10 $km \cdot h^{-1}$ for 1 min, with increments of 1 $km \cdot h^{-1}$ per minute until exhaustion. The inclination was kept constant at 1% (Harling et al., 2003).

Gas exchange was continuously measured and averaged every 30 s (VO_{2000}, MedGraphics, Minnesota, USA). Expired O_2 and CO_2 concentrations were determined using a galvanic fuel cell for the oxygen and a non-dispersive infrared analyzer for the carbon dioxide. Flow was determined using a bi-directional differential pressure pneumotach (preVent™). Heart rate (HR) was recorded with a Polar S810i monitor in the RR mode, but averaging HR at 10 s intervals (Polar Electro, Kempele, Finland) (Vanderlei et al., 2008).

At least two of the following criteria were required to ensure that VO_{2max} had been attained: 1) the occurrence of a plateau (i.e., < 150 $mL \cdot min^{-1}$ increase between two successive stages); 2) a respiratory exchange ratio above 1.10; and/or 3) HR in excess of 90% of age-predicted maximum. The MAS was recorded as the speed corresponding to the last complete stage on the incremental test (Noakes et al., 1990).

Constant Velocity Exhaustive Test

After arriving to the laboratory, subjects were required to lay supine for 20 min and breathe spontaneously for the recording of resting R-R intervals. After the subjects had performed the aforementioned standardized warm-up, the treadmill velocity was set at each individual's MAS and the participant ran until volitional exhaustion. Strong verbal encouragement was provided throughout the test. Exhaustion was determined by the inability

to maintain the exercise intensity or by clinical signs, such as dizziness, paleness and nausea, accompanied by the attainment of maximal HR obtained during the maximal incremental test.

Recovery Interventions

After the completion of the constant velocity exhaustive test at MAS, the subjects were submitted to three different recovery methods (separated by seven-day intervals) on different occasions in a random order (raffle card): passive un-immersed rest (PR), AR and CWI. The participants were allowed 1 min immediately following the completion of the running test to perform the transition to the recovery mode (e.g., removing shoes for CWI). The duration of each intervention was 6 min.

During PR, the subjects stood in the orthostatic position without any attempt to accelerate recovery; this condition was considered the control. Within the first minute post-exercise, subjects could incline their trunk and support their weight on the handrails of the treadmill. No participants passed out or experienced any ill sensations in the remaining six minutes of PR. Thus, the participants tolerated PR and the other recovery modes relatively well.

During the AR, the subjects walked/ran on the treadmill at a low intensity (30% of VO_{2max}) (Gordon et al., 2003). During the CWI intervention, the subjects were submerged in the orthostatic position in cold water at a level that covered the anterior superior iliac spine (Bastos et al., 2012). For this study water temperature was maintained constant at 11 ± 2^{o} C, which is within the 10-15^{o} C range suggested in the review of Halson (2011) and previously used in several investigations (Al Haddad et al., 2012; Bastos et al., 2012; Buchheit et al., 2009; Peiffer et al., 2009; Vaile et al., 2011). According to Wilcock et al. (2006) and Halson (2011), very cold water (< 5^{o} C) temperature is not suitable for post-exercise recovery of performance, but for treatment of acute injuries and reduces inflammation and oedema. In

addition, studies (Leeder et al., 2015; Al Haddad et al., 2012; Buchheit et al., 2009) showing the effectiveness of whole-body CWI on post-exercise biological markers have used 14-15^{o} C of water temperature. After each of the recovery methods, the subjects laid supine for an additional 83 min. Recovery was performed at 90 min following the post-exercise testing session, and at 24, 48, and 72 h post-exercise.

Methods of analysis of biological variables

Obtaining the blood samples

All blood samples were analyzed in duplicate. To avoid interassay variation, all samples were analyzed in a single batch at the end of the study, with the exception of hematological measures, which were performed on the day of the collection.

Venous blood samples were collected pre-exercise (Baseline) and at each of the four post-exercise time-points at 90 min, 24, 48 and 72 h. Each blood sample (5 mL) was collected from a superficial forearm vein using standard venipuncture techniques. All samples were collected directly into serum separator collection tubes and serum separated by centrifugation at 3,000 x g for 10 min at 4°C. Serum samples were stored frozen at -80°C until analysis.

Erythrocyte chemiluminescence

Systemic lipoperoxidation was evaluated in erythrocytes by chemiluminescence (CL). Erythrocytes was obtained from heparinized blood and washed three times with 0.9% saline solution at 4ºC. An aliquot of heparinized blood was used to determine hemoglobin (Coulter STKSÒ, Hialeah, USA). Erythrocytes lipoperoxidation was evaluated according Gonzalez-Flecha et al. (1991). Briefly, 30 µL of packed erythrocytes was added to 3 mL of phosphate buffer, and 1 mL of this solution was diluted in 12.3 mL of the same buffer. The chemiluminescent reaction was initiated by the addition of tert-butyl (10 µL) at a final

concentration of 3 mM in 1 mL of erythrocytes samples. CL curves were obtained in a GloMax® luminometer 20/20 (Promega, Madison, USA), and the results are expressed in relative light units (RLU) x g Hb $^{-1}$. The obtained curve was used as a qualitative indicator of lipoperoxidation, and quantitative results were obtained after area under curve integration using GraphPad Prism version 5.0 (GraphPad Software).

Plasma creatine kinase activity

The investigation of possible muscle damage generated by exhaustion test was performed by assessing the concentration of the two isoforms of CK, with CKmb and CKmm (Chen et al., 2009) (it is, by subtracting the myocardium) using was added 0.05 mL of sample pre prepared (kits CK-NAC, CK-NAC, Bioliquid) to read in a spectrophotometer (spectrophotometer SP-220, Biospectro).

Analysis of circulating cytokines

The levels of IL-1β and TNF-α in the serum were determined using an enzyme-linked immunosorbent assay (ELISA) with specific monoclonal antibody (MAb) pairs. Microplates (Nunc, Roskilde, Denmark) were sensitized overnight with purified anti-human IL-1β capture antibody or purified anti-human TNF-α capture antibody. Nonspecific binding was prevented by incubating the plates with 2% bovine serum albumin (Sigma, St. Louis, Mo.) in phosphate-buffered saline (PBS). The plates were incubated overnight with 100 μL of a 1:2 dilution of serum samples in PBS, 1% bovine serum albumin, and standard cytokines. The plates were then washed five times with 0.05% Tween in PBS and incubated with detection antibody biotin anti-human IL-1β or TNF-α for 1 h. The plates were washed and incubated for 30 min with the enzyme reagent streptavidin-horseradish peroxidase conjugate (SAv-HRP). Finally, the plates were washed seven times and incubated with p-nitrophenyl phosphate (BD). The

A450-A630 was read in a microplate reader. The intra-assay coefficient of variation of the methods for IL-1β and TNF-α were less than 10%. The results were expressed as pg/mL.

