Renan Felipe Hartmann Nunes

## EFFECTS OF FAR-INFRARED EMITTING CERAMIC MATERIALS AND COLD-WATER IMMERSION ON RECOVERY AFTER DIFFERENT EXERCISE-INDUCED MUSCLE DAMAGE PROTOCOLS

Tese submetida ao Programa de Pós-Graduação em Educação Física da Universidade Federal de Santa Catarina para a obtenção do Grau de Doutor em Educação Física. Orientador: Prof. Dr. Luiz Guilherme Antonacci Guglielmo.

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Esta Tese foi julgada adequada para obtenção do Título de "doutor" e aprovada em sua forma final pelo Programa de Pós-Graduação em Educação Física da Universidade Federal de Santa Catarina.

Florianópolis, 22 de fevereiro de 2019.

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Este trabalho é dedicado a Mayara Thays Muller por todo o seu amor e suporte incondicional.

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"Nosso grande medo não é o de que sejamos incapazes. Nosso maior medo é que sejamos poderosos além da medida. É nossa luz, não nossa escuridão, que mais nos amedronta. Nos perguntamos: Quem sou eu para ser brilhante, atraente, talentoso e incrível? Na verdade, quem é você para não ser tudo isso? Bancar o pequeno não ajuda o mundo. Não há nada de brilhante em encolher-se para que as outras pessoas não se sintam inseguras em torno de você. E à medida que deixamos nossa própria luz brilhar, inconscientemente damos às outras pessoas permissão para fazer 0 mesmo".

(Nelson Mandela, 1994)

#### **RESUMO**

O uso de materiais cerâmicos emissores de infravermelho (MCEIF) e a imersão em água fria (IAF) têm sido sugeridos como uma estratégia capaz otimizar a recuperação pós exercício. Assim, o principal objetivo desse estudo foi analisar os efeitos dos MCEIF e IAF na recuperação após diferentes protocolos de dano muscular induzido pelo exercício. Esse trabalho foi dividido em três estudos, cada um buscando responder uma questão específica. No primeiro estudo, o objetivo foi determinar se o uso de calças com materiais cerâmicos emissores de infravermelho durante duas horas ao longo de 72 horas após máximo exercício excêntrico proporciona melhora do desempenho neuromuscular, bioquímico e marcadores perceptuais em homens moderadamente ativos separados em tratamento (n=11 biocerâmica: BIO) ou (n=11 placebo: PL). Atividade plasmática da creatina quinase e lactato desidrogenase. dor muscular de início tardio, recuperação percebida e contração voluntária máxima foram mensurados nos momentos pré, 2, 24, 48 e 72 horas pós exercício. O exercício excêntrico induziu a um dano muscular evidenciado pelo aumento significativo da dor muscular de início tardio e da creatina quinase ao longo do período pós exercício (p>0.05). No entanto, o tamanho do efeito foi menor para o lactato desidrogenase em 24 h (TE= 0,50), mas maior em 48 h (ES= -0,58) no grupo tratamento comparado ao placebo. Em conclusão, o uso diário de MCEIF ao longo de 72 horas não facilita a recuperação após máximo exercício excêntrico. No segundo estudo, por sua vez, analisou-se o efeito do MCEIF durante o sono noturno nos marcadores neuromusculares. bioquímicos e perceptuais em atletas de futsal durante duas semanas no período de pré-temporada. Os atletas dormiram usando calças de biocerâmica (BIO; n=10) ou placebo (PL; n=10). O desempenho neuromuscular e os marcadores bioquímicos foram obtidos nos momentos pré (basal), pós primeira e segunda semana de treinamento. Dor muscular de início tardio (DMIT) e o stress do treino (ST) foram monitorados ao longo das semanas. Ambas, estatística tradicional (p<0,05) e a magnitude baseada em inferências (qualitativa) foram utilizadas para analisar as mudanças ao longo do tempo e entre grupos. Maiores mudanças no delta countermovement jump e squat jump nas semanas 1 e 2 foram reportados no grupo BIO. Ambos os grupos demonstraram melhora no tempo no teste de velocidade de 5-m na semana 2 comparado com a basal, porém, o BIO apresentou melhor desempenho no teste de velocidade de 10-m (semana 2) comparado ao PL. O percentual do delta do fator de necrose tumoral, interleucina-10 e

carbonila foi maior no grupo BIO versus o PL ao longo das semanas. Além disso, o percentual do delta das substâncias reativas ao ácido tiobarbitúrico, superóxido dismutase e catalase foram alterados ao longo das semanas comparado ao basal. O ST (semana 1) e DMIT (sete sessões) foram menores no grupo BIO comparado ao PL. Os resultados sugerem que o uso diário de vestuários com MCEIF pode facilitar a recuperação, principalmente nos marcadores perceptuais. Por fim, no terceiro estudo investigou-se o efeito da imersão em água fria (IAF) pós jogo nos marcadores de dano muscular, fadiga neuromuscular e nas respostas perceptuais ao longo de 72 horas pós partida de Rugby em atletas profissionais separados pelos grupos IAF (10°C/10min; n=11) ou controle (CON:30min sentado; n=11). O controle da carga interna (percepção subjetiva de esforço) e externa (sistema de posicionamento global) foram mensurados durante e pós jogo. Respostas bioquímicas, neuromusculares e perceptuais foram obtidas nos momentos pré, pós, 30 min, 24, 48 e 72 horas após o jogo. Magnitude baseada em inferências e o tamanho do efeito (TE) proposto por Cohen's foram mensurados para comparação das diferentes respostas ao longo do tempo e entre os grupos. Mudancas foram consideradas incertas com relação a carga de jogo, tempo dos testes de velocidade e as repostas perceptuais entre os grupos. Maior mudança do delta percentual do salto no squat jump (24 h), pico de potência (48 h) e taxa de desenvolvimento de força (após, 30 min e 24 h) foram reportados no grupo IAF comparado ao CON. Menores valores do percentual do delta do fator de necrose tumoral alfa (pós, 24 e 72 h) e rigidez (30 min e 48 h) foram demonstrados no grupo IAF versus CON. Além disso, diferentes efeitos dentro dos grupos foram relatados ao longo do pós-jogo. Em conclusão, a implementação de estratégias baseadas em IAF pós-jogo otimizou a recuperação dos marcadores de inflamação e fadiga em atletas de Rugby. Como uma síntese conclusiva dos três estudos, nota-se que o uso noturno e crônico do MCEIF e da IAF pós-jogo, pode ser considerado como uma estratégia efetiva nos diferentes marcadores de recuperação em atletas de esporte coletivo.

**Palavras-chave:** Dano muscular; esportes coletivos; desempenho; estratégias de regeneração; neuromuscular.

Linha de pesquisa: Exercício físico e desempenho no esporte.

**Projeto de pesquisa:** Efeito de diferentes técnicas de recuperação após dois modelos de dano muscular em atletas de rugby de elite; Efeitos da

impregnação em calça esportiva de um material cerâmico emissor de infravermelho no dano muscular em indivíduos saudáveis.

# **RESUMO EXPANDIDO**

# Introdução

O sucesso da melhora do desempenho e prevenção de lesões depende da qualidade e quantidade de estímulos prescritos durante o exercício físico. Quando a distribuição da carga de treino é excessiva e o tempo de recuperação inapropriado, o organismo pode apresentar estados de fadiga e adaptações inadequadas. Estudos têm demonstrado que modalidades de esportes coletivos que apresentam alta intensidade incluindo atividades excêntricas e exercícios de longa duração induzem a uma resposta inflamatória aguda. Este processo causa danos às fibras musculares e/ou ao tecido conjuntivo, queda na produção de força, diminuição da amplitude de movimento, aumento da rigidez, inchaço e edema, implicados pelo aumento da dor muscular de início tardio (DMIT). Nesse sentido, diversos estudos têm utilizado diferentes métodos de recuperação no esporte com o intuito de acelerar a recuperação de atletas nas diferentes modalidades esportivas. Um destes métodos é a terapia baseada na redução da temperatura através da imersão em água fria (IAF), amplamente discutida na literatura no que se refere à recuperação muscular pós sessão de exercício em atletas de alto rendimento. Além disso, outra técnica que tem recebido atenção dos pesquisadores da área da saúde e do esporte são os emissores de infravermelho longo (EIL). Além de formas tradicionais de emissão desse modelo de radiação (saunas e lâmpadas), recentemente, peças de vestuário, como calças, têm sido fabricadas com fibras impregnadas com um material cerâmico emissor de infravermelho longo (MCEIL), ativados por meio de resistências elétricas, com o propósito de fins terapêuticos, reduzindo a dor e otimizando a recuperação no esporte. Entretanto, para nosso conhecimento, nenhum estudo utilizou o MCEIL durante diferentes estratégias de tempo na melhora da recuperação, enquanto que o uso da IAF permanece questionável sobre alguns marcadores específicos de recuperação.

# Objetivos

O objetivo do presente estudo foi analisar o efeito do MCEIL impregnado em calças e IAF na recuperação após diferentes protocolos de danos muscular induzido pelo exercício. Os objetivos específicos foram separados em três estudos; 1) Verificar se o uso do MCEIL emitido por roupas por duas horas ao longo de três dias pode melhorar o desempenho neuromuscular e os marcadores bioquímicos e perceptuais em indivíduos saudáveis após máximo exercício excêntrico; 2) Verificar se o uso de MCEIL durante o sono noturno pode melhorar os marcadores bioquímicos de dano muscular e inflamação, assim como a influência do desempenho muscular esquelético e DMIT em atletas de futsal de elite durante um período de duas semanas de pré-temporada; 3) Determinar o efeito da IAF pós jogo nos marcadores de inflamação, fadiga neuromuscular, dor perceptual e recuperação dentro de 72 hora após jogo de Rugby.

## Metodologia

No primeiro estudo, o qual foi designado como randomizado, duplocego e controlado por placebo, os participantes foram alocados no grupo biocerâmica (n=11: BIO) ou placebo (n=11: PL). Atividade plasmática da creatina quinase e lactato desidrogenase, dor muscular de início tardio, recuperação percebida e contração voluntária máxima foram mensurados nos momentos pré, 2, 24, 48 e 72 horas pós exercício. O segundo estudo, caracterizado como randomizado, duplo-cego e controlado por placebo, os atletas utilizaram as calcas de biocerâmica (BIO; n=10) ou placebo (PL; n=10) durante o sono noturno. O desempenho neuromuscular e os marcadores bioquímicos foram obtidos nos momentos pré (basal), pós primeira e segunda semana de treinamento. Dor muscular de início tardio (DMIT) e o stress do treino (ST) foram monitorados ao longo das semanas. Por fim, no terceiro estudo, os atletas de Rugby profissionais foram separados pelos grupos IAF (10°C/10min; n=11) ou controle (CON:30min sentado; n=11). O controle da carga interna (percepção subjetiva de esforço) e externa (sistema de posicionamento global) foram mensurados durante e pós jogo. Respostas bioquímicas, neuromusculares e perceptuais foram obtidas nos momentos pré, pós, 30 min, 24, 48 e 72 horas após o jogo.

## Resultados e Discussão.

No estudo 1, o exercício excêntrico induziu a um dano muscular evidenciado pelo aumento significativo da dor muscular de início tardio e da creatina quinase ao longo do período pós exercício. No entanto, o tamanho do efeito foi menor para o lactato desidrogenase em 24 h (TE= 0,50), mas maior em 48 h (ES= -0,58) no grupo tratamento comparado ao placebo. Em conclusão, o uso diário de MCEIF ao longo de 72 horas não facilita a recuperação após máximo exercício excêntrico. Embora o tratamento cFIR não tenha sido extensivamente explorado, estudos prévios têm sido sugeridos como um método de recuperação pós-

mudanças exercício. No segundo estudo, maiores no delta countermovement jump e squat jump nas semanas 1 e 2 foram reportados no grupo BIO. Ambos os grupos demonstraram melhora no tempo no teste de velocidade de 5-m na semana 2 comparado com a basal, porém, o BIO apresentou melhor desempenho no teste de velocidade de 10-m (semana 2) comparado ao PL. O percentual do delta do fator de necrose tumoral, interleucina-10 e carbonila foi maior no grupo BIO versus o PL ao longo das semanas. Além disso, o percentual do delta das substâncias reativas ao ácido tiobarbitúrico, superóxido dismutase e catalase foram alterados ao longo das semanas comparado ao basal. O ST (semana 1) e DMIT (sete sessões) foram menores no grupo BIO comparado ao PL. Embora os resultados não sejam conclusivos sobre o desempenho do músculo esquelético e marcadores bioquímicos, o uso de cFIR pode ter contribuído para a redução dos marcadores perceptuais em jogadores de elite do futsal, especialmente durante as fases iniciais do treinamento intensivo (por exemplo, semana de treinamento). Por fim, no terceiro estudo, as mudanças foram consideradas incertas com relação a carga de jogo, tempo dos testes de velocidade e as repostas perceptuais entre os grupos. Maior mudanca do delta percentual do salto no squat jump (24 h), pico de potência (48 h) e taxa de desenvolvimento de força (após, 30 min e 24 h) foram reportados no grupo IAF comparado ao CON. Menores valores do percentual do delta do fator de necrose tumoral alfa (pós, 24 e 72 h) e rigidez (30 min e 48 h) foram demonstrados no grupo IAF versus CON. Além disso, diferentes efeitos dentro dos grupos foram relatados ao longo do pós-jogo. Embora os achados sejam divergentes na literatura, a eficácia do resfriamento pós-exercício na melhora dos marcadores bioquímicos e neuromusculares, podem indicar uma melhora na recuperação nos atletas de Rugby.

## **Considerações Finais**

De acordo com o objetivo específico do estudo 1, observou-se que o uso de MCEIL emitido por roupas por duas horas ao longo de três dias não foi capaz de melhorar o comportamento de produção de força muscular, dano muscular e marcadores perceptuais; Esses achados não apoiam convincentemente a hipótese de uma relação causal entre o método de recuperação da MCEIL e a melhora no desempenho. Quanto ao objetivo principal do estudo 2, o presente trabalho demonstrou que o uso de MCEIL durante a recuperação do sono durante um período curto e crônico contribuiu para reduzir a DMIT e a carga de treinamento. No entanto, os resultados não foram conclusivos sobre marcadores

bioquímicos e desempenho neuromuscular; apoiando em partes a hipótese deste estudo de uma relação positiva entre o uso de material biocerâmico e a recuperação perceptual. Finalmente, respondendo ao objetivo do estudo 3, o efeito da IAF pós jogo reportou efeitos positivos sobre a função neuromuscular medida a partir dos marcadores neuromuculares e inflamação. Estes resultados confirmam uma relação de "causa e efeito" entre a IAF e uma melhora no desempenho, mas não em relação aos marcadores perceptuais.

**Palavras-chave:** Dano muscular; esportes coletivos; desempenho; estratégias de regeneração; neuromuscular.

#### ABSTRACT

Far-infrared emitting ceramic materials (cFIR) and cold-water immersion (CWI) have been suggested as an efficient strategy to aid post-exercise recovery. Thus, the main purpose of this study was to analyze the effects of cFIR materials and CWI on recovery following different exerciseinduced muscle damage protocols. This work was divided into three studies, each one seeking to answer one specific question. First study aimed to determine whether the use of cFIR pants during two hours within after maximal eccentric exercise protocol 72 hours improve neuromuscular performance, biochemical and perceptual markers in moderately active men separated by treatment (n=11 bioceramic: BIO) or (n=11 placebo: PL). Plasma creatine kinase and lactate dehydrogenase activity. delayed-onset muscle soreness (DOMS), perceived recovery status, and maximal voluntary contraction were measured during preexercise and 2, 24, 48, and 72 hours post-exercise. Eccentric exercise induced muscle damage as evident in significant increases in delayedonset muscle soreness and creatine kinase throughout post-exercise (P>0.05). Despite the increased delayed-onset muscle soreness and creatine kinase values, no effect of treatment was evident (P>0.05). However. the standardized difference was lower for lactate dehydrogenase in 24 h (ES= 0.50), but higher in 48 h (ES= -0.58) in treatment compared to the placebo group. In conclusion, the daily use of cFIR clothing during over 72 hours did not facilitate recovery after maximal eccentric exercise. Second study sought to investigate the effects of cFIR during overnight sleep on neuromuscular, biochemical and perceptual markers in futsal players during a 2-week preseason training program. The athletes sleep wore bioceramic (BIO; n=10) or placebo pants (PL; n=10). Neuromuscular and biochemical markers were obtained at baseline, and after the first and second week of training. DOMS and training strain (TS) were monitored throughout. Both, traditional statistical and magnitude-based inference were used to analyze change over time and between-groups. Higher changes in delta countermovement jump and squat jump on weeks 1 and 2 were reported in BIO group. Both groups were faster in 5-m sprint in week-2 compared to baseline, furthermore, BIO was faster in 10-m on week-1 than PL. Delta percentage in tumor necrosis factor alpha, interleukin 10 and carbonyl was higher in BIO compared PL over weeks. Further, delta percentage in thiobarbituric acid reactive species, carbonyl, superoxide dismutase and catalase were modified across weeks compared to baseline. TS (week-1) and DOMS (seven sessions) was lower in BIO than PL. The results suggest that the

daily use of cFIR clothing could facilitate recovery, especially on perceptual markers. At last, third study aimed to analyze the effect of post-match cold-water immersion (CWI) on markers of muscle damage, neuromuscular fatigue and perceptual responses within 72 hours after a Rugby match in professional players separated into CWI (10°C/10min; n=11) or Control (CON:30min seated; n=11) groups. Internal (rating of perceived exertion) and external load (global positioning satellite) were and post-match. Biochemical, neuromuscular measured during performance and perceptual markers were obtained at pre, post, 30 min, 24, 48 and 72 h post-match. Magnitude-based inference and Cohen's effect size (ES) were used to analyze change over time and betweengroups. Changes were unclear for the match loads, sprint times and perceptual markers between-groups. Higher changes in delta percentage in squat jump (24 h), peak power (24 h) and rate of force development (post, 30 min and 24 h) were reported in CWI than in CON. Lower delta percentage in tumor necrosis factor alpha (post, 24 and 72 h) and stiffness (30 min and 48 h) were showed in CWI compared CON group. In addition, different within-groups effects throughout post-match were reported. Implementing post-match CWI-based strategies improved the recovery of markers of inflammation and fatigue in Rugby players. As a concluding remark from the three studies, it is worth mentioning that daily and overnight use of cFIR and CWI post-match, may be considered as an effective strategy in different recovery markers in team sports athletes.