Statistical Analysis

This study was performed using a randomized crossover design. Data normality was tested with the Shapiro-Wilk's test. The variables presented skewed distributions and were log-transformed for analysis. The method of magnitude-based inferences was applied to detect the chances of an increase/inconclusive/decrease for both groups. For this purpose, the smallest worthwhile change (SWC – 0.2 multiplied by the between subject standard deviation) was obtained for each variable. The chance of the changes was greater than the SWC was obtained and compared between groups. The quantitative chances of a positive/trivial/negative effects were interpreted as follows: <1% almost certainly not; >1–5% very unlikely; >5–25% unlikely; >25–75% possible; > 75–95% likely; > 95–99 very likely; >99% almost certain. If the chances were higher than 5% for positive and negative effects, the true difference was interpreted as unclear. The confidence interval was 90%. The magnitude-based inferences were calculated with spreadsheets available at http://www.sportsci.org/.

RESULTS

Constant Velocity Exhaustive Test: Performance and Physiological Responses

No significant differences were detected in the duration of the constant velocity tests (15.1 ± 1.1 $km \cdot h^{-1}$) under the PAS (3.9 ± 0.8 min), ACT (3.9 ± 0.9 min) and CWI (3.9 ± 0.7 min) conditions ($p = 0.92$). All tests were terminated by voluntary exhaustion, without clinical signs of adverse health conditions.

Data were described as mean and standard deviation in table 1.

 # INSERT TABLE 1 HERE #

Effect of Recovery Modes on Post-Exercise Cytokines

In the PAS group the IL1- β blood concentration possibly decreased 90 min post exercise compared with the baseline concentration values and it was unclear if the IL1-β blood concentration changed at 24, 48 and 72h post exercise. In ACT group it was unclear if the blood concentration of IL1-β changed post exercise compared with baseline values. In CWI group the blood concentration of IL1-β likely decreased 72h post exercise compared with baseline values and was unclear if IL1-β blood concentration changed 90 min, 24 and 48h and post exercise. It was unclear if exist effect when the IL1-β blood concentration was compared between different groups.

 # INSERT FIGURE 1 HERE #

In PAS group the TNF-α blood concentration likely and possibly decreased 90 min and 72h post exercise respectively compared with baseline values and was unclear if TNF-α blood concentration changed 24, 48h post exercise. In ACT group the TNF-α blood concentration possible increase 90 min and 48h post exercise respectively compared with baseline values and was unclear if TNF-α blood concentration changed 24, 72h post exercise. In CWI group the TNF-α blood concentration possible decreased 48 and 72h post exercise compared with baseline values and was unclear if TNF-α blood concentration changed 90min and 24h post exercise. When the differences between groups in TNF-α was analyzed, it was found likely negative effect to ACT group compared with PAS group 90 min post exercise, likely negative effect to CWI group compared with PAS group 90 min post exercise, and possible positive effect to CWI group compared with ACT group 48 h post exercise.

 # INSERT FIGURE 2 HERE #

Effect of Recovery Modes on Post-Exercise Creatine Kinase

In PAS group the CKmb blood concentration almost certain increased 90 min and 24 h post exercise, very likely and possible increased 48 and 72 h post exercise respectively compared with baseline values. In ACT group CKmb almost certain increased 90 min, very likely increased 24h and likely decreased 72h post exercise compared with baseline. It is unclear if CKmb changed in ACT group 48h post exercise compared with baseline. In CWI group, CKmb likely increased 90min, possible increased 24h and almost certain decreased 72 post exercise compared with baseline values. It is unclear if CKmb changed in CWI group 48h post exercise compared with baseline. When the differences between groups in CKmb was analyzed, it was found likely, very likely and almost certain positive effect to ACT group compared with PAS group 24, 48 and 72h post exercise respectively, it was found likely, almost certain, likely and almost certain positive effect to CWI group compared with PAS group 90 min, 24, 48 and 72 h post exercise respectively. Furthermore, it was found likely positive effect to CWI group compared with ACT group 90 min, 24 and 72 h post exercise.

 # INSERT FIGURE 3 HERE #

 # INSERT FIGURE 4 HERE #

Effect of Recovery Modes on Post-Exercise Erythrocyte chemiluminescence

In PAS group the CLpeak blood concentration possible increased 90 min, very likely increased 24h and likely increased 48h post exercise compared with baseline values and was unclear if changed 72h post exercise. In ACT group the CLpeak blood concentration likely decreased 90min and 72h post exercise compared with baseline values and was unclear if changed 24, 48h post exercise. In CWI group the CLpeak blood concentration very likely decreased 72h post exercise compared with baseline values and was unclear if changed 90min, 24 and 48h post exercise. When the differences between groups in CLpeak blood

concentration was analyzed, it was found likely positive effect to ACT group compared with PAS group 90min, 24 and 48h post exercise, likely, likely and very likely positive effect to CWI group compared with PAS group 24,48 and 72h post exercise respectively, and was unclear if exist effect when CWI group and ACT group was compared.

 # INSERT FIGURE 5 HERE #

DISCUSSION

The present study investigated possible harmful effects provided by CWI, ACT and PAS techniques, after high-intensity effort exercise, by oxidative stress, muscle damage and inflammatory variables. It was found that CWI may, in long-term, influence the decline of oxidative stress levels after intense exercise. Therefore, the primary hypothesis that CWI could cause deleterious effects was not sustained; the opposite was observed.

The results point to a similar behavior profile in most of the analyzed moments, in all the techniques. Differently from the initial hypothesis, no damage was observed in CWI groups, fact that points to the nonoccurrence of harmful effects in comparison to PAS. It should also be noticed that although there was no loss considered to be an important damage to tissue membrane due to inflammatory marker levels; both CWI and ACT seem to minimize physical exercise deleterious effects. Yet, CWI seems to provide more protection in comparison to PAS - both will be analyzed later.

The use of CWI is controversial due to the restriction of evidence related to its application, different physical stress levels and many different technique application models. Time and water temperature – among other variables - compromise the interpretation of damages and benefits. Based on the findings, the concern related to deleterious effects of oxidative stress is not relevant. On the other hand, although the responses of CKmb magnitude was optimized by the group performing it, - the group was better than competitor

groups – the assumption that such finding is an effective benefit from the technique is considered to be premature.

As for the CLpeak variable, its behavior was observed over time and it is similar to that of recovery techniques. Besides, CLpeak rose in the first 90 minutes and, consequently, decreased in subsequent moments. Significant reduced CLpeak favorable to CWI are highlighted at 24, 48 and 72 hours after physical effort, in comparison to PAS.

According to Buchheit et al., (2009) the compression effect from water immersion is applied to develop fluid displacement from peripheral to central body regions. The hydrostatic pressure results in several physiological changes, including substrate transport and cardiac output raise as well as peripheral resistance and extracellular fluid decrease by intracellular and intravascular osmotic gradients (WILCOCK et al., 2006). Besides, CWI induces a series of physiological and biochemistry responses, such as vasoconstriction, venous return stimulation, metabolite removal after exercise (BASTOS et al., 2012), muscle ache and edema reduction (LEEDER et al, 2012). Such events may somehow interfere in mechanisms that influence total antioxidant capacity, because of lower ERO values in CWI group.

In a systematic review, Bleakley et al., (2010) mention aggression potential related to oxidative stress raise due to low temperature applied to post-exercise recovery. Such responses were not observed in the present study, fact that may be explained by the different temperatures used by them (11 ± 2^{o} C) and the studies consulted for review (<4ºC) (Bleakley et al., 2010). Thus, before inferring about immediate response losses from CWI, it is worth considering the dose-response relation in the technique; in other words, exposure time and application temperature.

Particularities were observed in CK serum levels. Specifically for enzyme to skeletal muscle harm (CKmm), CWI and ACT techniques hit their peak 24 hours after the effort,

while PAS peaks at 48 hours. Based on results and comparisons among techniques, CWI and ACT seem to accelerate CKmm removal at 48 and 72 hours when they are compared to PAS.

Two hypotheses may help understanding the results from the technique comparisons. The first hypothesis refers to the protective effect both techniques could provide after high intensity exercise. The other one refers to the hemodynamic response provided by both: low intensity physical exercise and flow redirection provided by CWI. Such hypotheses shall be tested in further specific studies, as they are not the focus of the current experiment.

Some authors (Ascensão et atl., 2011; Pournot et al., 2011) describe that in post-exercise periods, CWI assists reducing intramuscular proteins liberation in the lymphatic system and tissue damage. Yet, they state that muscle damage restoration may be associated to cell permeability reduction, thereat CWI may attenuate possible grievances throughout this period. Ascensão et al. (2011) evaluated CWI effects (10 minutes at 10ºC) in athletes after soccer matches in comparison to water immersion at 35ºC. They got significant total CK concentration reduction in the CWI group, thus corroborating the findings in the current study.

On the other hand, Takeda et al., (2014) evaluated CWI effects (15ºC for 10 minutes) in athletes after rugby matches. They used muscle power and biochemical markers of muscle damage as variables. The authors found a better perception of function and recovery in the CWI group. Besides, there were no significant differences between CWI and the control group after intervention and 24 hours after the rugby match regarding aspartate transaminase, creatinine, total CK, lactate dehydrogenase and lactate markers. Therefore, the difference between the studies mentioned above may have occurred because of the used methodologies, which are different in terms of variable control. Thus, attention should be paid to the water temperature used in the experiment, effort model and to the exposure time to the technique.

So, an effective comparison is obtained and it may raise more concrete hypotheses about the herein found incongruities.

The present study shows that besides the fact that absence of loss in CKmb (specific to cardiac muscle damage) resulted from the use of CWI, this technique shows to be better than ACT and PAS. CKmb peak concentration in PAS occurred 24 hours after effort; as for CWI and ACT, peak concentration happened 90 minutes after exercise. With regards to the comparison of techniques, it is highlighted that CKmb lowest values were found at 24, 48 and 72 hours for CWI and ACT when they are compared to PAS. Besides, CWI presents lowest values at 90 minutes, 24 and 72 hours in comparison to ACT.

There is evidence that one of CWI main mechanisms is the baroreceptors stimulation through hydrostatic pressure (Buchheit et al., 2009; Wilcock et al., 2006). Such event is responsible for influencing the autonomic nervous system and it is measured by cardiac rate variability analysis (Bastos et al., 2012; Buchheit et al., 2009). According to Bastos et al., (2012) CWI may positively influence catabolites removal and cardiac autonomic activity; because it causes parasympathetic reactivation right after the technique is applied. Thus, CWI seems to provide cardioprotective effects on the low CKmb sericeous levels in the current study. As such phenomenon is speculative, testing such hypothesis in specific experiments is suggested.

With regard to inflammatory markers IL-1β and TNF-α, possible evidence of deleterious effects were not observed, because there were no significant differences among the applied techniques. The observed similar response profile indicates actions predominantly resultant from effort, not from the applied recovery techniques. The applied high-intensity exercise model seems to be insufficient to develop important inflammatory states. In a recent study, Leeder et al., (2015) investigated CWI effects (14ºC for 14 minutes) on seated and standing positions in comparison to PAS. They used 24 high-performance athletes after high-

intensity effort exercises, for the evaluation of IL-6 levels. The authors did not observe differences among groups in the analyzed variable, thus corroborating the findings in the current study.

On the other hand, White, Rhing and Wells (2014) investigated the effects of different protocols in CWI from inflammatory and functional responses after high-intensity exercises. A sample of 8 physically active individuals completed 5 repeated sprint tests. CWI protocols (CWI-10 minutes at 20ºC, CWI-30 minutes at 20ºC, CWI-10 minutes at 10ºC or CWI-30 minutes at 10ºC) were compared to PAS. The authors concluded that CWI seems to facilitate skeletal muscle recovery, but not in activities that demand power exercises for concentric contraction. Besides, these changes do not seem to be related to inflammatory cytokine levels. On the other hand, although discreetly, the authors state that the 30 minutes CWI protocol, regardless of temperature, may cause circulating inflammatory cytokine increase. Such condition alerts to possible harmful CWI effects.

The main limitation of the current study is that no functional or performance analyses were done, although it was not the major objective. Due to shortage of studies aiming to investigate possible deleterious effects from the techniques, the discussion on the findings could be headed towards the association between the behavior of analyzed markers and the functional responses from the participants in the research. Thus, exploring the cost-benefit relation of the technique application may be an interesting theme to sport and health sciences.

The present study is the first to monitor oxidative stress levels by CL and subtypes of CK in post-exercise recovery processes. However, based on discussions about the results, lack of standardization for technique use and variable control could be observed, fact that would determine difficulties to the comparison of results among studies about the same subject. Therefore, the demand for better definition of parameters related to the management of each technique should be highlighted, such as exposure time, temperature and application intensity.

Further researches shall analyze CWI real effects by using tools that measure series of events related to signaling pathways in different cellular tissues.

After all, according to the problem and findings herein presented, CWI application is suggested whenever evidence point out benefits such as the delayed onset muscle soreness (Bleakley et al., 2012; Leeder et al., 2012) and the cardiac autonomic nervous system (Bastos et al., 2012; Buchheit et al., 2009), with no undesirable effects, by considering cell ultrastructure damage in the presence of oxidative stress.