**Keywords:** Muscle damage; team sports; performance; inflammation; regeneration strategies; neuromuscular.

# LIST OF FIGURES

Figure 1	
Figure 2	
Figure 3	
Figure 4	
Figure 5	61
Figure 6	61
Figure 7	
Figure 8	63
Figure 9	64
Figure 10	77
Figure 11	85
Figure 12	86
Figure 13	
Figure 14	88
Figure 15	89

# LIST OF TABLES

Table 1. Training program of preseason at Week one
Table 2. Training program of preseason at Week two.    53
Table 3. Training models during 2-weeks preseason.    54
Table 4. Mean ( $\pm$ SD) value of vertical jumping height and 5, 10 and 15
m sprint running times in the bioceramic and placebo groups throughout
the weeks training period
Table 5. Mean ( $\pm$ SD) value of inflammatory and oxidative stress in the
bioceramic and placebo groups throughout the weeks training period. 60
Table 6. Internal and external load between CWI and CON group during
a Rugby Union matches
Table 7. Effect size and quantitative chances for inflammation,
neuromuscular fatigue and perceptual markers within-CWI group
throughout 72 h post-match
Table 8. Effect size and quantitative chances for inflammation,
neuromuscular fatigue and perceptual markers within-CON group
throughout 72 h post-match

# LIST OF ABBREVIATIONS AND SYMBOLS

Arbitrary units
Baseline
Bioceramic
Catalase
Far-infrared emitting ceramic materials
Confidence interval
Control
Creatine kinase
Countermovement jump
Cyclooxygenase-2
Coefficients of variance
Cold-water immersion
Delayed onset muscle soreness
Effect sizes
Exercise-induced muscle damage
Functional circuit-training
Far infrared
Flexibility Training
Global positioning system
Ground reaction force
Hamstrings
High-intensity interval training
Hydrogen peroxide
Infrared
Interleukin 1 beta
Interleukin 6
Interleukin 8
Interleukin 10
Isometric
Jump height
Lactate dehydrogenase
Logarithms transformation
lipopolysaccharide
Maximal eccentric exercise
Michigan cancer foundation-10A
Maximal voluntary contraction
Nicotinamide adenine dinucleotide phosphate oxidase

NO	Nitric oxide
PGE2	Prostaglandin E2
PL	Placebo
PPO	Peak power output
PRS	Perceived recovery status
Qua	Quadriceps
ROS	Reactive oxygen species
RFD	Rate of force development
RM	Repetition maximum
RSS	Repeated-sprint sequence
s-RPE	Session rating of perceived exertion
SIT	Sprint interval training
ST	Strength training
TBARS	Thiobarbituric acid reactive species
TD	Total distance
TNF-α	Tumor necrosis factor alpha
TS	Training strain
SJ	Squat jump
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor alpha
vVO <sub>2max</sub>	Minimal running speed required to elicit maximal
	oxygen uptake
~	Approximately
%	Percentage
$\Delta$	Delta

# TABLE OF CONTENTS

1	CHAPTER ONE	
1.1 INT INFRAF WATER	FRODUCTION – CURRENT PERSPECTIVES ON RED EMITTING CERAMIC MATERIALS AND R IMMERSION ON RECOVERY	FAR- COLD- 
1.2 OBJ	ECTIVES	
1.2.1	Main objective	
1.2.2	Specific objectives	
1.3	HIPOTHESIS	
1.3.1	Hypothesis Study 1	
1.3.2	Hypothesis Study 2	
1.3.3	Hypothesis Study 3	
2	CHAPTER TWO	
2.1 CERAM MAXIM	STUDY ONE: EFFECTS OF FAR-INFRARED EM IIC MATERIAL CLOTHING ON RECOVERY IAL ECCENTRIC EXERCISE	ITTING AFTER 29
2.1.1	Abstract	
2.1.2	Introduction	
2.1.3	Methods	
2.1.4	Results	
2.1.5	Discussion	
2.1.6	Conclusion	
3	CHAPTER THREE	
3.1 CERAM PRESEA	STUDY TWO: EFFECTS OF FAR-INFRARED EM IIC MATERIALS ON RECOVERY DURING 2 ASON OF ELITE FUTSAL PLAYERS	ITTING -WEEK 43
3.1.1	Abstract	
3.1.2	Introduction	

3.1.3	Methods
3.1.4	Results
3.1.5	Discussion
4	CHAPTER FOUR71
4.1 UNION I MARKE	STUDY THREE: RECOVERY FOLLOWING RUGBY MATCHES: EFFECTS OF COLD-WATER IMMERSION ON RS OF FATIGUE AND DAMAGE71
4.1.1	Abstract
4.1.2	Introduction74
4.1.3	Materials and Methods75
4.1.4	Results
4.1.5	Discussion
4.1.6	Conclusion
5	CONCLUSION
6	REFERENCES
7	ATTACHMENT 109
7.1	A – Parecer consubstanciado do CEP 109
7.2	B – Parecer consubstanciado do CEP 112

#### **1 CHAPTER ONE**

## 1.1 INTRODUCTION – CURRENT PERSPECTIVES ON FAR-INFRARED EMITTING CERAMIC MATERIALS AND COLD-WATER IMMERSION ON RECOVERY.

Improved performance and injury prevention depend on the quality and quantity of training prescription during physical exercise. Additionally, when training load management/distribution is exceeded and the recovery time is not appropriated, it presents excessive fatigue and maladaptation's (NAKAMURA et al., 2016; SCHNEIDER et al., 2018; TAVARES; SMITH; DRILLER, 2017). In this line, attenuating the time to restructure physical demands before inducing a new stimulus, it becomes an inadequate condition, since it prevents the organism from being in an optimal condition for athletic performing and it may increase the risk of injuries (HALSON, 2014; NÉDÉLEC et al., 2015; TAVARES; SMITH; DRILLER, 2017).

Several studies have been reported that sports as high-intensity running, impacts and eccentric characteristic commonly associated with futsal and rugby players, may result in exercise-induced muscle damage (EIMD) (JOHNSTON; GABBETT; JENKINS, 2014; MOREIRA et al., 2013; ROBERTS et al., 2008; TEIXEIRA et al., 2017). It is a condition characterized by transient ultrastructural myofibrillar disruption, loss of muscle strength and power, delayed onset muscle soreness (DOMS), swelling, reduced range of motion of the affected limb, systemic efflux of myocellular enzymes, proteins or a combination of these (HYLDAHL, RD; HUBAL, 2014). The mechanical alterations and metabolic stress associated with EIMD stimulate various cell types that comprise skeletal muscle to initiate subsequent tissue repair and remodeling (HYLDAHL, RD; HUBAL, 2014).

In this regard, the implementation of recovery strategies to counter the deleterious effects of EIMD and fatigue is popular to promote faster recovery (DUFFIELD et al., 2014; HALSON, 2014; LINDSAY et al., 2015; NÉDÉLEC et al., 2015; TAVARES; SMITH; DRILLER, 2017; WEBB et al., 2013). Of those many and varied recovery interventions, cold-water immersion (CWI) has become ubiquitous in many professional sports, though questions regarding logistical use and efficacy in team sports remain (BROATCH; PETERSEN; BISHOP, 2014; DE FREITAS et al., 2017; GARCIA; DA MOTA; MAROCOLO, 2016; HIGGINS; CLIMSTEIN; CAMERON, 2013; LEEDER et al., 2012). Furthermore, recently, the role of passive heating through use of far-

infrared emitting ceramic materials (cFIR) therapy has been suggested as an effective recovery method in team sports (LOTURCO et al., 2016), though minimal supporting evidence exists. Hence the efficacy and optimal use of post-match recovery interventions remains of ongoing research interest to aid player preparation.

In addition, therapies based on temperature reduction through CWI have been used with team sports to promote recovery after training and competition (ASCENSÃO et al., 2011; DE FREITAS et al., 2017; POINTON; DUFFIELD, 2012; TAVARES et al., 2018). This recovery strategy is wifely utilized among athletes of all levels in both hot and normal environments in an attempt to ameliorate hyperthermia-induced fatigue and reduced EIMD (MINETT et al., 2014; PEIFFER et al., 2010; TAVARES; SMITH; DRILLER, 2017). Indeed, post-exercise CWI has been shown to maintain subsequent exercise performance (PEIFFER et al., 2018) and in some (VAILE; GILL; BLAZEVICH, 2007) but not all cases (POINTON; DUFFIELD; CANNON, 2011) attenuate the increase in indirect markers of muscle damage.

CWI is suggested to ameliorate EIMD via several mechanisms associates with localized cooling, hydrostatic pressures and redistribution of blood flow (LEEDER et al., 2012; WILCOCK; CRONIN; HING, 2006). Briefly, the main beneficial effect of CWI is cold-related vasoconstriction that may limit cellular, lymphatic, and capillary permeability and thus the inflammatory processes, reducing cell necrosis, neutrophil migration, secondary damage, edema and muscle pain (BAILEY et al., 2007; CHEUNG; HUME; MAXWELF, 2003; WILCOCK; CRONIN; HING, 2006). Recently, Machado et al. (2016) in a systematic review and meta-analysis research concluded that CWI is marginally better than passive recovery in the management of muscle soreness; reporting water temperature of 11-15°C and immersion duration of 11-15min to provide the best outcomes.

Several possibilities of evaluations have been used to monitor CWI effects in sports (BYRNE; TWIST; ESTON, 2004). However, field tests of neuromuscular function have been suggested as suitable and practical means of neuromuscular assessment available to practitioners (BYRNE; TWIST; ESTON, 2004; GATHERCOLE et al., 2015). Sprint and vertical jump testing, such as the countermovement jump (CMJ) are popular isoinertial field tests of neuromuscular function. Measures of sprint performance are typically impaired after prolonged intermittent activity and might provide insight into movement-specific fatigue (TWIST; HIGHTON, 2013), specially post-cold therapy. In addition,

Gathercole et al. (GATHERCOLE et al., 2015) verified that CMJ is a valid and sensitive test for neuromuscular fatigue related to team-sport performance. According to the authors, besides the performance assessment, the usefulness of jump tests also seems enhanced by the convenient and detailed analysis of kinetic variables provided by force plate (e.g., rate of force development [RFD], peak power output [PPO] and stiffness etc.), which may permit greater insight into the post CWI effects on neuromuscular responses. These parameters may be able or sensitive in analyzing players with distinct physical characteristics. However, no previous studies have examined the effects of these specific markers (i.e., provide by force plate) in Rugby players. Furthermore, additional details regarding inflammatory effects of CWI (i.e., interleukin 6 [IL-6], tumor necrosis factor alpha [TNF- $\alpha$ ]) within 72-h after match play may be of paramount practical relevance to coaches and strength and conditioning professionals, which it would allow a better understanding of how CWI affect the recovery post-matches.

Besides CWI, far infrared (FIR) ray have been proposed as a post-exercise recovery method to reduce pain (LOTURCO et al., 2016) and improve performance (LEUNG et al., 2013), although it is not extensively explored in sports science. FIR consist of short electromagnetic waves with lengths within the infrared (IF) spectrum ranging from 5.6 to 1000 µm. This therapy is usually applied thought light-emitting devices or saunas (HAUSSWIRTH et al., 2011: KO: BERBRAYER, 2002; VATANSEVER; HAMBLIN, 2012). However, recently garments manufactured of FIR emitting ceramic (cFIR) or polymers materials, have been incorporated into sports clothing fabric, which facilitates their application (CIAN et al., 2015; LEUNG et al., 2012a; LOTURCO et al., 2016). cFIR are produced by a combination of different mineral oxides (VATANSEVER; HAMBLIN, 2012) (i.e., powder composed of micro-sized particles), which emit heat and radiation to produce anti-oxidative, anti-inflammatory and analgesic activities observed in *in vitro* and animal model studies (LEUNG et al., 2009; LIN: LEE; LUNG, 2013; VATANSEVER; HAMBLIN, 2012). Furthermore, bioceramics are refractory, inorganic, nonmetallic polycrystalline compounds that due to their inertness in aqueous conditions are highly biocompatible (SEGOVIA: GORDILLO; FIGUEROA, 2003).

Thus, the use of cFIR for recovery modality has become a new method to reduce pain and improve the performance in post-exercise recovery (KO; BERBRAYER, 2002; LEUNG et al., 2012a, 2013; VATANSEVER; HAMBLIN, 2012). For instance, Loturco et al. (2016) reported the effects of FIR emitting clothes used for three days during

sleep on indirect markers and physical performance recovery after a plyometric bout in soccer players. The results suggested that FIR clothes might reduce perceived DOMS, however the authors did not find any effect on physical performance recovery. Cian et al. (2015) showed that it improves postural control in young non-athlete participants and expert gymnasts, suggesting that bioceramic fabrics do have an effect in postural control and improve postural stability. In addition, ceramic coated clothing increased blood flow during a 30 min exercise on a bicycle ergometer in a cool environment (KATSUURA et al., 1989). Moreover, there were tendencies toward decreased tiredness and reduced skin temperature in subjects wearing a bioceramic shirt while running a steady velocity (LEUNG et al., 2013).

Although different cFIR methods have been used for exercises models, the time response and biological effects to induce beneficial effects after EIMD are still uncertain. However, to our knowledge, no studies have used cFIR during different time strategy (i.e., short period and short-term chronic recovery) to verify if this method improve the recovery status. Preliminary evidence exists to support the use of cFIR as a tool for post-exercise recovery, whilst CWI remains an equivocal regarding specific markers but often used recovery mode. Thus, the aim of this study was to analyze the effects of cFIR materials in different time conditions and CWI on specific recovery markers following different exercise-induced muscle damage protocols

## **1.2 OBJECTIVES**

#### 1.2.1 Main objective

To analyze the effects of cFIR materials and CWI on recovery following different exercise-induced muscle damage protocols.

#### 1.2.2 Specific objectives

# 1.2.2.1 (Study 1)

To verify whether the use of cFIR emitting clothes for two hours over three days could improve the neuromuscular performance and biochemical and perceptual markers in healthy individuals after maximal eccentric exercise;

#### 1.2.2.2 (Study 2)

To verify whether daily use of cFIR during overnight sleep could improve biochemical markers of muscle damage and inflammation, as well as influence skeletal muscle performance and DOMS in elite futsal players during a 2-week preseason training period;

## 1.2.2.3 (Study 3)

To determine the effect of post-match CWI on markers of inflammation, neuromuscular fatigue, perceptual soreness and recovery within 72 h after a Rugby match

## 1.3 HIPOTHESIS

The hypothesis of the present study is that cFIR and CWI will optimize the recovery after different exercise-induced muscle damage protocols improving the blood-based, neuromuscular and perceptual markers.

1.3.1 Hypothesis Study 1

H1 - The use of cFIR emitting clothes for two hours over three days after maximal eccentric exercise would be accompanied by attenuation of falls in neuromuscular performance, muscle damage and perceptual markers.

## 1.3.2 Hypothesis Study 2

H1 - The daily use of cFIR during overnight would improve the biochemical markers of muscle damage and inflammation, accompanied by attenuation of falls of skeletal muscle performance and perceptual markers during a 2-week preseason training period.

## 1.3.3 Hypothesis Study 3

H1 - The CWI after Rugby matches will result in reduction of the inflammatory markers, neuromuscular fatigue and perceptual responses after 72 h.

## 2 CHAPTER TWO

# 2.1 STUDY ONE: EFFECTS OF FAR-INFRARED EMITTING CERAMIC MATERIAL CLOTHING ON RECOVERY AFTER MAXIMAL ECCENTRIC EXERCISE

This first paper was accepted to publish in one of the following issues of the Journal of Human Kinetics.

## Original research

Title

Effects of far-infrared emitting ceramic material clothing on recovery after maximal eccentric exercise

#### Running title

Far-Infrared Emitting Ceramic Clothing on Recovery

*Key words* muscle damage, delayed-onset muscle soreness, bioceramic, neuromuscular performance, post-exercise recovery.