CONCLUSION

According to findings in the current study, we can conclude that CWI and ACT present less deleterious effects in comparison to PAS, because of the lower liberation of free radicals and muscle damage markers, differently from the previously established hypothesis.

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Table 1. Mean and standard deviation of the blood concentration of CKmb, CKmm, IL-1β, TNF-α and CLpeak at different moments analyzed.

	Baseline	90 min	24 h	48 h	72 h
CKmb (IU)					
PAS	8.68±6.37	14.08±7.93	17.16±11.22	12.39±8.96	9.74±6.93
ACT	7.82±5.29	15.42±11.05	10.16±3.69	6.84±3.14	5.65±2.47
CWI	7.94±4.75	9.55±2.38	7.92±2.67	7.75±3.59	4.90±2.63
Ckmm (IU)					
PAS	53.17±36.17	58.64±51.01	82.36±56.83	93.08±41.13	76.38±38.45
ACT	90.99±73.42	95.37±80.10	127.09±104.12	87.94±71.29	95.36±76.88
CWI	105.51±94.03	115.63±100.82	147.97±122.01	139.62±125.42	119.02±108.61
IL-1β (pg/mL)					
PAS	31.67±20.07	27.12±14.12	33.69±15.42	33.90±16.48	31.58±18.25
ACT	33.83±18.58	32.79±14.81	30.24±12.95	31.90±17.09	31.64±17.93
CWI	28.62±8.66	28.18±10.20	34.30±15.45	29.22±10.51	25.56±13.79
TNF-α (pg/mL)					
PAS	25.91±17.39	21.54±19.67	25.29±18.09	29.75±22.78	24.97±20.91
ACT	27.22±21.92	30.88±22.51	29.15±21.98	29.21±20.71	26.98±23.46
CWI	30.54±24.58	30.47±20.84	28.50±23.67	23.49±15.23	21.26±18.42
CL peak (RLU) x g Hb $^{-1}$					
PAS	4.89±2.11	5.90±3.58	7.92±4.19	7.49±4.51	6.00±4.03
ACT	7.74±4.97	4.69±1.63	7.68±4.15	6.97±2.91	4.42±1.55
CWI	7.32±4.18	7.04±3.83	6.15±1.27	5.88±2.72	4.32±1.45

Note: CKmb: creatine kinase in isoform mb; CKmm: creatine kinase in isoform mm; IL-1β: interleukin-1β; TNF-α: tumor necrosis factor-α; CL peak: chemiluminescet peak; PAS: passive recovery; ACT: active recovery; CWI: cold water immersion

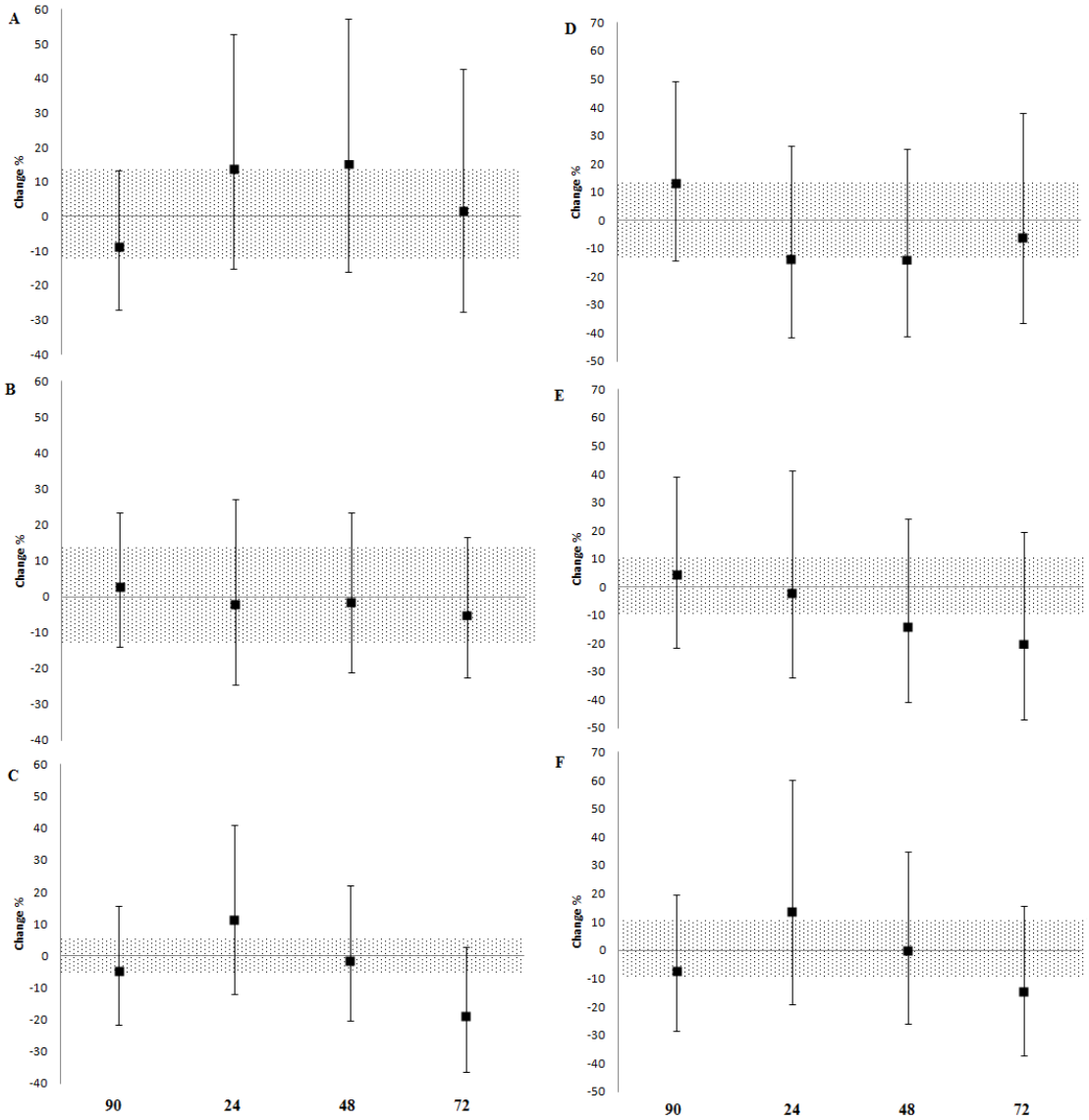

Figure 1 – Differences in IL1-β following exercise compared with baseline. A) PAS group - 90 min (5/56/39), 24h (50/43/7), 48h(52/40/8), 72h (29/48/23); B) ACT group - 90 min (16/77/7), 24h (16/62/23), 48h(13/69/18), 72h (7/69/24); C) CWI group - 90 min (16/39/45), 24h (62/27/11), 48h(26/39/35), 72h (3/12/85). Comparing the differences between groups: D) ACT – PAS - 90 min (48/45/7), 24h (11/35/53), 48h(11/36/54), 72h (37/42/39); E) CWI – PAS - 90 min (37/43/20), 24h (29/35/36), 48h(13/28/60), 72h (16/21/70); F) CWI – ACT - 90 min (12/45/43), 24h (55/33/13), 48h(28/44/28), 72h (18/31/62).