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## 2.1.1 Abstract

The purpose of this study was to determine whether Far-Infrared Emitting Ceramic Materials worn as Bioceramic pants improve neuromuscular performance, biochemical and perceptual markers in healthy individuals after maximal eccentric exercise. Twenty-two moderately active men were randomized into Bioceramic (n=11) or Placebo (n=11) groups. To induce muscle damage, three sets of 30 maximal isokinetic eccentric contractions of the quadriceps were performed at 60°.s<sup>-1</sup>. Subjects wore the bioceramic or placebo pants for 2 hours immediately following the protocol, and then again for two hours prior to each subsequent testing session at 24, 48 and 72 hours post. Plasma creatine kinase and lactate dehydrogenase activity, delayed-onset muscle soreness, perceived recovery status, and maximal voluntary contraction were measured pre-exercise and 2, 24, 48, and 72 hours postexercise. Eccentric exercise induced muscle damage as evident in significant increases in delayed-onset muscle soreness at 24 - 72 hours (P < 0.05) and creatine kinase between Pre to 2, 24, 48 and 72 hours (P < 0.05). Despite the increased delayed-onset muscle soreness and creatine kinase values, no effect of Bioceramic was evident (P>0.05). Furthermore, decreases in maximal voluntary contraction between Pre and immediately, 2, 24, 48 and 72 hours post (P < 0.05) were reported. However, the standardized difference was *moderate* lower for lactate dehydrogenase in 24 h (ES= 0.50), but higher in 48 h (ES= -0.58) in Bioceramic compared to the Placebo group. Despite inducing muscle damage, the daily use of Far-Infrared Emitting Ceramic Materials clothing over 72 hours did not facilitate recovery after maximal eccentric exercise.

*Key words* muscle damage, delayed-onset muscle soreness, bioceramic, neuromuscular performance, post-exercise recovery.

## 2.1.2 Introduction

High-intensity and eccentric exercise can induce deleterious effects on skeletal muscle fibers, which might result in muscle soreness, swelling, and reduction of muscle strength and power for several days after exercise (PAULSEN G, MIKKELSEN UR, RAASTAD T, 2012; PROSKE; MORGAN, 2001). Exercise-induced muscle damage (EIMD) is typically assessed by indirect markers, including delayed-onset muscle soreness (DOMS), and by the appearance of muscle-specific proteins in the blood, such as creatine kinase (CK) and lactate dehydrogenase (LDH) (HODY et al., 2013; PAULSEN G, MIKKELSEN UR, RAASTAD T, 2012; PROSKE; MORGAN, 2001). Considering that high mechanical loads can result in considerable muscle damage, and inadequate recovery may affect subsequent physical performance and increase the risk of injury/illness, it is necessary to investigate strategies to improve the post-exercise recovery process (DUFFIELD et al., 2014; HALSON, 2014; VAILE; GILL; BLAZEVICH, 2007).

In this regard, a host of different strategies are proposed to aid post-exercise recovery with varying degrees of effectiveness and logistical practicality (DUFFIELD et al., 2014; HALSON, 2014; LAURENT et al., 2011; VAILE; GILL; BLAZEVICH, 2007). Strategies that aid skeletal muscle recovery from damage and promote antiinflammatory responses via practical methods are deemed critical. Hence, recently the role of bioceramic materials, also called Far-Infrared Emitting Ceramic Materials (cFIR), have been proposed as a postexercise recovery method (HAUSSWIRTH et al., 2011; LOTURCO et al., 2016). Previous in vitro and animal model studies report that the emitted heat and radiation from FIR materials can increase blood circulation, facilitate cell growth (VATANSEVER; HAMBLIN, 2012) and tissue regeneration (SEGOVIA; GORDILLO; FIGUEROA, 2003), leading to calcium-dependent nitric oxide (NO) and calmodulin upregulation in different cell lines (LEUNG TK, LIN YS, CHEN YC, SHANG HF, LEE YH, SU CH, 2009). Given the proposition that such properties can positively influence anti-oxidative, anti-inflammatory and analgesic activities (VATANSEVER; HAMBLIN, 2012), it is suggested that cFIR may have role as a post-exercise recovery tool.

Bioceramics are produced by a combination of oxides (VATANSEVER; HAMBLIN, 2012), which reflect/emit highperformance far-infrared rays (FIR) (SEGOVIA; GORDILLO; FIGUEROA, 2003). FIR-emitting polymers or ceramic nanoparticles have recently been incorporated into sports apparel to aid the facilitation and practical application of their use (CIAN et al., 2015; LOTURCO et al., 2016). Accordingly, cFIR has become a promising method to reduce pain and induce tissue repair (KO; BERBRAYER, 2002; LEUNG et al., 2012a; LOTURCO et al., 2016; VATANSEVER; HAMBLIN, 2012) however, conflicting results were reported regarding post-exercise recovery improvement (HAUSSWIRTH et al., 2011; LEUNG et al., 2013; LOTURCO et al., 2016).

For instance, FIR treatments with small far-infrared radiators (e.g., lamps, ceramic disks, plaster, and pads) have been shown positive effects in chronic diseased populations (BAGNATO et al., 2012; LAI et al., 2014; SILVA et al., 2009), though the exact mechanisms of cFIR remain unknown. In exercise models, Hausswirth et al. (2011) reported positive effects on neuromuscular performance after 30 min of FIR (via lamps) in endurance runners, but any decrease in blood-based markers were observed. In this line, although Loturco et al. (2016) suggested that FIR emitting clothes used for three days during sleep may reduce perceived DOMS after an intense plyometric session in professional soccer players, none positive effects was showed regarding indirect markers (i.e., CK) and physical performance recovery. The results suggested that FIR clothes might reduce perceived DOMS, despite the absence of improved physical performance in these soccer players. Hence the role of a placebo effect remains present, though the further application of cFIR therapy following EIMD remains to be investigated. Nevertheless, to our knowledge, no studies have used the bioceramic material as a recovery strategy during a short period. Furthermore, according to a pilot study (data not shown), this material used for two hours on rats was adequate to reduce inflammation induced by Complete Freud's Adjuvant (EMER et al., 2014). Thus, the aim of this study was to verify whether the use of cFIR emitting clothes for two hours over three days could improve the neuromuscular performance and biochemical and perceptual markers in healthy individuals after maximal eccentric exercise.

## 2.1.3 Methods

## **Participants**

A total of twenty-two moderately active, healthy men (mean age:  $25.7 \pm 3.8$  y, height: cm  $177.3 \pm 0.01$ , body weight:  $77.2 \pm 9.6$  kg) volunteered to participate in this study. Participants had no personal history of lower limb injury and were not involved in regular training programs before the study. The sample size was determined using power

analysis software G\*Power software (V.3.0.10). Accordingly, each group should comprise 11 participants to provide 80% power at the 0.05 level of significance. The inclusion criteria were: to attend 100% of physical tests and blood sample collections on each occasion, to be free from chronic diseases, not to be taking any medication (e.g. painkillers, antiinflammatory), nutritional supplements, illegal substances or technical recovery (e.g. cold-water immersion, massage, therapeutic exercise) and to wear the bioceramic (BIO) or placebo (PL) impregnated pants for ~2 h during the experimental approach. The subjects were also instructed to avoid heavy exercise and alcoholic and caffeinated products within 48 h preceding the tests. Each individual signed a written informed consent after being informed about the purpose, experimental procedures, possible risks, and benefits of the study. The experimental procedures were approved by the Institutional Review Board, according to the Helsinki Declaration.

## Experimental overview

The study was designed as a randomized double-blind placebocontrolled trial (Figure 1). Participants were randomly allocated in the BIO (n=11) group or PL (n=11) group, according the first generator randomizer where each subject was allocated to a single treatment by using the method of randomly permuted blocks (available at http://www.randomization.com) Participants undertook testing sessions at a standardised time of day  $(\pm 2 h)$  within a 1-week period. The first visit consisted of a familiarization session for all tests and procedures, including a modified (shorter) version of the eccentric exercise protocol. In the second session, participants performed the maximal eccentric exercise protocol to induce skeletal muscle damage. Before and after this session maximal voluntary contractions, blood samples and perceptual markers were recorded at Pre, and 2 h, 24 h, 48 h, and 72 h post exercise. The BIO or PL pants were worn for 2 h immediately post-exercise and then again for 2h prior to each of the ensuing measurement time points. Although the individuals were instructed about how to adapt their meal calories (approximately 60% carbohydrates, 15% proteins, and 25% lipids), no recalls of foods or diets were performed.



#### Figure 1.

Experimental protocol. MVC= maximal voluntary contraction; MEE= maximal eccentric exercise; CK = creatine kinase; LDH= lactate dehydrogenase; DOMS= delayed-onset muscle soreness; PRS= perceived recovery status.

#### Treatments

Both the BIO and PL pants were made of polyester (81%) and spandex (19%) and came in three different sizes (M, L, and XL). The bioceramic material used in this study was composed of microscopic particles including kaolinite, tourmaline, and aluminum oxide. A radiant power emission analysis in the infrared region in the range between 9 and 11 microns was conducted with a Scientech calorimeter (Boulder, CO, USA), Astral model (S AC2500S series), reporting the following value: 1) Fabric with bioceramic emissivity 0.91; 2) Fabric without bioceramic emissivity 0.83. For any given size, BIO and PL pants were identical in size, appearance, and elasticity. The only difference between bioceramic and placebo pants was a garment label indicating 1 or 2. All subjects wore the pants for two hours after the exercise protocol, and then again for two hours over three successive days at the same time during which they remained seated at rest in the laboratory. To eliminate any potential bias, participants in the placebo condition were led to believe that their pants were also a beneficial active treatment for recovery. Of note, the use of cFIR in two hours interventions was deemed appropriate from an ecological perspective for athletes, though it has been previously demonstrated that acute exposure to FIR (i.e. 30 min) (HAUSSWIRTH et al., 2011) improved indirect markers of EIMD.

## Maximal eccentric exercise (MEE)

The MEE protocol was performed using an isokinetic dynamometer Biodex IV (Shirley Corporation, New York, USA). A standardized warm-up protocol consisting of a 10-min cycling on a bicycle ergometer (Lode, Excalibur) at 70-80 rpm and 50 W. Participants were then placed in a supine position to induce a maximal lengthening of the rectus femoris (the only biarticular muscle of the knee extensor muscles) as muscle damage mainly occurs at longer muscle lengths (KANDA et al., 2013). Subjects were strapped across the femur distal extremity to avoid compensation from the hip joint. Initially two submaximal voluntary isometric knee extensions of 5 s with 30 s of recovery were performed as part of a warm up. Then three sets of five submaximal concentric contractions of the extensor muscles at an angular velocity of 120°.s<sup>-1</sup> throughout a constant motion range (100° of flexion from the maximal active extension) were performed. The contraction intensity was progressively increased across repetitions until they reached their own maximal performance. Afterwards, the subjects performed three sets of five eccentric contractions (at  $60^{\circ}$ .s<sup>-1</sup> angular velocity) progressively intensified until reaching their own maximal performance. After the familiarization, the subjects performed the eccentric contraction bout, which consisted of three sets of 30 maximal eccentric contractions of the quadriceps muscle group at 60°.s<sup>-1</sup> angular velocity throughout the same range of motion used for their familiarization. Each set was separated by a 30 s rest (HODY et al., 2013). The choice of the leg for this unilateral eccentric bout was randomly assigned (HODY et al., 2013). During each contraction, the subjects were verbally encouraged to produce their maximal performance, and the examiner checked the temporal evolution of the isokinetic curves on the screen to ensure that the subjects worked at their maximal intensity.

## Blood sampling CK and LDH activity

Blood samples (5mL) were drawn from an antecubital vein using a standard venipuncture technique with an ethylene diamine tetra acetic acid (EDTA) tube. Samples were centrifuged for 10 minutes at 1500 rpm to separate the plasma. Samples were extracted, stored at -80 °C until analysis according to specialized laboratory conditions for the following variables: CK and LDH. CK and LDH activity was determined spectrophotometrically by using commercial kits (Cobas Mira Plus, Roche, Basel, Switzerland and Sigma Diagnostics, St. Louis, MO for CK and LHL, respectively). CK and LDH intra- and inter-assay CVs were 3.8% and 2.8%, and 3.2% and 1.6% respectively.
## Delayed-onset muscle soreness (DOMS)

Each participant was asked to complete a questionnaire about the perceived leg muscle soreness on a scale from 0 ("absence of soreness") to 10 ("very intense soreness"). This method has been previously used as a non-invasive way to monitor changes in perceived pain following muscle damage protocols (VAILE; GILL; BLAZEVICH, 2007). Prior to reporting their DOMS ranking, the participants were required to perform a standardized half squat to ensure that all subjects were experiencing the same movement/sensation.

#### Perceived recovery status scale (PRS)

Perceived Recovery Status scale (PRS) consists of scores ranging between 0 and 10, in which 0–2 means very poorly recovered and with anticipated declines in performance, 4–6 means low to moderately recovered and expected similar performance, and 8–10 representing high perceived recovery with expected increases in performance (LAURENT et al., 2011). The subjects were asked to draw a vertical line that intersected the horizontal descriptor scale at the position that best described their perceived recovery level.

#### Statistical Analysis

The Shapiro-Wilk and Levene's tests were used to verify normality and data homogeneity, respectively. The isometric peak torque, blood variables, and perceptual marker data were analyzed using a two-way repeated measures ANOVA (group × time), followed by the Bonferroni post-hoc test. The statistical analyses were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, USA). The effect sizes (ES) were calculated and classified following the scale proposed by Cohen (1988): small (ES from 0.2 to 0.5), moderate (ES from 0.5 to 0.8), and large (ES  $\geq 0.8$ ). Values are reported as mean values and standard deviation (SD).

### 2.1.4 Results

The % $\Delta$ MVC for BIO and PL groups are shown in Figure 2. There were no significant main effects of group or interaction (P > 0.05). Nevertheless, a time main effect was found for % $\Delta$ MVC between Pre compared to Post, 2 h, 24 h, 48 h and 72 h and immediately Post compared to 24 h, 48 h and 72 h conditions (F=18,087; P < 0.05).



Figure 2.

Perceptual delta changes of maximal voluntary contraction (MVC) between interventions (Bioceramic [BIO] and Placebo [PL]) throughout 72 hours post maximal eccentric exercise (MEE). \* Statistically significant differences in relation to Pre (P < 0.05); \* Statistically significant differences in relation to Post (P < 0.05).

The % $\Delta$ CK and % $\Delta$ LDH in BIO and PL groups at different time points are presented in Figures 3A and 3B. There was no between-group significant effect (P > 0.05), although a main effect for time was evident for % $\Delta$ CK between Pre compared to 2 h, 24 h, 48 h and 72 h, and 2 h compared to 24 h, 48 h and 72 h (F=13,046; P < 0.05). There were no significant main effects in the groups, time, nor group × time interaction at % $\Delta$ LDH. However, the standardized difference was *moderate* lower in 24 h (ES= 0.50), but higher when compared to the PL group in 48 h (ES= -0.58) (Figure 3B).





Perceptual delta changes of creatine kinase (CK) (A) and lactate dehydrogenase (LDH) (B) between interventions (Bioceramic [BIO] and Placebo [PL]) throughout 72 hours post maximal eccentric exercise (MEE). \* Statistically significant differences in relation to Pre (P < 0.05); <sup>#</sup> Statistically significant differences in relation to 2h (P < 0.05). <sup>a</sup> Moderate effect size compared with Placebo group.

There were no significant main effects for group or interaction (P < 0.05) for either DOMS or PRS (Figures 4A and 4B, respectively). That said, DOMS was significantly increased at 24 h, 48 h, 72 h compared to Pre, and 48 h compared to 2 h (F=29,821; P < 0.05). Similarly, a significant decrease was reported in the PRS scale at 24 and 48 h compared to Pre (F=6,765; P < 0.05).



Figure 4.

Quadriceps delayed-onset muscle soreness (DOMS) (A) and perceived recovery status scale (PRS) (B) between interventions (Bioceramic [BIO] and Placebo [PL]) throughout 72 hours post maximal eccentric exercise (MEE). \* Statistically significant differences in relation to Pre (P < 0.05); <sup>#</sup> Statistically significant differences in relation to 2h (P < 0.05).

## 2.1.5 Discussion

The present study was designed to evaluate the effect of using cFIR immediately and in the days following eccentric exercise period on neuromuscular performance, muscle damage, and on the perceived recovery. The MEE bout was effective at inducing significant reductions in MVC and increases in CK and DOMS. However, neither treatment nor time x treatment interactions were observed on isometric peak torque (MVC), biochemical measures (CK and LDH), or perceptual markers (DOMS and PRS). Consequently, the use of BIO was not effective at improving recovery following muscle damaging exercise.

Although cFIR treatment has not been extensively explored, previous studies has been suggested as a post-exercise recovery method (HAUSSWIRTH et al., 2011; LEUNG et al., 2013; LOTURCO et al., 2016). Hausswirth et al. (2011) performed a running protocol to induce muscle damage in runners, and applied FIR (via lamps) immediately 24 h and 48 h post-exercise. The results showed that trained runners exposed to 30 min of FIR in the whole body were able to recover knee extensor MVC by 48 h post. Conversely, Loturco et al. (2016) did not show any positive effects of cFIR clotting in male soccer players who performed damaging drop jumps and used FIR for ten hours sleeping over three successive nights. Nevertheless, the authors have reported that athletes appeared to be well-trained, adapted, and hence resilient to the damaging effects of plyometric exercise, which may have attenuated possible cFIR effects.

Whilst no differences were observed for post-exercise CK or LDH responses between groups, the effect size in LDH was lower at 24 h but higher in 48 h in the BIO group compared to the PL. This finding is in agreement with previous studies that demonstrated that FIR sauna and clothes applied during three consecutive days did not decrease CK levels in highly-trained endurance runners (HAUSSWIRTH et al., 2011) and in elite soccer players (LOTURCO et al., 2016). One of the proposed effects of FIR is to improve local microcirculation, increasing the blood flow development of leukocyte migration into tissues (YANG et al., 2010) and irradiation of endothelial cells (PARK et al., 2013), resulting in vasodilatation and a rise in tissue temperature to facilitate neutrophil migration into the muscle (KANDA et al., 2013). Whilst such mechanisms were not investigated in the present study, the lack of difference in CK or LDH responses following BIO suggest insufficient emissivity to reflect/emit FIR ray by the clothing into human tissues, or

was insufficient to promote biological effects and improve recovery in the current population.

Although scientific evidence has been reported regarding the biological and hyperthermic effects of cFIR, its mechanisms on muscle performance are still unclear. The loss of maximal voluntary contraction force is considered one of the best clinical methods for quantifying muscle damage (MILES et al., 2008). Thus, the EIMD assessed by indirect markers (e.g. inflammatory mediators), may concur with the inhibition of force development in skeletal muscles (WILCOX; OSBORNE; BRESSLER, 1992) and partially explain the muscle strength loss. Therefore, it has been suggested that cFIR therapy may modulate cytokine expression during the muscle repair process, inducing a decrease in pro-inflammatory responses (LIN et al., 2007). However, our results showed no significant difference between the two groups.

Previous studies in clinical trials have also demonstrated that FIR reduced pain (BAGNATO et al., 2012; KO; BERBRAYER, 2002; LIN et al., 2007; SILVA et al., 2009), intolerance to cold (SILVA et al., 2009), muscle stiffness (LAI et al., 2014) and serum IL-6 and endothelin-1 concentrations in subjects following total knee arthroplasty (WONG et al., 2012). However, in the field sports few studies have investigated the cFIR effect on perceptual markers, such as DOMS and PRS. Loturco et al. (2016) were the first to report that FIR emitting clothes over three successive nights may have contributed to reduced DOMS within 72 h (moderate and large effect sizes, respectively) after the plyometric exercise bout. The authors have suggested FIR clothes may be used to accelerate muscle pain recovery after eccentrically-biased exercises in soccer players. Hausswirth et al. (2011) observed that the perception of muscle pain was significantly reduced after 48 h in relation to the nontreatment condition. In the present study, DOMS and PRS did not differ between conditions, either due to insufficient exposure or lack of perceptual or biological effect of the biocermic pants given the placebo effect was masked by the PL condition.

Despite these findings, certain limitations should be acknowledged. These include the short time and possible insufficient microscopic particles emissivity to reflect/emit cFIR from clothing into human tissues. Consequently, insufficient biological effects and acceleration of the recovery of skeletal muscle function and decreasing pain was evident. Furthermore, a longer time lapse between the end of the exercise protocol and the time the participants start wearing the cFIR pants can prevent cFIR acute effects upon neutrophil migration (LOTURCO et al., 2016). So, it is suggested that the use of cFIR during the sleep period, under more controlled exposure time and ambient conditions makes the treatment more effective (LOTURCO et al., 2016). Finally, our study also was limited by the sole use of scales to quantify DOMS.

# 2.1.6 Conclusion

In conclusion, the selected eccentric exercise stimulus used in our study induced muscle damage in the quadriceps muscles, However, the use of cFIR emitting clothes for two hours over three days did not contribute to improve MVC, perceptual scales and blood-based markers (although lower ES in 24 h in BIO than PL for LDH was observed) after a maximal eccentric exercise. Despite scarce research regarding cFIR in exercise models, the present findings suggested that the use of this material for two hours on a daily basis did not facilitate recovery in health individuals. Although, the use of recovery clothes could be practical and applicable, longer exposure time has to be considered, since this method does not affect the day routine of individuals. Future studies should investigate the effects of FIR-emitting clothes on other blood markers in order to understand its biological mechanisms after exercise. Additionally, further studies should be conducted to evaluate the required time for cFIR use, different protocols, and individuals that are involved to induce beneficial effects on human tissues and its possible effects when associated to different recovery strategies.

## Conflict of Interest

None of the authors of this paper has a competing interest.

## References

The references of the paper are at "references section" page 98.

## **3 CHAPTER THREE**

# 3.1 STUDY TWO: EFFECTS OF FAR-INFRARED EMITTING CERAMIC MATERIALS ON RECOVERY DURING 2-WEEK PRESEASON OF ELITE FUTSAL PLAYERS

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## Original research

### Title

Effects of far-infrared emitting ceramic materials on recovery during 2-week preseason of elite futsal players

Running title

Far-infrared emitting ceramic on recovery of futsal players

Key words Team sports, muscle damage, inflammation, performance

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## 3.1.1 Abstract

We investigated the effects of Far-Infrared Emitting Ceramic Materials (cFIR) during overnight sleep on neuromuscular, biochemical and perceptual markers in futsal players. Twenty athletes performed a 2week preseason training program and during sleep wore bioceramic (BIO; n=10) or placebo pants (PL; n=10). Performance (countermovement jump-CMJ; squat jump-SJ; sprints 5-m, 10-m, 15-m) and biochemical markers (tumor necrosis factor alpha-TNF-α, interleukin 10-IL-10, thiobarbituric acid reactive species-TBARS, carbonyl, superoxide dismutase-SOD, catalase-CAT) were obtained at baseline, and after the first and second week of training. Delayed-onset muscle soreness (DOMS) and training strain (TS) were monitored throughout. Changes in  $\Delta$ CMJ and  $\Delta$ SJ were *possibly* (60/36/4 [week-1]) and *likely* (76/22/2 [week-2]) higher in BIO. Both groups were faster in 5-m sprint in week-2 compared to baseline (P=0.015), furthermore, BIO was likely faster in 10-m (3/25/72 [week-1]). Significant group×time interaction in %ΔTNF- $\alpha$  were observed (P=0.024 [week-1]; P=0.021 [week-2]) with values possibly (53/44/3 [week-1]) and likely (80/19/1 [week-2]) higher in BIO. The  $\Delta IL-10$  decreased across weeks compared to baseline (P=0.019) [week-1]; P=0.026 [week-2]) showing values likely higher in BIO (81/16/3 [week-1]; 80/17/3 [week-2]). Significant weekly increases in % $\Delta$ TBARS (P=0.001 [week-1]: P=0.011 [week-2]) and % $\Delta$ Carbonvl (P=0.002 [week-1]; P<0.001 [week-2]) were observed compared to baseline, showing likely (91/5/4 [week-1]) and possibly (68/30/2 [week-2]) higher changes in BIO. Significant weekly decreases in  $\Delta$ SOD was observed compared to baseline (P=0.046 [week-1]; P=0.011 [week-2]), and between week-2 and week-1 (P=0.021), in addition to significant decreases in % $\Delta$ CAT compared to baseline (P=0.070 [week-1]; P=0.012 [week-2]). TS (P=0.021; very-likely [0/2/98]; week-1) and DOMS was lower in BIO (likely; seven sessions) with different over time (P=0.001). The results suggest that the daily use of cFIR clothing could facilitate recovery, especially on perceptual markers during the early phases of an intensive training period.

Key words team sports, muscle damage, inflammation, performance.

## 3.1.2 Introduction

During the preseason period, team sports athletes deal with large physical and physiological demands as a result of training sessions and friendly matches (MILOSKI et al., 2016; NAKAMURA et al., 2016). The high-intensity efforts typically found in team sports matches and training sessions, encompassing a high load upon the eccentric phase of muscle contraction, have been shown to induce inflammatory and oxidative stress responses (LEEDER et al., 2012; PAULSEN G, MIKKELSEN UR, RAASTAD T, 2012), as well as increased passive stiffness, swelling and edema. All of the above contribute to some extent to delayed-onset muscle soreness (DOMS), which may result in reduction in physical performance and disruption in athlete's regular training routine (PAULSEN G, MIKKELSEN UR, RAASTAD T, 2012; SMITH, 2000).

Athletes with inadequate recovery are prone to an increased risk of injury, illness, and overtraining (NÉDÉLEC et al., 2015; WATSON et al., 2017). Therefore, it is accepted that implementation of effective recovery strategies to counteract post-exercise muscle damage manifestations and prolonged impairment in performance is desired in order to optimize physiological adaptations to training (LEEDER et al., 2012; NÉDÉLEC et al., 2015). From a practical standpoint, it is desirable that the recovery method minimally disrupts the athletes' habits and causes no discomfort during its use.

Recently, Far-Infrared Emitting Ceramic Materials (cFIR) have attracted attention for therapeutic purposes, reducing pain and inducing tissue repair (VATANSEVER; HAMBLIN, 2012). cFIR are produced by a combination of oxides such as magnesium, silica, aluminum, tourmaline and mica, among others (VATANSEVER; HAMBLIN, 2012) and may be especially manufactured for performance enhancement and recovery purposes in competitive sports (LEUNG et al., 2013; LOTURCO et al., 2016). Infrared ray is a non-ionizing radiation emitted when a molecule de-excites from a higher vibrational or rotational quantum level, which is physically expressed as heat (KARSTENS; BOBELA; SMITH, 2006). Far-infrared is a region in the infrared spectrum of electromagnetic radiation from 3 to 100 µm (International Commission on Illumination Classification of IR Radiation) which penetrates up to 1.5 inches (almost 4 cm) beneath the skin (VATANSEVER; HAMBLIN, 2012). Particularly in the range of 8-14 mm, FIR is thought to have many biological effects (KIMURA et al., 2008).

The emitted heat and radiation from FIR materials influences cell membrane potentials and mitochondrial metabolism (VATANSEVER;

HAMBLIN, 2012), and can increase blood circulation (LIN; LEE; LUNG. regeneration (SEGOVIA: 2013). tissue GORDILLO: FIGUEROA, 2003), induce upregulation of calcium-dependent nitric oxide (NO) and calmodulin in different cell lines (LEUNG TK, LIN YS, CHEN YC, SHANG HF, LEE YH, SU CH, 2009), having positive effects anti-oxidative, anti-inflammatory and analgesic activities on (VATANSEVER; HAMBLIN, 2012). Recent studies in support of the analgesic effect of cFIR demonstrated that treatment produced significant inhibitory effects on both prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) under lipopolysaccharide (LPS) stimulation (LEUNG et al., 2012a), which suggest a possible anti-inflammatory and pain relief mechanism. However, these studies have been conducted in vitro and using animal models.

In exercise models, some evidence has led to speculation of performance enhancement and recovery benefits in competitive sports. Recently, Loturco et al. (2016) suggested that FIR clothes may reduce perceived DOMS after an intense plyometric session in professional soccer players. Furthermore, improvements in posture control and stability (CIAN et al., 2015), resting energy expenditure and recovery following endurance exercise (LEUNG et al., 2013) have been observed with the use of this material.

However, to our knowledge, no studies have used cFIR as a short-term chronic recovery strategy during a training period, especially during the preseason of team sports such as futsal, which is characterized by high training loads and sometimes accumulated fatigue due to insufficient recovery (MILOSKI et al., 2016). Thus, the aim of this study was to verify whether daily use of cFIR during overnight sleep could improve biochemical markers of muscle damage and inflammation, as well as influence skeletal muscle performance and DOMS in elite futsal players during a 2-week preseason training period.

## 3.1.3 Methods

## Experimental Approach to the Problem

In this study, we evaluated the effects of daily use of cFIR recovery on skeletal muscle performance, biochemical and perceptual markers in twenty futsal players during a two-week preseason training. The research was performed with an elite team of the Brazilian Futsal League during a randomized double-blind placebo-controlled trial experimental design. Skeletal muscle performance and biochemical

markers of each player was assessed at baseline, week one and week two of the training programme. The DOMS and training strain were used to monitor the perception of soreness and training load daily.

### Subjects

A total of twenty elite futsal players (mean age:  $24.0 \pm 4.7$  yr, height:  $176.0 \pm 0.06$  cm, body mass:  $72.3 \pm 6.0$  kg) from a Brazilian professional team volunteered to participate in the study. These selected futsal players are considered elite players because: i) their team competes in the first division of the Brazilian Futsal League; ii) in the recent years this team has reached the finals of the Brazilian Futsal Championships, iii) two of the volunteering athletes have played for the Brazilian National futsal team; iv) all players had at least 5 years of experience in futsal training and competition. The inclusion criteria were: to attend 100% of monitored training sessions during the study, to perform all physical tests on each occasion, to participate in all fasting blood sample collections, to be free from chronic diseases, not to be taking any medication (e.g. painkillers, anti-inflammatory), nutritional supplements with intracellular buffers (i.e. beta alanine and creatine), illegal substances or technical recovery (e.g. cold-water immersion, massage, therapeutic exercise) and to wear the bioceramic (BIO) or placebo (PL) pants for ~8 h during sleep time during the experimental period.

Before commencement of the study, each athlete was instructed to refrain from any exercise (48 hours before baseline tests), avoid alcoholic (48 hours before all tests) and caffeinated products preceding the tests day. All players were familiar with the study procedures before the beginning of the investigation. Each player gave their written informed consent after the explanation of the purpose, experimental procedures, possible risks and benefits of the study. The experimental procedures were approved by the Institutional Review Board, according to the Declaration of Helsinki.

#### Procedure

The study was designed as a randomized double-blind placebocontrolled trial. Briefly, participants were separated by position and randomly allocated to BIO (n=10; mean age:  $24.3 \pm 3.3$  yr, height: 178.6  $\pm 0.05$  cm, body mass:  $72.6 \pm 5.7$  kg) or PL (n=10; mean age:  $25.5 \pm 4.1$ yr, height:  $175.6 \pm 0.03$  cm, body mass:  $71.9 \pm 6.7$  kg) group, using the method of randomly permuted blocks (available at http://www.randomization.com). Due to the stratification based on playing position, each group was homogenous, equally balanced in the

variables described above. After randomization, the participants received a code alert with their allocation. Treatment allocation was masked from the study personnel during follow-up. However, two athletes were excluded from the PL group due to injury during the first week. Therefore, eight participants completed the study in this group. One week prior to commencement of the study, players were familiarized with the physical tests performed (jumps first and sprints on sequence), perceptual scales and testing procedures. During the experimental period, in order to minimize the impact of confounding factors, the athletes completed only the training program prescribed by the team's coaching staff, whilst all the prescribed standardized meals were taken at the same place and time. Furthermore, although the players were instructed how to adapt their meal calories (approximately 60% carbohydrates, 15% proteins, and 25% lipids), no information was received regarding dietary intakes. The consumption of water during and post training was ad libitum, however energy drinks (e.g. hypotonic or drinks containing carbohydrates) were not allowed (DE FREITAS et al., 2017).

Skeletal muscle performance (jumps and sprints) and the biochemical markers (inflammatory and oxidative stress) were obtained at baseline (BAS), and after the first (Week 1<sup>st</sup>) and second (Week 2<sup>nd</sup>) weeks of training. All measures were performed on Saturday morning. Athletes performed the entire procedure from 08:00 a.m to 11:30 a.m, in the following order: a) fasting blood sample collection; b) breakfast; c) countermovement jump (CMJ) and squat jump (SJ); d) 5-m, 10-m and 15-m sprints. Physical tests procedures started 90 minutes after breakfast and were recorded and reproduced to all athletes on the testing occasions. The suggested breakfast included: cereal (90 g), cow's milk (300 ml), bread (200 g), cheese (30 g), ham (30 g), flavoured yoghurt (200 g), orange juice (200 ml) and fruits (banana and papaya). However, the breakfast ingested was not relative to body mass.

During the training program, DOMS scores were registered before the training session and session rating of perceived exertion (s-RPE) was obtained after 15-30 minutes of each session to quantify the internal training load. The players wore the BIO or PL pants during the sleep time for five consecutive days (Monday to Friday night) but not during the weekend (Saturday and Sunday night) throughout the two weeks of observation. The weekend was not included in the experimental procedure due to the difficulty to standardize sleep habits of the players. All participants were instructed to continuously wear the pants between 10:00-11:00 p.m. and to 6:00-07:00 a.m. (~8 h). Every night the athletes were reminded through a mobile application about wearing the study supplied pants, moreover, every morning before the training session they were asked about the time they wore the garments during the night. No player wore the pants for less than 7 h or for more than 9 h during any given night for the duration of the study.

## Bioceramic material

The bioceramic formulation used in this study was composed of microscopic particles produced from various cFIR ingredients, which include kaolinite, tourmaline, and aluminum oxide (Patented Formulation by M.E.T. LLC, USA). A radiant power emission analysis in the infrared region in the range between 9 and 11 microns was conducted in the Spectroscopy and Laser Laboratory of the Institute of Exact Sciences at the Fluminense Federal University with a Scientech calorimeter (Boulder, CO, USA), Astral model (S AC2500S series) connected to a power and measurement unit detector Scientech, Astral model (S AI310D series); 1) Fabric with bioceramic emissivity 0.91; 2) Fabric without bioceramic emissivity 0.83. Both types of pants (BIO and PL) were made of polyester (81%) and spandex (19%) and came in three different sizes (M, L and XL) and the size worn by any given participant was always the same for the bioceramic and placebo pants. For any given size, BIO and PL pants were identical in size, appearance and elasticity. The only difference between bioceramic and placebo pants was a garment label indicating 1 or 2. The Biopower garments were commercially available (www.redwaveglobal.com).