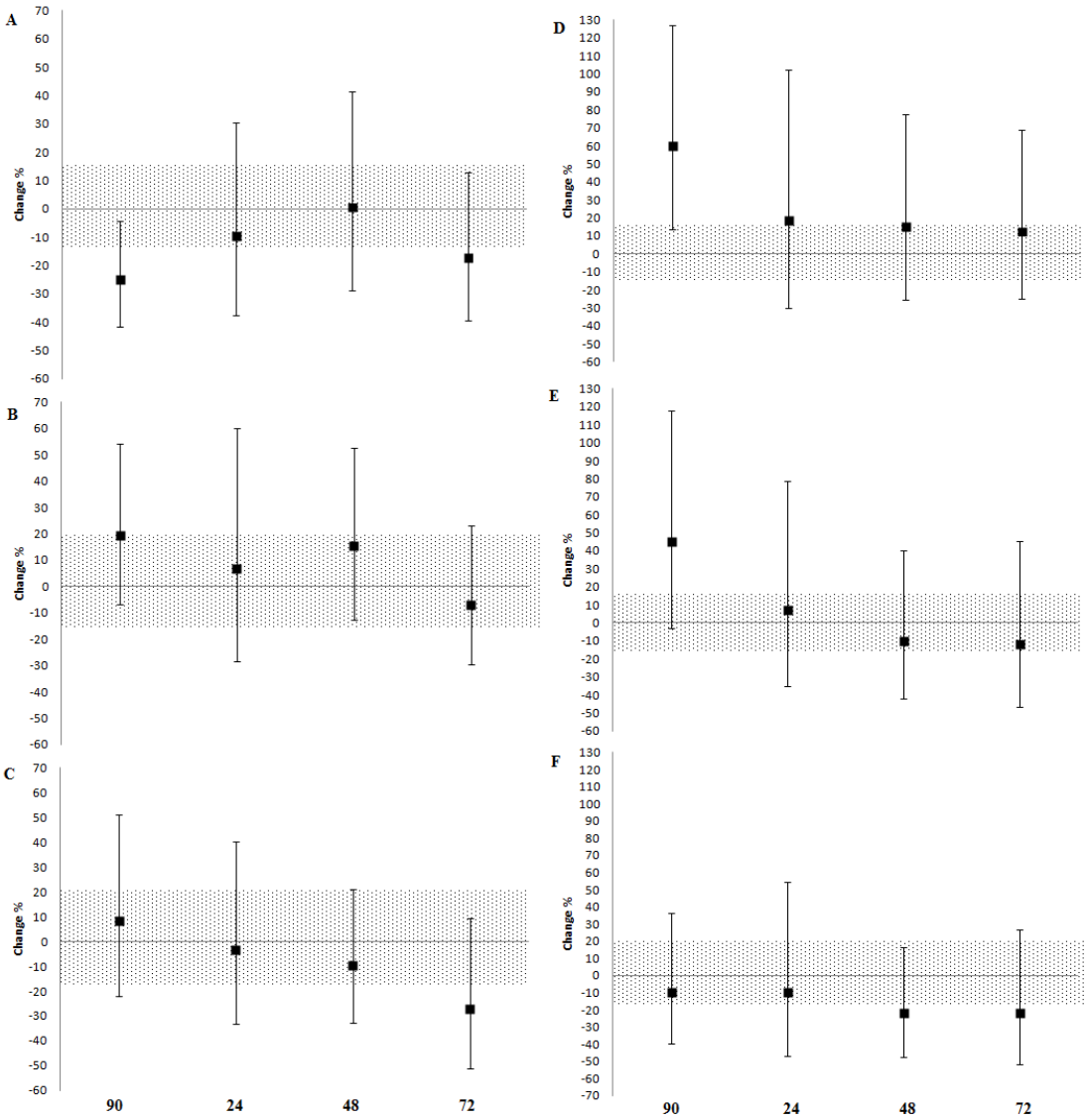

Figure 2 – Differences in TNF-α following exercise compared with baseline. A) PAS group - 90 min (0/16/84), 24h (13/45/42), 48h(24/52/23), 72h (4/36/60); B) ACT group - 90 min (50/49/1), 24h (32/53/15), 48h(41/56/3), 72h (7/67/26); C) CWI group - 90 min (29/63/8), 24h (15/61/23), 48h(5/65/30), 72h (2/28/70). Comparing the differences between groups: D) ACT – PAS - 90 min (93/7/0), 24h (51/34/15), 48h(47/41/12), 72h (67/45/13); E) CWI – PAS - 90 min (80/18/1), 24h (38/41/22), 48h(15/44/40), 72h (32/40/44); F) CWI – ACT - 90 min (13/51/36), 24h (19/42/40), 48h(4/36/60), 72h (19/35/58).