### Training program

The training program during the second and third week of the preseason phase (i.e., study period) was planned by the team's coaching staff, without any interference from the investigators. During the first week (BAS week), the players were subjected to different test protocols (anthropometric, aerobic and anaerobic power, strength, speed and agility) to determine the team's baseline characteristics and the ensuing training loads and the training was prescribed with emphasis on metabolic and neuromuscular adaptations as determined by the team's staff. During this period, the training load was not monitored by the investigators. After the BAS week, all training program of 2 sessions per day for 5 days of the week. The players woke up at ~6:00-7:00 a.m. to attend a training session starting at 9:00 a.m. and lasting for ~ 90-120 min. After a ~ 4 h of recovery (at 3:00 p.m), players attended another training session lasting for ~ 90-120 min. Specifically, training aimed at developing muscle

strength, aerobic power and tolerance to lactic acidosis, neuromuscular power/speed, technical-tactical abilities, joint mobility, self-myofascial release and flexibility, as reported in table 1, e and 3.

Saturday (BAS)	Sunday	Week 1	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Fasting Blood CMJ SJ Sprints (5, 10, 15m)	Training off	Morning	DOMS Warm-up FCT (1 Set 10 exer 40s 10s rest) ST (2 Sets 10 exer 10RM ~80% load 60s rest) + Core (3 Sets 4 exer 10 rep) HIIT (1 Set 7 rep of 400m ~100/110%vVO2max 60- 90s passive recovery)	DOMS Warm-up Goalkeepers: specific technical training Specific technical with power (sprints with ball possession; time reaction with ball) Attack and defense exercise (1x1+1; 2x2+1; 3x2+1; 4x3+1)	DOMS Self-myofascial release Warm-up FCT (1 Set 8 exer 40s 10s rest) + Core (3 ISO exer 1 set 40s 10s rest) Sprints and jumps at sand (4 sets 8 sprints 10 m; 4 sets 8 jumps+sprint 5m+jumps+sprint 5m; 20s rest between efforts and 60s between series)	DOMS Warm-up Goalkeepers: specific technical training SIT (1 Set 10 rep of 200m all-out 90s passive recovery)	DOMS Self-myofascial release Warm-up ST (3 Sets 10 exer 10RM ~80% load 60s rec) + Core (3 Sets 4 exer 10 rep) FLEX-T (1 Sets 8 Exercise 30-60s)	Fasting Blood CMJ SJ Sprints (5, 10, 15m)	Training off
			s-RPE	s-RPE	s-RPE	s-RPE	s-RPE		
	Training off	Afternoon	DOMS Self-myofascial release Warm-up Goalkeepers: specific technical training Specific technical and tactical training; Attack and defense exer (2x2+1; 3x2+1; 4x3+1); Small-Sided Games	DOMS Warm-up FCT (3 Sets 6 exer 10 rep 10s rest) + Core (2 ISO exercise 30s 10s rest) Attack and defense exer (1x1+1; 2x2+1; 3x2+1; 4x3+1) Small-Sided Games	DOMS Self-myofascial release Warm-up Goalkeepers: specific technical training Specific technical and tactical training Attack and defense exer (3x2+1; 4x3+1; 5x5+1)	DOMS Warm-up Self-myofascial release FCT (1 Set 10 exer 40s 10s rest) Specific technical and tactical training Small-Sided Games	DOMS Warm-up Simulated Game	Training off	Training off
			s-RPE	s-RPE	s-RPE	s-RPE	s-RPE		
		Sleep	~8 hours BIO/PL pants	~8 hours BIO/PL pants	~8 hours BIO/PL pants	~8 hours BIO/PL pants	~8 hours BIO/PL pants	No	No

Table 1. Training program of preseason at Week one.

DOMS: Delayed-onset muscle soreness; FCT: Functional Circuit-Training; ST: Strength Training; HIIT: high intensity interval training; SIT: sprint interval training; FLEX-T: Flexibility Training; RM: repetition maximum; vVO<sub>2max</sub>: minimal running speed required to elicit maximal oxygen uptake; ISO: isometric; s-RPE: session rating of perceived exertion; CMJ: countermovement jump; SJ: squat jump; BIO: bioceramic; PL: placebo; exer: exercise; s: second; rep: repetition; mt: meters; %: percentage; ~: approximately; BAS: baseline.

Week 2	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	DOMS Warm-up ST (3 Sets 10 exer 10RM ~80% load 60s rest) +	DOMS Self-myofascial release Warm-up	DOMS Warm-up CRS (3 Sets 11 exer 10RM	DOMS Self-myofascial release (Foam Roller) CCT	DOMS Self-myofascial release (Foam Roller) FLEX-T (1 Sets 8	Fasting Blood CMJ SJ Sprint (5, 10, 15m)	
Morning	Core (3 Sets 4 exer 10 rep) SIT (1 set 10 rep of 40m; 1 set 10 rep of 30m; 1 Set 10 rep of 10m all-out 10s rest between efforts and 60-90s passive recovery between encovers)	FCT (1 Set 10 exer 40s 10s rest) + Core (4 ISO exer 40s 10s rest) Specific technical and tactical training	~60% load 30s rest) FLEX-T (1 Sets 8 Exercise 30-60s)	(6 exercise: 3 Sets 30-60s; 30-60s rest) Specific technical and tactical training; Attack and defense exer (1x1+1; 2x2+1; 3x2+1; 4x3+1);	Exercise 30-60s) tactical training		Training off
	s-RPE	s-RPE	s-RPE	₅-RPE	s-RPE		
Afternoon	DOMS Self-myofascial release Goalkeepers: specific technical training (30min) Specific tactical training;	DOMS Warm-up Attack and defense exercise (1x1+1; 2x2+1; 3x2+1; 4x3+1) Small-Sided Games	DOMS Self-myofascial release Warm-up Goalkeepers: specific technical training (30min) Simulated Game	DOMS Warm-up Friendly Game	DOMS Warm-up Friendly Game	Training off	Training off
	s-RPE	s-RPE	s-RPE	s-RPE	s-RPE		
Sleep	~8 hours BIO/PL pants	~8 hours BIO/PL pants	~8 hours BIO/PL pants	~8 hours BIO/PL pants	~8 hours BIO/PL pants	No	No

Table 2.	Training	program of	of preseason	at Week two.
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DOMS: Delayed-onset muscle soreness; FCT: Functional Circuit-Training; ST: Strength Training; SIT: sprint interval training; CRS: Circuit Resistance Strength; CCT: Core Circuit-Training; FLEX-T: Flexibility Training; RM: repetition maximum; ISO: isometric; s-RPE: session rating of perceived exertion; CMJ: countermovement jump; SJ: squat jump; BIO: bioceramic; PL: placebo; exer: exercise; s: second; rep: repetition; mt: meters; %: percentage; ~: approximately.

Training aimed	Exercises
Dynamic and isometric resistance strength: weightlifting equipment and free weights	Squats, leg press, stiff leg deadlift, standing calf raise, leg curl, hip adductors and abductors, bench press, lat pull down, shoulder press.
Core	Planks, hollow position, superman, t-stabilization, L-sit, glute bridge hold, sit ups, bird dog, ham curl, cable horizontal trunk rotation.
Circuit	Proprioception exercise at stable and unstable conditions, pushup, pistol, wall ball, one- warm swing, burpee and core exercises.
Aerobic and anaerobic	High-intensity interval training (HIIT) (~100-120% vVO <sub>2max</sub> ), SIT (~30s all-out) and RSS (~120-160% vVO <sub>2max</sub> at anaerobic speed reserve).
Power and speed	Horizontal, vertical and drop jumps, straight-line sprints, sprints with changes of direction at the sand and indoor court, resistance sprints using exercises with and without a ball performed at maximal speed.
Technical-tactical skills	Specific futsal ball-drill, attack and defense exercise (superiority and inferiority numerical), standard of the game, small-sided, simulated and friendly games.
Joint mobility, self-myofascial release and flexibility	Banded distraction and self-myofascial release with foam roller to calf, medial/anterior shin, knee, posterior chain (hamstring), medial chain (adductor), anterior high chain (hip flexor, quadriceps), anterior high chain (glutes, hip capsule), trunk (psoas, low back, oblique), thoracic spine (upper back, neck, scapula) area).

Table 3. Training models during 2-weeks preseason.

HIIT: High-intensity interval training; vVO<sub>2max</sub>: minimal running speed required to elicit maximal oxygen uptake; SIT: sprint interval training; RSS: repeated-sprint sequence.

## **Biochemical markers**

Venous fasting blood samples were collected at the same time of the day during each session. Blood samples were collected from a superficial forearm vein using standard venipuncture techniques. All samples were allowed to clot at room temperature for 30 min and subsequently were collected directly into serum separator collection tubes (Greiner Bio-one; Frickenhausen, Germany) and serum separated by centrifugation at 3.000 g and 4°C for 15 min. The resulting serum was placed into 0.5 ml Eppendorf tubes and frozen at -80 °C until the time of assaying (BRÜGGEMANN et al., 2017).

Circulating concentrations for total serum of interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF- $\alpha$ ) were determined using commercially available DuoSet Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits (DuoSet, R&D Systems, Minneapolis, MN, USA). The concentrations of cytokines were estimated by interpolation from a standard curve by colorimetric measurements in an ELISA plate reader (Perlong DNM-9602, Nanjing Perlove Medical Equipment Co, Nanjing, China). All results were expressed in pg·ml. Samples and provided standards were analyzed in duplicate according to manufacturers' instructions. Interassay coefficients of variance (CV) ranged from 4.8% and 5.7%, respectively (BRÜGGEMANN et al., 2017).

To determine oxidative stress, we measured the formation of Thiobarbituric acid reactive species (TBARS) during an acid-heating reaction (DRAPER; HADLEY, 1990). Briefly, the samples were mixed with 1 mL of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67%. They were then heated in a boiling water bath for 15 minutes with the addition of butylated hydroxytoluene. TBARS measurement was determined by a spectrophotometer (Hitachi U-1900, Tokyo, Japan) with absorbance at 535nm using 1,1,3,3-tetramethoxypropane as an external standard. Results are expressed as malondialdehyde equivalents per milligram of protein.

Protein carbonyls method detects the determination of the carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) (LEVINE et al., 1990). Proteins were precipitated by the addition of 20% trichloroacetic acid and reacted with DNPH. The samples were then redissolved in 6 mol·L<sup>-1</sup> guanidine hydrochloride, and the carbonyl contents were determined through a spectrophotometer (Hitachi U-1900, Tokyo, Japan) with absorbance at 370 nm using a molar absorption coefficient of 22.000 M<sup>-1</sup>. The intra- and inter-assay CVs were 3.6% and 4.8%, respectively.

Superoxide dismutase (SOD), was determined in erythrocytes according to the method suggested by Bannister and Calabrese (1987). Specific activity was expressed as units per milligram ( $U \cdot mg^{-1}$ ) of protein. One unit is estimated by 50% inhibition of adrenaline autooxidation read at 480 nm using a spectrophotometer (Hitachi U-1900; Tokyo, Japan). The intra- and inter-assay CVs were 7.7% and 8.2%, respectively.

Catalase (CAT), was determined in erythrocytes according to the method suggested by Aebi (1984). The samples were sonicated in 50 mmol·L<sup>-1</sup> phosphate buffer, and the resulting suspension was centrifuged at 3000g for 10 min. The supernatant was used for the enzyme assay. CAT activity was measured by the rate of decrease in hydrogen peroxide (10 mmol·L<sup>-1</sup>) absorbance at 240 nm using a spectrophotometer (Hitachi U-1900; Tokyo, Japan). The intra- and inter-assay CVs were 6.1% and 6.9%, respectively.

#### Performance tests

All participants performed three experimental sessions starting with 10 to 15 minutes of standard warm-up stage that consisted of stretching, jogging, jumps and sprints with changes of direction (MILOSKI et al., 2016). The CMJ and SJ were performed to assess jumping height. In the SJ, subjects were required to remain in a static position with a 90° knee flexion angle for 2 s prior to jumping, without any preparatory movement. In the CMJ, the players were instructed to execute a downward movement followed by a complete extension of the legs and were free to determine the countermovement amplitude to avoid changes in jumping coordination. Both jumps were executed with the hands fixed on the hips. All jumps were performed using an Ergojump contact mat (Cefise, Brazil) processed by the software (Jump fit 1.0, Cefise, Brazil). The dipping phase had a self-selected depth to avoid disturbing the athletes' coordination. The athletes were required to jump as high as possible. Three attempts were allowed for each jump. Successive attempts of the same jump mode were interspersed with ~15 s.

Five minutes after the jumps, the athletes performed three maximal sprints along the course of 0-m, 5 m, 10 m, and 15 m, with a 90 s passive rest interval between each sprint. Each sprint time was recorded using a photocell system (Cefise, Brazil), with timing gates placed at the 0-m (i.e., starting gate), 5-m, 10-m, and 15-m marks (i.e., finishing gates). The same equipment was used to automatically control the passive rest

interval between each sprint. All sprinting tests were conducted in an indoor court, thus eliminating any potential negative effect of the environmental conditions. The best two jumping and sprinting results were averaged and used for analysis (CRONIN; HANSEN, 2005).

#### Delayed-onset muscle soreness (DOMS)

Each participant was asked to complete a muscle soreness questionnaire for the lower limbs before each training session, in which they were required to rank their perception of soreness on a scale from 0 ("absence of soreness") to 10 ("very intense soreness"). This method has been used previously used as a non-invasive way to monitor changes in perceived pain following muscle damaging protocols (VAILE; GILL; BLAZEVICH, 2007). Prior to reporting their DOMS ranking, subjects were required to perform a standardized half squat with a 90° knee flexion angle and the hands fixed on the hips to ensure that all subjects were experiencing the same movement/sensation.

## Quantification of internal training load

The internal training load was recorded using training strain (TS) through the s-RPE method, as previously used in futsal players (MILANEZ et al., 2014). Approximately 15-30 minutes after the completion of every training session, the players were required to report the intensity of the entire session by means of a modified 10-point RPE scale (FOSTER et al., 2001). This value of RPE was multiplied by the total duration of every training session. On sequence, The RPE loads were computed as daily average units and total weekly mean (daily average sum). Thereafter, the "monotony" was calculated weekly by dividing the weekly mean RPE by the standard deviation ([total weekly mean RPE  $\div$  7 days week]  $\div$  standard deviation of total weekly mean RPE). Finally, the training strain was determined as the overall weekly RPE multiplied by monotony (total weekly mean RPE x monotony) (FOSTER et al., 2001).

#### Statistical analysis

The Shapiro-Wilk and Levene's tests were used to verify normality and homogeneity of the data, respectively. For the statistical analysis, biochemical markers [Delta percentage = (Post-values (week 1 and 2) – BAS / BAS X 100)] and skeletal muscle performance [Delta = (Post values (week 1 and 2) – BAS)] were transformed to reduce bias arising from uniformity errors. The data from biochemical and perceptual markers of muscle damage and skeletal muscle performance (blood markers, DOMS, jump and sprint performances) were analyzed using two-way repeated measures ANOVA (group  $\times$  time), followed by the Bonferroni post-hoc test. The independent test was used to examine possible differences in TS between groups. Significance was assumed at 5% (P < 0.05) a priori. Values are reported as mean values  $\pm$  and standard deviation (SD). The statistical analyses were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, USA). Magnitude based inference analysis was used to examine the differences in biochemical and perceptual markers and skeletal muscle performance during the preseason conducted using a customized spreadsheet (HOPKINS, 2006). We used this qualitative approach because traditional statistical approaches often do not take into account the magnitude of an effect, which is typically more relevant to therapeutic prescription than a statistically significant effect. The smallest worthwhile change was calculated (i.e., 0.2 x by the between-subjects standard deviation SD) and 90% confidence intervals (CI) were also determined to characterize biochemical, performance and perceptual indices (BATTERHAM; HOPKINS, 2006; HOPKINS et al., 2009). The quantitative chances of higher, similar or lower differences were evaluated qualitatively as follows: <1%, almost certainly not; 1% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possible; 75% to 95%, likely; 95% to 99%, very-likely; >99%, almost certain. The true difference was assessed as unclear when the chances of having positive and negative results were both >5%. Threshold values for Cohen's ES statistics were >0.2 (small), >0.5 (moderate), and >0.8 (large). In this study, two outliers (three standard deviations around the mean) (HOWELL, 1998) were removed from the TBARS analyses (an individual of each group; BIO n=9; PL n=7).

# 3.1.4 Results

The mean values of skeletal muscle performance and biochemical markers are reported in tables 4 and 5 respectively.

Table 4. Mean  $(\pm$  SD) value of vertical jumping height and 5, 10 and 15 m sprint running times in the bioceramic and placebo groups throughout the weeks training period

	BAS	Week 1	Week 2		
SJ (cm)					
Bioceramic	$36.6\pm3.2$	$36.2\pm4.0$	$37.2 \pm 5.4$		
Placebo	$35.7\pm3.6$	$35.6\pm4.6$	$34.5\pm5.2$		
CMJ (cm)					
Bioceramic	$39.4\pm3.4$	$39.1\pm3.9$	$40.1\pm4.4$		
Placebo	$38.6\pm3.9$	$38.7\pm5.4$	$39.3\pm3.3$		
Sprint 5 m (s)					
Bioceramic	$1.07\pm0.04$	$1.05\pm0.05$	$0.92\pm0.29$		
Placebo	$1.06\pm0.05$	$1.03\pm0.14$	$0.99\pm0.08$		
Sprint 10 m (s)					
Bioceramic	$1.39\pm0.04$	$1.39\pm0.03$	$1.37\pm0.03$		
Placebo	$1.37\pm0.05$	$1.39\pm0.06$	$1.36\pm0.05$		
Sprint 15 m (s)					
Bioceramic	$2.52\pm0.06$	$2.54\pm0.09$	$2.44\pm0.06$		
Placebo	$2.52\pm0.10$	$2.54\pm0.19$	$2.45\pm0.10$		
BAS=Baseline; SJ= Squat Jump; CMJ= Countermovement Jump; m= meters; s=					

seconds

bioectanile and placebo groups throughout the weeks training period.					
	BAS	Week 1	Week 2		
TNF-α (pg·ml)					
Bioceramic	$13.0\pm9.03$	$13.1 \pm 8.1$	$16.6\pm12.9$		
Placebo	$9.1\pm8.5$	$7.5\pm 6.6$	$8.5\pm9.6$		
IL-10 (pg·ml)					
Bioceramic	$4.3 \pm 2.1$	$4.2 \pm 2.3$	$3.9 \pm 2.0$		
Placebo	$4.6\pm4.9$	$3.2 \pm 2.9$	$2.8 \pm 3.4$		
TBARS (nmol·mg)					
Bioceramic	$0.006\pm0.001$	$0.007\pm0.001$	$0.009\pm0.001$		
Placebo	$0.006\pm0.001$	$0.008 \pm 0.001$	$0.009 \pm 0.0009$		
Carbonyl (nmol·mg	g)				
Bioceramic	$0.015\pm0.004$	$0.016\pm0.004$	$0.018\pm0.004$		
Placebo	$0.015\pm0.005$	$0.016\pm0.005$	$0.017\pm0.006$		
SOD $(U \cdot mg^{-1})$					
Bioceramic	$0.015\pm0.002$	$0.014\pm0.004$	$0.011\pm0.004$		
Placebo	$0.031\pm0.021$	$0.024\pm0.016$	$0.021\pm0.015$		
CAT $(U \cdot mg^{-1})$					
Bioceramic	$0.016\pm0.014$	$0.012\pm0.014$	$0.008\pm0.007$		
Placebo	$0.028\pm0.015$	$0.019\pm0.010$	$0.016\pm0.015$		
Bioceramic Placebo SOD (U·mg <sup>-1</sup> ) Bioceramic Placebo CAT (U·mg <sup>-1</sup> ) Bioceramic Placebo	$\begin{array}{c} 0.015 \pm 0.004 \\ 0.015 \pm 0.005 \\ \hline \\ 0.015 \pm 0.002 \\ 0.031 \pm 0.021 \\ \hline \\ 0.016 \pm 0.014 \\ 0.028 \pm 0.015 \end{array}$	$\begin{array}{c} 0.016 \pm 0.004 \\ 0.016 \pm 0.005 \end{array} \\ \\ 0.014 \pm 0.004 \\ 0.024 \pm 0.016 \end{array} \\ \\ 0.012 \pm 0.014 \\ 0.019 \pm 0.010 \end{array}$	$\begin{array}{c} 0.018 \pm 0.004 \\ 0.017 \pm 0.006 \\ \hline \\ 0.011 \pm 0.004 \\ 0.021 \pm 0.015 \\ \hline \\ 0.008 \pm 0.007 \\ 0.016 \pm 0.015 \end{array}$		

Table 5. Mean  $(\pm SD)$  value of inflammatory and oxidative stress in the bioceramic and placebo groups throughout the weeks training period.

BAS= Baseline; TNF- $\alpha$ = Tumor necrosis factor alpha; IL-10= Interleukin 10; TBARS= Thiobarbituric acid reactive species; SOD= Superoxide dismutase; CAT= Catalase.

There were no significant main effects in the groups, time, nor group × time interaction for  $\Delta$ SJ (*P*=0.91) and  $\Delta$ CMJ (*P*=0.93). However, changes in  $\Delta$ SJ and  $\Delta$ CMJ were *likely* higher in BIO than in PL group in Week 2 (Fig 5A) and was *possibly* higher in Week 1 (Fig 5B), respectively, with small effect size in both cases.



Figure 5.

Delta change between interventions (Bioceramic and Placebo) throughout the weeks training period for (panel A) squat jump and (panel B) countermovement jump. Likely and possibly small effect between groups were reported at week 2 (ES = 0.38) (panel A) and week 1 (ES = 0.26) (panel B). Dashed line with circle represents Bioceramic. Dashed line with solid square represents Placebo. BAS = baseline.

Significant improvement over time was observed in  $\Delta 5$ -m sprint in Week 2 compared to BAS (P=0.015), but no effects in  $\Delta 10$ -m (P=0.24) and  $\Delta 15$ -m sprint (P=0.60) were detected. Though, the  $\Delta 10$ -m revealed that BIO was *likely* faster than PL group in Week 1, with small effect size (Fig 6B), but *unclear* differences were noticed to  $\Delta 5$ -m (Fig 6A) and  $\Delta 15$ -m (Fig 6C).



Figure 6.

Delta change between interventions (Bioceramic and Placebo) throughout the weeks training period for (panel A) sprint 5-m, (panel B) sprint 10-m and (panel C) sprint 15-m. \* Statistically significant differences to baseline (BAS) (P <

0.05). Possibly small effect between groups were reported at week 1 (ES = -0.35) (panel B). Dashed line with circle represents Bioceramic. Dashed line with solid square represents Placebo.

There was statistical difference on group × time interaction in  $\Delta TNF-\alpha$  in Week 1 (*P*=0.024) and Week 2 (*P*=0.021). Furthermore, the BIO showed values *possibly* and *likely* higher in Week 1 and Week 2 than in PL, respectively, with small standardized difference (Fig 7A). Compared with BAS,  $\Delta IL-10$  decreased significantly across weeks 1 (*P*=0.019) and 2 (*P*=0.026) for the groups. The values reported in BIO were *likely* higher than in PL in Week 1 and Week 2, with a small effect size (Fig 7B).



Figure 7.

Percentage change between interventions (Bioceramic and Placebo) throughout the weeks training period for (A) TNF- $\alpha$  and (B) IL-10. #Statistically significant differences on group × time interaction (P < 0.05); \*Statistically significant differences to baseline (BAS) (P < 0.05). Possibly and Likely small effect at week 1 and week 2 (ES = 0.21 and ES = 0.34 (panel A) and Likely small effect at week 1 and week 2 (ES = 0.48 and ES = 0.45) (panel B) were reported between groups. Dashed line with circle represents Bioceramic. Dashed line with solid square represents Placebo. TNF-  $\alpha$  = Tumor necrosis factor alpha; IL-10 = Interleukin 10.

Significant effects over time in % $\Delta$ TBARS were seen in Week 1 (*P*=0.001) and Week 2 (*P*=0.001) compared to BAS, in Week 2 compared to Week 1 (*P*=0.011), and the changes were *likely* higher in BIO than in PL in Week 1, with a large increase (Fig 8A). An increase was observed in % $\Delta$ Carbonyl in Week 1 (*P*=0.002) and Week 2 (*P*<0.001) compared to BAS, and in Week 1 compared to Week 2 (*P*<0.001), for both groups.

Moreover, the values were *possibly* higher in BIO than in PL in Week 2, with small effect size (Fig 8B). A decrease was observed in % $\Delta$ SOD in Week 1 (*P*=0.046) and Week 2 (*P*=0.011) compared to BAS, in Week 2 compared to Week 1 (*P*=0.021), and in % $\Delta$ CAT in Week 1 (*P*=0.070) and Week 2 (*P*=0.012) compared to BAS. Magnitude-based inference analysis showed *unclear* for all conditions (Fig 8C and 8D).



Figure 8.

Percentage change between interventions (Bioceramic and Placebo) throughout the weeks training period for (panel A) TBARS, (panel B) protein carbonyl, (panel C) SOD and (panel D) CAT. \*Statistically significant differences to baseline (BAS) (p<0.05). \*\*Statistically significant differences to Week 1 (P < 0.05). Very-likely large effect (ES = 1.25) (panel A) and possibly small effect (ES = 0.29) (panel B) between groups were reported at week 2. Dashed line with circle represents Bioceramic. Dashed line with solid square represents Placebo. TBARS = Thiobarbituric acid reactive species; SOD = Superoxide dismutase; CAT = Catalase.

Figure 9A displays the DOMS monitored during 20 training sessions. Results demonstrated significant effects over time (P=0.001).

The BIO showed a *likely* lower DOMS at session 6 (4/15/82; ES= -0.56), 8 (1/4/94; ES= -1.02), 9 (3/13/84; ES= -0.63), 10 (1/7/91; ES= -0.74), 16 (5/19/77; ES= -0.48), 17 (5/17/78; ES= -0.52) and 20 (3/8/89; ES= -0.85) compared to PL group.

The TS during 2-week preseason training is reported in figure 9B. The results demonstrated lower weekly TS during Week 1 in BIO compared to PL ( $3565.3 \pm 582.3$  vs  $4226.2 \pm 491.9$  A.U. *P*=0.021), but not in Week 2 ( $4108.8 \pm 1302.5$  vs  $3553.7 \pm 1144.1$  A.U. *P*=0.351). The analysis for the training strain showed that the values reported in BIO were *very-likely* lower than in PL group in Week 1 with large difference, but *unclear* at Week 2.



Figure 9.

DOMS scale (0-10 arbitrary units, panel A) before each session training between groups. Training strain (arbitrary units, panel B) during two-week preseason training. a = Statistically significant differences to 2, 4 to 10, 15 to 20 sessions; b = 2 to 20 sessions; c = 4 to 6 and 15 to 20 sessions; d = 6 to 19 sessions; e = 17, 19 sessions; f = 2, 3, 5 sessions; g = 1, 2, 5, 6, 8 sessions; h = 2, to 6, 8 sessions (P < 0.05). & = 75% to 95%, likely. \*Statistically significant differences between groups (P < 0.05); Very-likely large effect (ES = -1.19) between groups were reported at week 1. Open solid square represents Bioceramic. Dashed solid square represent Placebo.

### 3.1.5 Discussion

The present study was designed to analyze the effect of cFIR emitting clothing during sleep recovery over a 2-week preseason period on neuromuscular performance, biochemical and perceptual markers in Brazilian elite futsal players. In the present study, we attempted to further characterize some of the mechanisms through which cFIR exerts its analgesic and anti-inflammatory action in Brazilian elite futsal players.

Recent studies have demonstrated that a higher training load during the preseason of futsal players seeks to reach an optimal physical and technical fitness status, which in turn may positively affect competitive performance and avoid an elevated incidence of injury during the in-season phase (MILOSKI et al., 2016; MOREIRA et al., 2013). However, when excessive training loads are sustained for consecutive weeks, the athletes may experience overreaching/overtraining-related symptoms (MOREIRA et al., 2013). Thus, different recovery strategies have been used in sports to optimize the physiological and neuromuscular adaptations to training, and although not extensively explored, FIR therapy has been suggested as a neuromuscular post-exercise recovery method (HAUSSWIRTH et al., 2011).

The results of the present study showed no significant differences in skeletal muscle performance between the groups through the traditional statistical analysis; however, based on the qualitative analyses by Hopkins (BATTERHAM; HOPKINS, 2006; HOPKINS, 2006; HOPKINS et al., 2009) our results demonstrated quantitative changes in SJ (likely higher at Week 2), CMJ (possibly higher at Week 1) and 10-m sprint time (likely faster at Week 1) in BIO compared to PL group; however, the effect size was considered small in these cases. In this line, Hausswirth et al. (2011) demonstrated that 30 min whole body FIR (lamps) therapy sessions conducted 24h and 48h following a running protocol to induce muscle damage in runners, facilitated knee extensor maximum voluntary contractions recovery 48h after performing the muscle damage protocol (HAUSSWIRTH et al., 2011). In contrast, Loturco et al. (2016) did not identify the potential effect of cFIR clothing on male soccer players during a 10h sleeping period over three successive nights after 100 drop-jumps observed for vertical jumps (SJ and CMJ) or maximal dynamic strength (leg press 1RM) performance. Nevertheless, the authors reported that athletes appeared to be well trained and adapted and hence resilient to the damaging effects of plyometric exercise, which may have attenuated possible cFIR effects (LOTURCO et al., 2016).

The hypothesis which could explain our results is related to the amount of exposure time (weeks) and the FIR emissivity of the clothing's into human tissues, which perhaps were not enough to promote statistically significant (traditional statistical analysis) recovery of skeletal muscle performance (although a trend is seen when the Hopkins statistical method is applied). Moreover, the severity of the muscle damage induced during preseason training on consecutive days may have disguised the effect of cFIR, evidenced by the reduction in inflammatory and oxidative stress markers during the two-week training period.

Likewise, no significant differences in DOMS were noted between groups; however, the magnitude-based analyses demonstrated that cFIR treatment was likely lower in seven training session (35% of the preseason) with a moderate and large effect. A considerable number of clinical trials have suggested that cFIR can be effective in reducing pain (BAGNATO et al., 2012; WONG et al., 2012). For example, cFIR reduced pain, intolerance to cold and periodic movements in post-polio syndrome patients (LAI et al., 2014); induced significant reduction in the pain after one week of intervention with FIR-emitting plasters applied to osteoarthritis patients (12h a day) (BAGNATO et al., 2012); reduced pain in patients with arthritis and peripheral vascular disease (KO; BERBRAYER, 2002); reduced muscle stiffness suggesting and application in the management pain, in a trial involving 48 patients with chronic myofascial neck pain (LAI et al., 2014); and decreased pain sensation and serum IL-6 and endothelin-1 concentrations after 15 minutes of FIR exposure per day for five consecutive days in subjects following total knee arthroplasty (WONG et al., 2012).

Nevertheless, despite the considerable amount of data regarding the analgesic and anti-inflammatory effects of cFIR, the precise mechanisms underlying its effect remains to be fully understood. Very recently, Loturco et al. (2016) did not find significant differences on CK activity between treated and placebo groups, but DOMS effect sizes were greater in the cFIR group, suggesting that cFIR clothes may reduce perceived DOMS after an intense plyometric session performed by soccer players (LOTURCO et al., 2016). Corroborating these findings, Hausswirth et al. (2011) observed that perception of muscle pain was significantly decreased after 48h in relation to non-treatment group (HAUSSWIRTH et al., 2011). However, muscle pain could have been partially biased due to the absence of blinding. These findings are in line with the present study, since we found that the daily use of cFIR during overnight sleep improved skeletal muscle performance and decreased

DOMS in Brazilian elite futsal players during a 2-week preseason training period.

In this study, we decided to apply cFIR during the sleep period, under more controlled exposure time and ambient conditions, as previously reported by Loturco et al. (2016), because the time lapse between the end of the exercise protocol and the time the participants start wearing the cFIR pants prevents cFIR acute effects upon neutrophil migration. Moreover, night time intervention makes the treatment more comfortable and increases compliance as opposed to wearing the clothes during daily activities (LOTURCO et al., 2016).

Interestingly, our results have shown that serum concentrations of TNF- $\alpha$  in the BIO group were higher (small effect size) when compared to PL group. Increased cytokine production in tissues causes pain, providing a crosstalk mechanism between the immune system and the brain. However, TNF- $\alpha$  can contribute to host defense or greater recovery by limiting the spread of pathogenic organisms into the circulation, promoting coagulation to localize the invader, and stimulating the growth of damaged tissues (TRACEY; VLASSARA; CERAMI, 1989). Thus, it is probable that in the current study the TNF- $\alpha$  increase may have been a contributing factor to the improvement in performance outcomes.

Furthermore, no differences were observed on oxidative stress variables. Our results are in agreement with previous studies that demonstrated that FIR sauna and clothes applied during three consecutive days did not improve the indirect markers of EIDM in highly-trained endurance runners (HAUSSWIRTH et al., 2011) and in elite soccer players (LOTURCO et al., 2016). One of the main effects of FIR is to improve local microcirculation, increasing leukocyte migration into tissues (PARK et al., 2013) through phosphorylation of e-NOS inducing nitric oxide (NO) release, resulting in vasodilatation and a rise in tissue temperature, facilitating neutrophil migration into the muscle (KANDA et al., 2013). However, the understanding of the behavior of these responses in athletes is undefined.

Additionally, it is still not clear whether inflammation leads to oxidative stress or vice versa. TNF- $\alpha$  and interleukin 1 beta (IL-1 $\beta$ ) activate neutrophils and macrophages and increase their accumulation in the inflammatory issue, where they produce more inflammatory cytokines and reactive oxygen species (ROS) (VERRI et al., 2010). Cytokines such as TNF- $\alpha$  and IL-1 $\beta$  can induce the production of hydrogen peroxide (H2O2) and superoxide, while H2O2 and superoxide and indirectly upregulates cytokine production (BOWIE; O'NEILL, 2000). Finally, pro-

inflammatory cytokines and superoxide anion may directly activate nociceptive neurons to induce pain and also contribute to increase tissue damage (SALVEMINI et al., 2011). For example, Lin et al. (2013) demonstrated that FIR radiation was associated with a significantly antioxidative effect in scavenging superoxide anions human blood (LIN; LEE; LUNG, 2013). Furthermore, Leung et al. (2009) treated Michigan cancer foundation-10A cells (MCF-10A) with hydrogen peroxide before incubating for a further 24 h beneath cFIR or control powder. Cells were also treated with ionizing radiation from a fluoroscopic X-ray source to induce cell damage, and after culture for a further 48h, beneath cFIR or control powder.