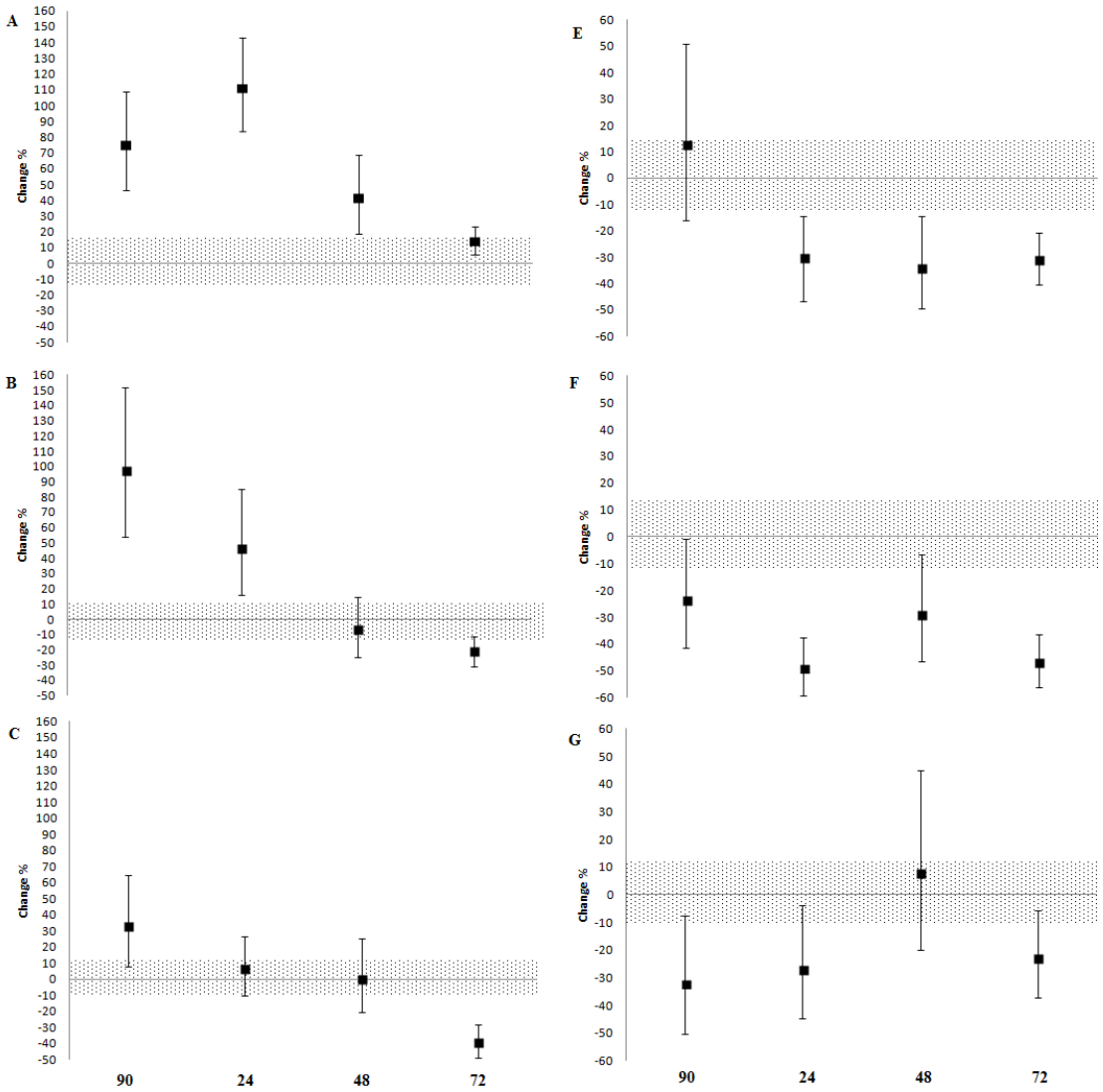

Figure 3 – Differences in CKmb following exercise compared with baseline. A) PAS group - 90 min (100/0/0), 24h (100/0/0), 48h(97/3/0), 72h (40/60/0); B) ACT group - 90 min (100/0/0), 24h (97/3/0), 48h(6/60/34), 72h (0/6/94); C) CWI group - 90 min (93/7/0), 24h (34/61/5), 48h(21/57/21), 72h (0/0/100). Comparing the differences between groups: D) ACT – PAS - 90 min (47/45/8), 24h (0/7/93), 48h(0/4/96), 72h (0/0/100); E) CWI – PAS - 90 min (1/16/83), 24h (0/0/100), 48h(0/9/91), 72h (0/0/100); F) CWI – ACT - 90 min (1/6/93), 24h (1/10/89), 48h(41/44/14), 72h (2/12/89).

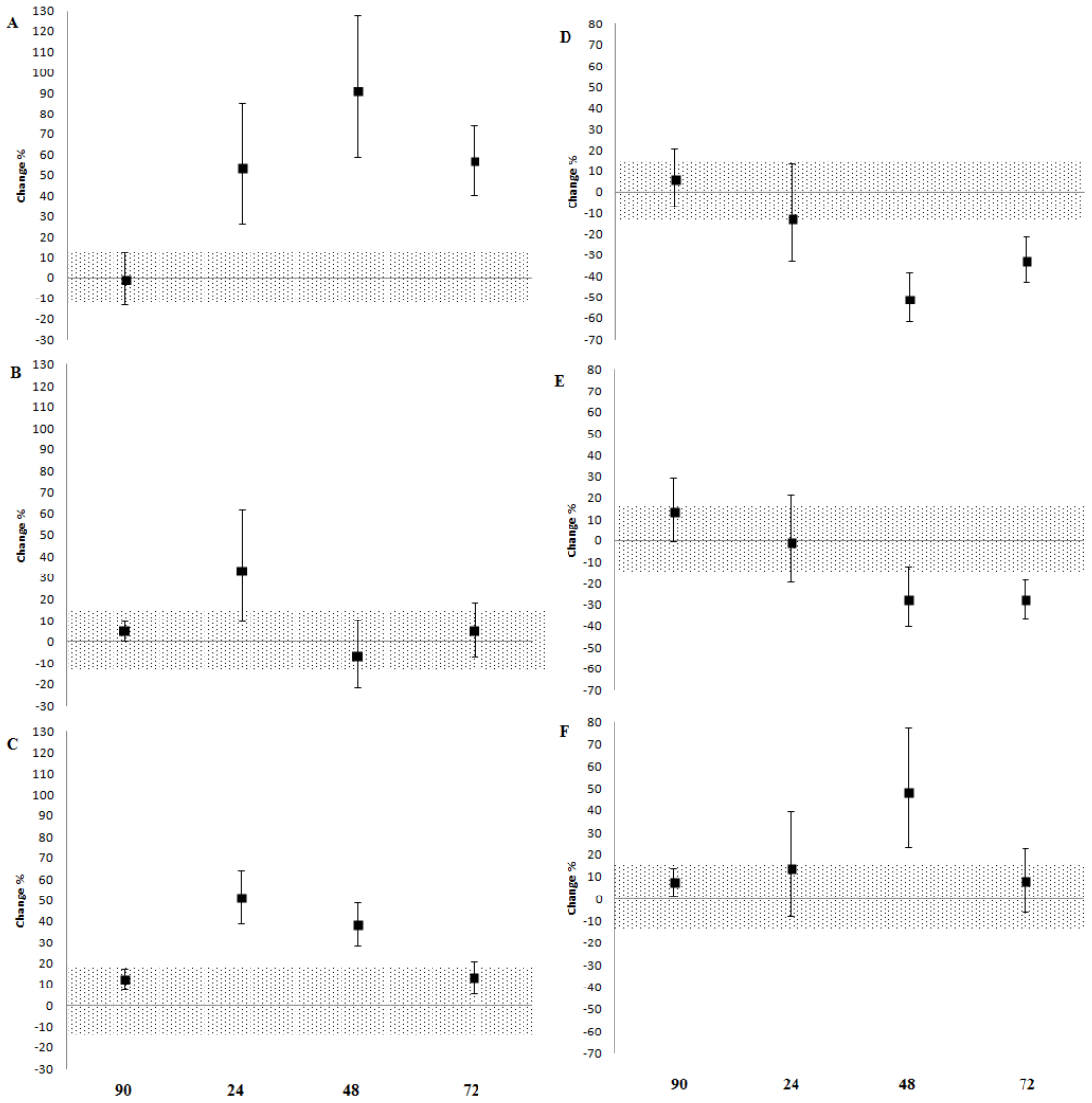

Figure 4 – Differences in CKmm following exercise compared with baseline. A) PAS group - 90 min (4/88/7), 24h (99/1/0), 48h(100/0/0), 72h (100/0/0); B) ACT group - 90 min (0/100/0), 24h (89/11/0), 48h(2/75/23), 72h (9/90/1); C) CWI group - 90 min (4/96/0), 24h (100/0/0), 48h(100/0/0), 72h (14/86/0). Comparing the differences between groups: D) ACT – PAS - 90 min (15/84/1), 24h (4/46/50), 48h(0/0/100), 72h (0/0/100); E) CWI – PAS - 90 min (38/62/0), 24h (9/79/12), 48h(0/7/93), 72h (0/1/99); F) CWI – ACT - 90 min (1/99/0), 24h (42/57/2), 48h(98/2/0), 72h (17/84/0).

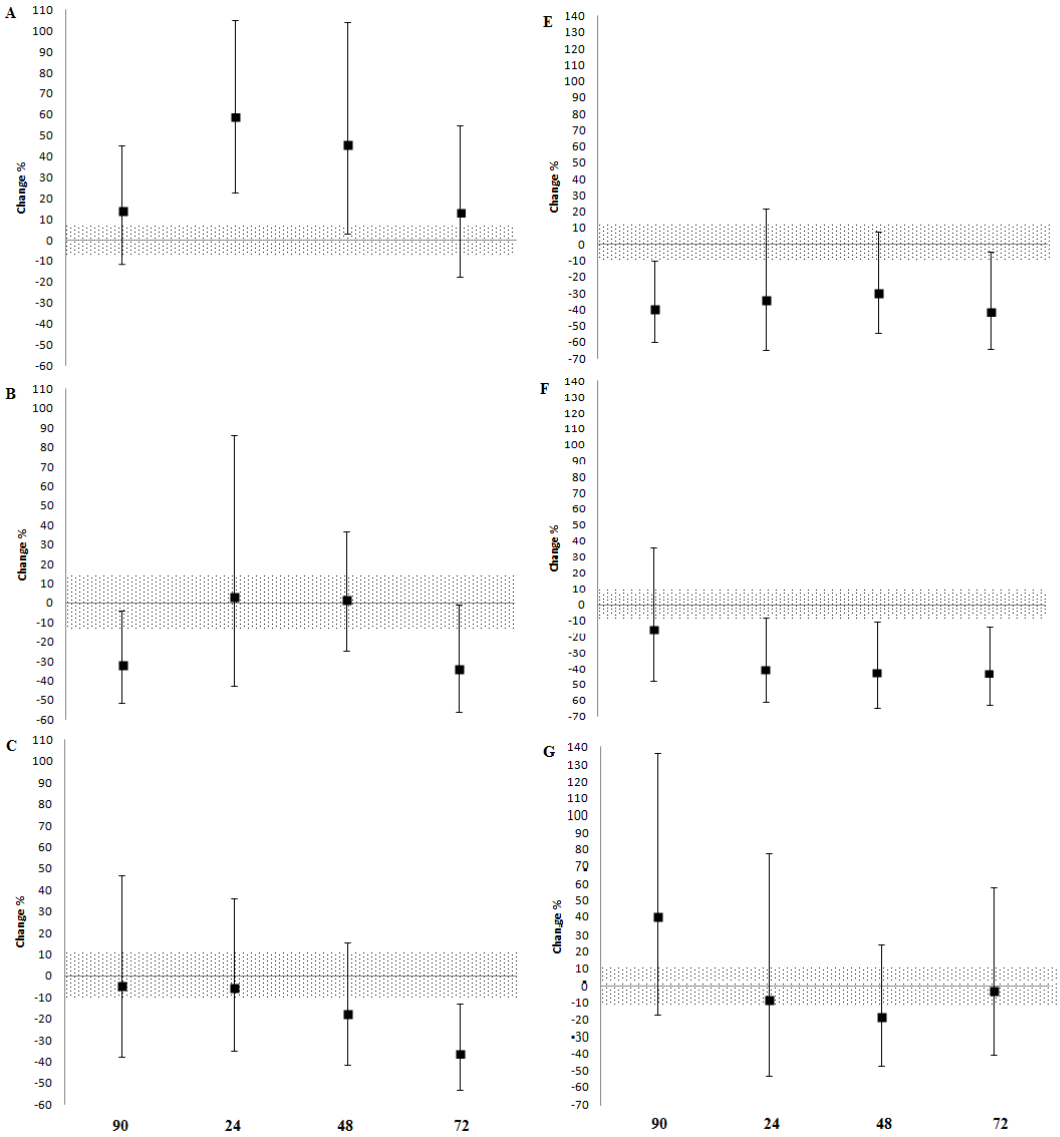

Figure 5 – Differences in CLpeak following exercise compared with baseline. A) PAS group - 90 min (63/30/8), 24h (99/1/0), 48h(92/6/2), 72h (59/28/13); B) ACT group - 90 min (1/10/89), 24h (38/31/31), 48h(25/56/19), 72h (2/10/88); C) CWI group - 90 min (27/33/40), 24h (22/37/41), 48h(7/25/68), 72h (0/3/96). Comparing the differences between groups: D) ACT – PAS - 90 min (1/5/94), 24h (8/12/80), 48h(4/13/83), 72h (3/6/92); E) CWI – PAS - 90 min (17/23/60), 24h (1/4/95), 48h(1/4/95), 72h (1/3/97); F) CWI – ACT - 90 min (76/16/8), 24h (30/23/48), 48h(10/25/65), 72h (44/33/38).

ANEXO C

Endereços eletrônicos dos guias para autores das revistas científicas *International Journal of Sports Medicine, e Journal of Sports Science*

Guia para autores da *International Journal of Sports Medicine*. Disponível em: < http://www.thieme.com/media/ita/pubid926886473.pdf >. Acessado em: 06 ago 2015.

Guia para autores da *Journal of Sports Science*. Disponível em: < http://www.tandfonline.com/action/authorSubmission?journalCode=rjsp20&page=instructions#mp_general >. Acessado em: 06 ago 2015.

ANEXO D

Parecer de aprovação do Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Paulista – UNESP

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Presidente Prudente

Presidente Prudente, 10 de dezembro de 2010.

Ilmo.(a) Sr.(a)
Prof. Dr. Carlos Marcelo Pastre.

Ref. Projeto intitulado: "Influência de diferentes tipos de recuperação sobre a modulação autonômica cardíaca e marcadores bioquímicos no sangue".

Processo 105/2010

Recebemos o projeto, o qual foi examinado pelo relator, tendo recebido o parecer anexo.