Another interesting finding of the present study is the demonstration, for the first time, that cFIR during preseason training resulted in a qualitative smaller decrease in IL-10 levels when compared with the PL group (but, with small effect size). IL-10 was the first antihyperalgesic cytokine to be described and exerts its action by inhibiting the production of hyperalgesic cytokines such as IL-1β, IL-6, IL-8 and TNFα (BORGHI et al., 2015). Evidence shows that IL-10 can suppress superoxide anion production, accompanied by downregulation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) subunit expression (KUGA et al., 1996). Borghi et al. (2015) showed the crucial role of endogenous IL-10 in controlling intense acute swimming-induced muscle mechanical hyperalgesia in mice. IL-10 production occurs as an early event of intense acute swimming, suggesting its importance in regulating the first events of muscle mechanical hyperalgesia (BORGHI et al., 2015). Reduction of proinflammatory cytokines and serum markers of oxidative induced by cFIR were not observed in this study.

Although no studies have evaluated the effect of cFIR for two weeks in athletes, it has been previously demonstrated that acute exposure to cFIR (i.e. 40 min) effectively reduced oxidative stress markers as well as inflammatory cytokine levels. However, despite the present data reporting positive results obtained with reduction of pain and TL (training strain), the physiological mechanisms are uncertain.

The lower TS found in BIO compared to PL group can be associated with cardiac autonomic adaptation. On this account, some researches have reported that the use of cFIR can be a strategy to activate parasympathetic responses during resting, and improve cardiac autonomic modulation after exercise (LEUNG et al., 2013). Grounded on pre-clinical data, bioceramic materials may be beneficial for normalizing the psychologically induced stress athletes may go through during training, which leads to elevated heart rate and increased oxidative stress (LEUNG et al., 2012b). It has been demonstrated (LEUNG et al., 2013) that the use of cFIR showed a tendency to activate parasympathetic responses in resting or after exercise; reduce resting metabolic rate; and even to decrease skin temperature or tiredness during sustained exercise in twenty-six men and five women. However, we cannot confirm these findings in our study.

Though the results are not conclusive about skeletal muscle performance and biochemical markers, the use of cFIR may have contributed to reduced perceptual markers in elite futsal players, especially during the early phases of intensive training period (i.e. week one of training program). Our study was limited by the sole use of visual analogue scales to quantify DOMS and TS, the short-term chronic period of the study, reduced number of subjects and the lack of dietary controls during the study. Additionally, possible effects derived from the use of cFIR on the other aspects of recovery (e.g. cardiac autonomic modulations, sleep-hygiene, metabolic and hormonal responses), including chronic period (e.g. competitive and training season) should also be investigated in future studies.

## Practical applications

To our knowledge, this is the first study to analyze the effects of cFIR during a training period in elite athletes. Further research is needed to test advantages and disadvantages of cFIR during recovery period after exercise, targeting the biological mechanisms associated with muscle repair. Despite scarce and conflicting research regarding cFIR in exercise models, the present findings suggest that use of the cFIR clothes for ~8 hours on a daily basis during heavy training periods, could facilitate recovery, mainly upon perceptual markers.

## Conflict of interest

The authors have no conflict of interest to declare.

## References

The references of the paper are at "references section" page 98.

## 4 CHAPTER FOUR

# 4.1 STUDY THREE: RECOVERY FOLLOWING RUGBY UNION MATCHES: EFFECTS OF COLD-WATER IMMERSION ON MARKERS OF FATIGUE AND DAMAGE.

This third paper was accepted and published online in its first version on the Applied Physiology Nutrition and Metabolism journal.

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## Original research

## Title

Recovery following Rugby Union matches: effects of cold-water immersion on markers of fatigue and damage.

## Running title

Cold water immersion on recovery of rugby players

*Key words* Team sports, performance analysis, regeneration strategies, neuromuscular, inflammation, muscle damage.

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# 4.1.1 Abstract

We investigated the effect of post-match cold-water immersion (CWI) on markers of muscle damage, neuromuscular fatigue and perceptual responses within 72 h after a Rugby match. Twenty-two professional male Rugby players were randomized into CWI (10°C/10min; n=11) or Control (CON:30min seated; n=11) groups. Activity profile from Global Positioning Satellite systems and post-match rating of perceived exertion were measured to determined match load. Biochemical (tumor necrosis factor alpha [TNF- $\alpha$ ], interleukin-6 [IL-6]), neuromuscular performance (squat and countermovement jump [SJ; CMJ], peak power output [PPO], rate of force development [RFD], stiffness, 10- and 30-m sprint time and perceptual markers (soreness, perceived recovery) were obtained at pre, post, 30 min, 24, 48 and 72 h post-match. Magnitude-based inference and Cohen's effect size (ES) were used to analyze change over time and between-groups. Changes were unclear for the match loads, sprint times and perceptual markers between-groups. Higher % $\Delta$ SJ at 24 h (very-likely [ES=0.75]) and in %∆PPO SJ at 48 h (likely [ES=0.51]) were observed in CWI than in CON. Values in % ARDF CMJ were higher at post (likely [ES=0.83]), 30 min (very-likely [ES=0.97]) and 24 h (likely [ES=0.93]) in CWI than in CON. Furthermore,  $\%\Delta LogTNF-\alpha$  were lower in CWI than in CON group at post (almost-certainly [ES:-0.76]), 24 h (very-likely [ES:-1.09]) and 72 h (likely [ES:-0.51]) and in Astiffness SJ at 30 min (likely [ES=-0.67]) and 48 h (very-likely [ES=-0.97]), as well as, different withingroups effects throughout post-match were reported. Implementing postmatch CWI-based strategies improved the recovery of markers of inflammation and fatigue in Rugby players, despite no change in markers of speed or perceptual recovery.

*Key words* team sports, performance analysis, regeneration strategies, neuromuscular, inflammation, muscle damage.

# 4.1.2 Introduction

Rugby is an intermittent, high-intensity, contact sport encompassing high-speed runs, sprints, acceleration/decelerations and collision-based activities such as tackling, scrums, rucks and mauls (JOHNSTON; GABBETT; JENKINS, 2014; JONES et al., 2015). This combination of physical load induces post-match fatigue, soreness and muscle damage (JOHNSTON: GABBETT: JENKINS. 2014: TAVARES; SMITH; DRILLER, 2017). Further, the timeline of postmatch suppression of performance and existence of damage, perceptual fatigue, and delayed-onset muscle soreness (DOMS) can occur for up to 48-h (POINTON; DUFFIELD, 2012; WEBB et al., 2013) and even ~4-5 days in Rugby players (JOHNSTON; GABBETT; JENKINS, 2014; MCLELLAN; LOVELL, 2013). Thus, the use of recovery strategies, particularly interventions such as cold-water immersion (CWI), are popular in an attempt to improve the recovery timeline and preparedness for ensuing training/competition.

One of the most common recovery strategies in professional sport is CWI, often used to reduce the symptoms of fatigue and allow faster recovery (POINTON; DUFFIELD, 2012; TAVARES; SMITH; DRILLER, 2017; WEBB et al., 2013). The physiological responses to CWI include decreased skin, tissue, core and muscle temperatures, leading to vasoconstriction and a theoretical reduction of swelling, edema and acute inflammation from muscle damage (COCHRANE, 2004; WILCOCK; CRONIN; HING, 2006). Furthermore, the use of CWI can also contribute to a reduction in nerve conduction properties and decrease in muscle spasm and pain (COCHRANE, 2004; WILCOCK; CRONIN; HING, 2006). However, despite widespread acceptance in sport, equivocal findings remain, with studies demonstrating beneficial effects of cold therapies on performance, damage and perceptual markers (GARCIA; DA MOTA; MAROCOLO, 2016; GILL; BEAVEN; COOK, 2006; POINTON; DUFFIELD, 2012); whilst others have failed to find benefits of CWI on recovery in Rugby players (HIGGINS; CLIMSTEIN; CAMERON, 2013; LINDSAY et al., 2015). In part, these differing outcomes have been related to the contrasting cooling methodological approaches used in the literature. In addition, the lack of sport-specific tests and sensitive markers of exercise-induced muscle damage (EIMD) and fatigue relevant to the sport limit some of the previous findings.

In this regard, Tavares et al. (2017) in a recent literature review study in Rugby players concluded that acutely (<48 h post-match), cold strategies have a beneficial effect on markers of muscle damage (i.e.,

creatine kinase [CK]), neuromuscular function (i.e., jump height and maximal voluntary contraction [MVC]) and DOMS. However, although different markers of EIMD and fatigue have been previously assessed after CWI (HIGGINS; CLIMSTEIN; CAMERON, 2013; LINDSAY et al., 2015; POINTON; DUFFIELD, 2012; TAVARES; SMITH; DRILLER, 2017; WEBB et al., 2013), to the best of our knowledge, no previous studies have examined the changes specifically in inflammatory (i.e., interleukin 6 [IL-6], tumor necrosis factor alpha [TNF- $\alpha$ ]) and neuromuscular function measured from the vertical ground reaction force (GRF) (ACHE-DIAS et al., 2016) (i.e., rate of force development [RFD], peak power output [PPO] and stiffness) in Rugby Union players. Consequently, more specific markers can provide additional information about neuromuscular responses following sports-specific fatigue to further understand the effect of CWI on recovery (KENNEDY; DRAKE, 2017; MAFFIULETTI et al., 2016; PEÑAILILLO et al., 2015; TILLIN: PAIN; FOLLAND, 2013).

Rugby matches are played with rest intervals of 6-8 days, although teams frequently train within this period and prior to subsequent matches. Therefore, recovery is a priority to allow optimal performance in the next training session or match. Given this 96 h timeline of recovery (JOHNSTON; GABBETT; JENKINS, 2014; MCLELLAN; LOVELL, 2013), methods to improve recovery are critical. Thus, the aim of this study was to determine the effect of post-match CWI on markers of inflammation, neuromuscular fatigue, perceptual soreness and recovery within 72 h after a Rugby match. We hypothesized that the CWI after Rugby match will result in reduction of the inflammatory markers, neuromuscular fatigue and perceptual responses after 72 h, due to the CWI's purported anti-inflammatory and/or analgesic properties.

# 4.1.3 Materials and Methods

#### **Participants**

Twenty-two male Rugby Union players  $(25.2 \pm 3.6 \text{ y}; 96.8 \pm 16.8 \text{ kg}; 182.2 \pm 6.3 \text{ cm})$ , consisting of 11 Backs  $(24.1 \pm 1.6 \text{ y}; 96.1 \pm 17.2 \text{ kg}; 181.5 \pm 6.8 \text{ cm})$  and 11 Forwards  $(25.6 \pm 2.6 \text{ y}; 97.7 \pm 17.1 \text{ kg}; 182.0 \pm 7.09 \text{ cm})$  from a Brazilian professional team volunteered to participate in the study. At the time, athletes were engaged in a training program of 1 session per day for 5 days/week and 1 match/week. Inclusion criteria included performing all testing measures, be free from chronic diseases, not to be taking any medication, nutritional supplements with intracellular buffers, illegal substances or undertaking recovery

techniques during the duration of the study. All participants were briefed about the experimental design and signed an informed consent form. The study was approved by a local Institutional Ethics Committee and followed the ethical guidelines of the Declaration of Helsinki.

# Experimental design

One week prior to the study, players were familiarized with the physical tests performed, rating scales and procedures and undertook anthropometric measurements. Briefly, in this balance crossover study, the players were separated by playing position (Backs and Forwards) and randomized into CWI (n=11), or Control (CON n=11) groups by random permute block (available at http://www.randomization.com). However, three athletes were excluded from the CON group due to injury during games, thus the CON group included eight participants. The other players and opposition team were of the same competitive level and participated solely in the friendly match without taking part in any other experimental test. The athletes were separated and randomized in two matches due to the limited number availability of the activity profile by Global Positioning System (GPS) units. During the experimental approach, athletes refrained from any exercise 24 h before and within 72 h after the match. The consumption of water during the match and post-match recovery period was *ad libitum*; however, players abstained from energy or sports drinks during this period.

Biochemical markers (inflammatory), physical performance (jumps and sprints) and perceptual markers (DOMS and Perceived Recovery Scale [PRS]) were obtained at pre, immediately post (not PRS), as well as 30 min (excluding biochemical markers) 24, 48 and 72 h after a one-off friendly Rugby match. Internal and external loads were measured using the rating of perceived exertion (RPE) and activity profile by GPS during the game. Environmental temperatures during the matches were similar (i.e.,  $22.5 \pm 0.8^{\circ}$ C,  $55.7 \pm 1.5^{\circ}$  humidity). On the match day, the players arrived at the University's facilities at 12 p.m. A blood sample was obtained after the participants had been resting for approximately 15 min. Subsequently, DOMS, PRS scale, jumps (squat jump [SJ] and countermovement jump [CMJ]) and sprint abilities (10 m and 30 m) were assessed before the beginning of the match (~3 h p.m), as reported in Figure 10.



#### Figure 10.

Experimental protocol.

IL-6= interleukin 6; TNF- $\alpha$ = tumor necrosis factor alpha; CMJ= countermovement jump; SJ= squat jump; PPO= peak power output; RFD= rate of force development; DOMS= delayed-onset muscle soreness; PRS= perceived recovery status; RPE= rating of perceived exertion.

#### Match performance

Game movement patterns were obtained from GPS devices sampling at 5 Hz (SPI Elite, GPSports Systems, Australia). Devices were fitted to the upper back of each player using an adjustable neoprene harness. Velocity ranges were selected based on a previous study (MCLELLAN; LOVELL, 2013). Match activities were divided into the following categories: total distance covered (m), walking (0-6.0 km.h<sup>-1</sup>), jogging (6.1-12.0 km.h<sup>-1</sup>), cruising (12.1-14.0 km.h<sup>-1</sup>), striding (14.1-18.0 km.h<sup>-1</sup>), high-intensity running (18.1- 20.0 km.h<sup>-1</sup>) and sprinting (>20.1 km.h<sup>-1</sup>). Furthermore, approximately 15-30 min after the end of the match, players were required to report the intensity using the session rating of perceived exertion (s-RPE method) (FOSTER et al., 2001).

#### **Recovery strategies**

After the post-match measures, players in the CWI group submerged their lower limbs up to the iliac crest in a stirred ~10°C coldwater bath for 10 min (LINDSAY et al., 2015), following which they undertook testing at 30 min post. The CON group remained seated for 30 min post-match in a controlled environment (22-23°C, 50–60% humidity) and undertook no other external recovery method before undertaking testing.

### **Biochemical markers**

Blood samples were collected from a superficial forearm vein using standard venipuncture techniques. All samples were allowed to clot at room temperature for 30 min collected directly into serum separator collection tubes (Greiner Bio-one; Frickenhausen, Germany) and serum separated by centrifugation at  $3.000 \times g 4^{\circ}C$  for 15 min. The resulting serum was aliquotted into 3 x 0.5 ml Eppendorf tubes and frozen at -80 °C until the time of assaying (BRÜGGEMANN et al., 2017).

Circulating concentrations for total serum of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-a) were determined using commercially available DuoSet Sandwich Enzyme-Linked Immunosorbent Assav (ELISA) kits (DuoSet, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The concentrations of cytokines were estimated by interpolation from a standard curve by colorimetric measurements in an ELISA plate reader (Perlong DNM-9602, Nanjing Perlove Medical Equipment Co, Nanjing, China). All results were expressed in pg·ml<sup>-1</sup>. Interassay coefficients of variance (CV) ranged from 4.8% and 5.7%. respectively (BRÜGGEMANN et al., 2017).

#### Neuromuscular fatigue assessments

Neuromuscular fatigue was assessed by (1) vertical jump variables, and (2) 10 m and 30 m linear speed. Vertical jumps were performed on a piezoelectric force platform (Kistler<sup>®</sup> Quattro Jump 9290AD, Winterthur, Switzerland), with a sampling frequency of 500 Hz. Each participant performed three jumps of SJ and CMJ, with a randomized order and a rest interval of 30s between jumps. Jump height, PPO, RFD (30 and 50 N·m·s<sup>-1</sup>) were calculated from GRF of the highest jump, according to Ache-Dias et al. (2016) and vertical stiffness from Morin et al. (2005) equations. In the CMJ protocol, subjects started from a static standing position and were instructed to perform a descent phase, free knee flexion followed by a rapid and vigorous extension of the lower limb joints (ascent phase). Subjects were instructed to jump as high as possible with trunk as vertically as possible and hands remaining on hips. SJ adopted the same instruction, despite of the difference that during the beginning movement all subjects started with knee flexed (90°).

Five minutes after the jumps, the athletes performed a singlesprint performance test consisted of three maximal sprints of 30 m, with a 90s passive rest interval. Each sprint time was recorded using a three pairs photocell system (CEFISE®, Speed Test 4.0, São Paulo, Brazil), with timing gates placed at the 0 m, 10 m and 30 m marks. All sprinting tests were conducted outside on grass play field.