Decorrente do exposto, este Comitê, em concordância com o parecerista, considera o projeto **APROVADO**.

Informamos, ainda, que diante do cronograma do desenvolvimento da pesquisa, fica estabelecido o seguinte prazo: até a última terça-feira útil do mês **Março de 2013** para entrega de **um relatório final sucinto** ao CEP (vide modelo na página da FCT), sendo que os Termos de Consentimento Livre e Esclarecido (TCLE), assinados, deverão permanecer em poder do pesquisador responsável pelo período mínimo de 5 anos após o encerramento do estudo, para eventual fiscalização da CONEP.

Atenciosamente,

Dra. Edna Maria do Carmo
COORDENADORA DO COMITÊ DE ÉTICA EM PESQUISA

Faculdade de Ciências e Tecnologia
Comitê de Ética em Pesquisa
Rua Roberto Simonsen, 305 - CEP 19060-900 - Presidente Prudente - SP
Tel: (18) 3229-5388 - ramal 5482 ou 3229-5372 – e-mail: cep@fct.unesp.br

ANEXO E

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

INFLUÊNCIA DE DIFERENTES TIPOS DE RECUPERAÇÃO SOBRE A MODULAÇÃO AUTONÔMICA CARDÍACA E MARCADORES BIOQUÍMICOS NO SANGUE

As informações contidas nesta folha, fornecidas por CARLOS MARCELO PASTRE tem por objetivo firmar acordo escrito com o voluntário para participação da pesquisa acima referida, autorizando sua participação com pleno conhecimento da natureza dos procedimentos que será submetido.

1) Natureza da pesquisa: Você é convidado a participar desta pesquisa, que tem como finalidade investigar as respostas do coração (batimentos do coração) e análise da presença de substâncias encontradas no sangue após exercício intenso a partir da técnica de imersão em tambor com água e gelo, recuperação ativa (exercício leve) e recuperação. Todos os procedimentos relacionados à coleta sangüínea serão realizados por profissionais capacitados e os procedimentos de exercício monitorados por profissionais fisioterapeutas.

2) Participantes da pesquisa: um total de 20 sujeitos, com características sócio-econômicas semelhantes, entre 18 e 23 anos, selecionados aleatoriamente e que realizam atividades físicas regularmente. Para fazer parte deste grupo você não pode ser tabagista, fazer consumo de bebida alcoólica, drogas ou de medicamentos e ter alguma doença.

3) Envolvimento na pesquisa: Ao participar deste estudo você deverá permitir que um exame físico (peso, altura e possíveis alterações dos batimentos cardíacos) seja realizado. Você deverá comparcer no local de coleta por 3 vezes não consecutivas para realização dos testes e também, 24, 48 e 72 horas após cada um dos testes para coleta de sangue, ou seja 1, 2 e 3 dias. Você permanecerá no local de coleta aproximadamente duas horas e poderá fazer qualquer pergunta em relação aos procedimentos e outros assuntos relacionados com esta pesquisa.

4) Sobre as coletas: As coletas serão marcadas com antecedência e serão realizadas no Laboratório de Fisioterapia Desportiva (LAFIDE) da Faculdade de Ciências e Tecnologia – FCT/UNESP, respeitando o horário das 13:00 às 19:00 horas.

5) Protocolo experimental: No primeiro dia, será realizado um teste em que você deve correr numa esteira até não agüentar mais. Vai ser medido o quanto de ar você respira neste teste por meio de uma máscara. O segundo dia será composto por um exercício de corrida na esteira para você se preparar para um novo esforço, seguido por um exercício intenso, como já dito, até você não agüentar mais. Após o teste, você deverá permanecer na esteira, realizando exercício leve por mais seis minutos. No terceiro dia, você repetirá o exercício intenso e, após o teste, permancerá imerso em tambor com agúa e gelo até a cintura, por mais seis minutos em repouso. No final do segundo e terceiro dia, será realizada coleta sanguínea após o exercício leve e a imersão em água e gelo. Será retirada quantidade mínima de sangue necessária para análise. Oito vezes por dia do lobo da orelha. Um único furo por dia será necessário. Também será coletado sangue do antebraço 5 vezes por dia. Uma cânula será colocada no seu braço e por ela o sangue será retirado com uma seringa. No total o correspondente a uma seringa média será coletado. As coletas

de sangue serão feitas em três dias. Você também usará uma cinta no tórax com um aparelho que registrará os batimentos do seu coração. Você permanecerá com ele antes, durante e até 90 minutos após os exercícios.

6) Riscos e desconforto: Os procedimentos utilizados nesta pesquisa obedecem aos Critérios da Ética na Pesquisa com Seres Humanos conforme resolução n. 196/96 do Conselho Nacional de Saúde – Brasília – DF. Você poderá ter um certo desconforto devido ao contato do gelo com a pele e durante a coleta sanguínea. Se você apresentar sensações como tontura, palidez, sudorese intensa, dor ou qualquer outro sinal ou sintoma o exercício será interrompido imediatamente Os riscos a sua saúde geral são mínimos, já que será monitorado durante todo procedimento. Em qualquer momento você poderá desistir de participar dos procedimentos.

7) Confidencialidade: Todas as informações coletadas neste estudo são estritamente confidenciais. Seus dados serão identificados com um código, e não com seu nome. Apenas os membros da pesquisa terão conhecimento dos dados, assegurando assim sua privacidade. 8) Benefícios: Ao participar desta pesquisa você não terá nenhum beneficio direto. Entretanto, esperamos que este estudo traga informações importantes sobre a recuperação do organismo após a realização de um exercício intenso e alterações provocadas no comportamento dos batimentos cardíacos e nas substâncias encontradas no sangue. No futuro, essas informações poderão ser usadas em beneficio de outras pesquisas.

9) Pagamento: Você não terá qualquer tipo de despesa para participar da pesquisa e, nada será pago por sua participação.

10) Liberdade de recusar ou retirar o consentimento: Você tem a liberdade de retirar seu consentimento a qualquer momento e deixar de participar do estudo sem

penalizações. 11) Quaisquer dúvidas entrar em contato com Prof. Dr. Carlos Marcelo Pastre: Fone (18) 9116 6364; (18) 32295365. Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto, preencha os itens que seguem:

CONSENTIMENTO LIVRE E ESCLARECIDO

Eu,_____,

RG_____ após a leitura e compreensão destas informações, entendo que minha participação é voluntária, e que posso sair a qualquer momento do estudo, sem prejuízo algum. Confiro que recebi copia deste termo de consentimento, e autorizo a execução do trabalho de pesquisa e a divulgação dos dados obtidos neste estudo.

Obs: Não assine esse termo se ainda tiver dúvida a respeito.

Presidente Prudente, __/___/__.

Telefone para contato: _____

Assinatura do Voluntário: _____

Assinatura do Orientador: _____