#### Delayed-onset muscle soreness (DOMS)

Each participant was asked to complete a muscle soreness questionnaire for the lower and upper limbs in which they were required to rank their perception of soreness on a scale from 0 ("absence of soreness") to 10 ("very intense soreness"). Subjects performed a standardized half squat for lower-body DOMS and a rotation of the trunk for both sides to rate the intensity of soreness in general (THOMPSON D, NICHOLAS CW, 1999; VAILE; GILL; BLAZEVICH, 2007).

#### Perceived recovery status scale (PRS)

The subjects were asked to draw a vertical line that intersected the horizontal descriptor by a visual analogue scale at the appropriate position that best described their perceived level of recovery from 0 ("very poorly recovered") and 10 ("high-perceived recovery") (LAURENT et al., 2011).

#### Statistical analysis

Data were analyzed for practical significance using magnitudebased inferences. The smallest worthwhile change (i.e., 0.2 x betweensubjects standard deviation SD) and 90% confidence intervals (CI) were determined for between-trials comparisons (HOPKINS et al., 2009). The quantitative chances of higher/beneficial, similar/trivial or lower/harmful differences were evaluated qualitatively as follows: <1%, almost certainly not; 1% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possible; 75% to 95%, likely; 95% to 99%, very likely; >99%, almost certainly. True difference was assessed as unclear when the chances of having positive and negative results were both >5%. Threshold values for Cohen's effect size (ES) statistics were >0.2 (small), >0.5 (moderate), and >0.8 (large). However, just differences classified as equal/higher *likely* and *moderate* effect size were considered for describing changes between groups. Delta changes were used for analysis to reduce bias arising from uniformity errors. Values are reported as mean values ± standard deviation (SD).

# 4.1.4 Results

Internal and external load during the match showed no clear and substantial difference for the all variables between groups (Table 6).

	CWI	CON	ES	CI (90%)	% of chance
	40761 1400 5	2702.1 1700.2	0.04		(rating)
TD (m)	$42/6.1 \pm 1428.5$	$3/83.1 \pm 1/99.3$	0.24	(-0.48  to  0.96)	54/31/15
					Unclear
Time match (%)	$83.8 \pm 19.1$	$80.6 \pm 20.0$	0.14	(-0.63 to 0.90)	44/33/22
					Unclear
RPE (a.u)	$557.4 \pm 156.0$	$601.0 \pm 235.3$	-0.16	(-0.90 to 0.58)	20/35/46
					Unclear
0-6 km.h <sup>-1</sup> (m)	$1886.6 \pm 703.8$	$1872.7 \pm 777.9$	-0.02	(-0.88 to 0.84)	33/31/36
					Unclear
6-12 km.h <sup>-1</sup> (m)	$1369.9 \pm 346.8$	$1130.9 \pm 639.6$	-0.62	(-1.92 to 0.68)	14/14/72
				· · · · · ·	Unclear
12-14 km.h <sup>-1</sup> (m)	$343.0 \pm 121.7$	$276.0 \pm 164.7$	-0.50	(-1.50 to 0.50)	12/18/70
				(	Unclear
14-18 km.h <sup>-1</sup> (m)	$361.7 \pm 187.3$	$321.3 \pm 205.5$	-0.19	(-1.05 to 0.66)	21/29/50
			0117	(1100 10 0100)	Unclear
$18-20 \text{ km h}^{-1}$ (m)	$124.8 \pm 105.4$	101 6 + 69 1	-0.20	(-0.86  to  0.47)	15/35/50
10 20 km.n (m)	$124.0 \pm 100.4$	101.0 ± 07.1	0.20	( 0.00 to 0.47)	Unclear
>20 km h <sup>-1</sup> (m)	$200.7 \pm 200.9$	$180.0 \pm 52.4$	-0.09	(-0.71  to  0.52)	20/42/38
>20 KIII.II (III)	$200.7 \pm 200.9$	$100.0 \pm 32.4$	-0.09	$(-0.71 \ 10 \ 0.52)$	20/42/38 Unalaar
NIQ Constants	$10.29 \pm 0.9$	04.20	0.19	(0.92 + 0.46)	15/27/49
N° Sprints	$10.38 \pm 9.8$	$8.4 \pm 3.8$	-0.18	(-0.82  to  0.46)	15/3//48
					Unclear

Table 6. Internal and external load between CWI and CON group during a Rugby Union matches.

Note – TD: total distance; RPE: rating of perceived exertion; a.u: arbitrary units; m: meter; N°: number; k.mh<sup>1</sup>: kilometer per hour; CI: confidence interval; ES: effect size; CWI: cold-water immersion; CON: control.

Within-group Comparisons

Quantitative chances for inflammation, neuromuscular fatigue and perceptual markers within-groups throughout 72 h post-match are showed in table 7 and 8. IL-6 was increased at post and 24 h compared to pre in CWI, and CON. TNF- $\alpha$  decreased at 24 h in CWI; and increased at post and 72 h above pre in the CON group.

CMJ was reduced at post, 30 min, 24, 48 and 72 h compared to pre in CWI, and CON. A decrease in SJ occurred at post, 30 min, 24, 48 and 72 h compared with pre in CWI, and CON.

PPO\_CMJ decreased at post and 30 min compared with pre in CWI, but it was unclear for CON, whilst PPO\_SJ was reduced at 24 h compared to pre in CWI, and in addition at 48 and 72 h in CON.

RDF\_30 CMJ decreased at 30 min and 48 h than pre in CWI, and in addition to post and 24 h in CON. Compared with pre, RDF\_30 SJ decreased at 30 min, 24, 48 and 72 h in CWI, and CON. A decrease in RDF\_50 CMJ occurred at 30 min, 24 and 48 h compared to pre in CWI, and in addition at post in CON, while RDF\_50 SJ was reduced in 30 min, 24, 48 and 72 h compared to pre in CWI, and CON.

Stiffness in CMJ decreased at post, but increased at 48 h compared to pre in CWI, but unclear for CON. A decrease in stiffness in SJ occurred at post, 30 min, 24, 48 and 72 h than pre in CWI, and CON at 72 h.

10 m sprints decreased at post, 30 min, 24, 48 and 72 h than pre in the CWI, and CON. Compared with pre, 30 m sprints decreased at post, 30 min, 24, 48 and 72 h in CWI, and CON, but unclear at 72 h.

Quadriceps DOMS increased at post, 30 min, 24 and 48 h than pre in CWI, and CON, except at 48 h. An increase for hamstrings DOMS occurred at post, 24 and 48 h compared to pre in CWI, and CON, except at 48 h. Trunk DOMS increased at post, 30 min and 24 h than pre in CWI, and CON, but unclear at 24 h. PRS decreased at post, 30 min, 24 and 48 h compared to pre in CWI, and CON.

Table 7. Effect size and quantitative chances for inflammation, neuromuscular fatigue and perceptual markers within-CWI group throughout 72 h post-match.

	Pre x Post Pre x 30 min			Pre x 24 h		Pre x 48 h		Pre x 72 h		
	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance
Log IL-6 (pg·ml <sup>-1</sup> )	6.72	100/0/0 Almost certainly			0.38	79/20/1 Likely	0.22	53/42/6 Unclear	0.21	52/42/6 Unclear
$Log TNF-\alpha (pg ml^{-1})$	-0.07	1/89/10 Trivial			0.21	0/49/51 Possible	0.10	21/77/11 Trivial	0.09	6/89/4 Trivial
CMJ (cm)	-0.38	1/13/87 Likely	-0.52	0/2/98 Very likely	-0.28	0/23/77 Likely	-0.20	0/55/45 Possible	-0.20	0/58/42 Possible
SJ (cm)	-0.47	0/6/94 Likely	-0.58	0/1/99 Very likely	-0.20	4/47/48 Possible	-0.55	0/3/97 Very likely	-0.33	0/19/81 Likely
PPO CMJ (w/Kg)	-0.20	2/54/44 Possible	-0.21	0/54/46 Possible	-0.22	0/43/57 Unclear	-0.13	0/76/24 Trivial	-0.12	3/69/29 Unclear
PPO SJ (w/Kg)	0.04	29/49/22 Unclear	0.00	15/70/15 Unclear	-0.20	0/61/39 Possible	-0.11	1/79/20 Trivial	-0.03	5/84/11 Unclear
$RDF\_30\ CMJ\ (N{\cdot}m{\cdot}s^{-1})$	-0.33	10/27/63 Unclear	-0.50	0/10/90 Likely	0.11	41/37/23 Unclear	-0.33	0/19/81 Likely	-0.17	7/49/44 Unclear
RDF_30 SJ (N·m·s <sup>-1</sup> )	-0.08	22/41/37 Unclear	-0.98	0/1/99 Very likely	-0.63	0/3/97 Very likely	-0.50	0/2/98 Very likely	-0.47	0/6/94 Likely
RDF_50 CMJ (N·m·s <sup>-1</sup> )	-0.17	20/33/47 Unclear	-0.42	0/14/86 Likely	-0.25	2/39/59 Possible	-0.36	1/20/79 Likely	0.22	56/42/5 Unclear
RDF_50 SJ ( $N \cdot m \cdot s^{-1}$ )	0.10	17/82/1 Trivial	-0.20	5/53/42 Possible	-0.49	0/8/92 Likely	-0.50	0/3/97 Very likely	-0.34	0/15/85 Likely
Stiffness CMJ (kN/m)	-0.21	5/44/51 Possible	-0.23	11/35/54 Unclear	0.17	47/36/17 Unclear	0.26	60/37/3 Possible	0.20	49/39/11 Unclear
Stiffness SJ (kN/m)	-2.22	2/2/96 Very likely	-2.21	3/1/96 Very likely	-2.22	4/3/93 Likely	-5.67	1/0/98 Very likely	-3.63	2/1/97 Very likely
Vel 10 m (m/s <sup>-1</sup> )	-0.38	0/12/88 Likely	-0.56	0/2/98 Very likely	-0.39	0/17/82 Likely	-0.42	0/10/90 Likely	-0.20	0/58/42 Possible
Vel 30 m (m/s <sup>-1</sup> )	-0.61	0/3/97 Very likely	-0.75	0/1/99 Very likely	-0.54	0/2/98 Very likely	-0.25	0/25/75 Likely	-0.22	0/39/61 Possible
DOMS Qua (a.u)	1.42	98/2/0 Very likely	1.04	95/1/1 Very likely	0.94	96/3/1 Very likely	0.28	62/34/9 Possible	0.12	41/41/18 Unclear
DOMS Ham (a.u)	0.82	88/9/3 Likely	0.30	68/20/12 Unclear	0.79	89/8/2 Likely	0.41	78/20/2 Likely	-012	28/28/44 Unclear
DOMS Trunk (a.u)	1.83	98/1/1 Very likely	1.37	98/2/0 Very likely	0.87	91/7/2 Likely	0.00	23/54/23 Unclear	0.25	54/29/17 Unclear
PRS scale (a.u)	-7.22	0/0/100 Almost certainly	-6.09	0/0/100 Almost certainly	-3.32	0/0/100 Almost certainly	-0.50	0/2/98 Very likely	0.00	22/57/22 Unclear

Note - Log IL-6: log transformation interleukin 6; Log TNF- $\alpha$ : log transformation tumor necrosis factor alpha; CMJ: countermovement jump; SJ: squat jump; PPO: peak power output; RFD: rate of force development in 30 and 50 m/s<sup>-1</sup>; Vel: velocity 10 and 30 meters; DOMS: delayed-onset muscle soreness; Qua: quadriceps; Ham: hamstrings; PRS: perceived recovery status; ES: effect size; a.u: arbitrary units; CWI: cold water immersion; CON: control.

Table 8. Effect size and quantitative chances for inflammation, neuromuscular fatigue and perceptual markers within-CON group throughout 72 h post-match.

	Pre x Post		Pre x 30 min		Pre x 24 h			Pre x 48 h	Pre x 72 h		
	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance	
Log IL-6 (pg·ml <sup>-1</sup> )	1.04	98/2/0 Very likely			0.20	57/42/1 Possible	-0.07	2/92/8 Trivial	-0.04	3/88/9 Trivial	
Log TNF-α (pg·ml <sup>-1</sup> )	4.59	98/1/1 Very likely			0.48	63/7/30 Unclear	0.16	48/17/35 Unclear	3.19	97/1/2 Very likely	
CMJ (cm)	-0.24	0/35/65 Possible	-0.21	0/52/48 Possible	-0.20	0/59/41 Possible	-0.25	0/26/74 Possible	-0.34	0/18/82 Likely	
SJ (cm)	-0.25	0/34/66 Possible	-0.20	0/55/45 Possible	-0.38	0/11/89 Likely	-0.37	0/13/87 Likely	-0.22	0/40/60 Possible	
PPO CMJ (w/Kg)	0.08	24/69/7 Unclear	-0.15	1/65/34 Unclear	-0.07	2/86/12 Trivial	-0.14	0/74/25 Unclear	-0.07	1/88/11 Trivial	
PPO SJ (w/Kg)	0.06	28/57/14 Unclear	-0.21	0/46/54 Unclear	-0.20	0/55/45 Possible	-0.20	0/52/48 Possible	-0.21	0/59/41 Possible	
RDF_30 CMJ (N·m·s <sup>-1</sup> )	-0.42	1/16/83 Likely	-0.59	0/3/97 Very likely	-0.39	0/12/88 Likely	-0.56	0/6/94 Likely	-0.20	19/31/50 Unclear	
RDF_30 SJ (N·m·s <sup>-1</sup> )	-0.16	16/38/46 Unclear	-0.27	1/34/65 Possible	-0.33	0/23/77 Likely	-0.30	0/21/76 Likely	-0.32	0/17/83 Likely	
RDF_50 CMJ (N·m·s <sup>-1</sup> )	-0.20	0/49/51 Possible	-0.41	0/8/92 Likely	-0.23	0/39/61 Possible	-0.20	1/49/50 Possible	0.14	38/57/5 Unclear	
RDF_50 SJ ( $N \cdot m \cdot s^{-1}$ )	0.12	15/82/3 Trivial	-0.33	0/13/78 Likely	-0.41	0/13/87 Likely	-0.41	0/11/87 Likely	-0.48	0/8/92 Likely	
Stiffness CMJ (kN/m)	0.25	54/26/20 Unclear	-0.09	29/30/41 Unclear	0.30	60/28/12 Unclear	0.20	50/38/12 Unclear	-0.18	47/42/11 Unclear	
Stiffness SJ (kN/m)	0.30	57/25/18 Unclear	-0.07	30/29/41 Unclear	0.19	49/23/28 Unclear	0.04	38/31/31 Unclear	-3.23	4/10/88 Likely	
Vel 10 m (m/s <sup>-1</sup> )	-0.27	0/36/61 Possible	-0.97	0/1/99 Very likely	-0.98	0/2/98 Very likely	-0.77	0/2/98 Very likely	-0.29	3/33/64 Possible	
Vel 30 m (m/s <sup>-1</sup> )	-0.29	0/2/73 Possible	-0.75	0/1/99 Very likely	-0.25	0/38/61 Possible	-0.22	0/43/57 Possible	-0.16	3/56/40 Unclear	
DOMS Qua (a.u)	0.50	93/6/1 Likely	1.02	88/10/1 Likely	0.89	92/6/2 Likely	0.20	51/45/4 Unclear	0.50	67/17/16 Unclear	
DOMS Ham (a.u)	0.32	69/29/2 Possible	0.67	82/12/5 Unclear	0.89	87/11/2 Likely	0.45	66/19/14 Unclear	0.00	27/46/27 Unclear	
DOMS Trunk (a.u)	1.58	94/3/3 Likely	1.18	94/4/2 Likely	0.66	73/14/14 Unclear	-0.15	26/28/46 Unclear	0.00	37/26/37 Unclear	
PRS scale (a.u)	-5.5	0/0/100 Almost certainly	-4.35	1/0/99 Very likely	-3.49	3/1/96 Very likely	-2.37	1/1/98 Very likely	0.02	24/56/20 Unclear	

Note - Log IL-6: log transformation interleukin 6; Log TNF- $\alpha$ : log transformation tumor necrosis factor alpha; CMJ: countermovement jump; SJ: squat jump; PPO: peak power output; RFD: rate of force development in 30 and 50 m/s<sup>-1</sup>; Vel: velocity 10 and 30 meters; DOMS: delayed-onset muscle soreness; Qua: quadriceps; Ham: hamstrings; PRS: perceived recovery status; ES: effect size; a.u: arbitrary units; CWI: cold water immersion; CON: control.

#### Between-group Comparisons

Changes in  $\%\Delta$ TNF- $\alpha$  for CWI were *almost certainly* lower than in CON group in post ([0/0/100]; ES: -0.76), very likely at 24 h ([1/3/96]; ES:-1.09), and *likely* at 72 h ([0/7/93]; ES:-0.51) (Fig 11B), while no clear and substantial difference between groups in  $\%\Delta$ IL-6 was observed (Fig 11A).



Figure 11.

Percentage delta change between interventions throughout the 72-h recovery period for (A) interleukin 6 and (B) tumor necrosis factor alpha. <sup>a</sup> Almost Certain moderate effect compared with control group. <sup>b</sup> Very Likely large effect compared with control group. <sup>c</sup> Likely moderate effect compared with control group. ^ Almost Certain large effect compared with pre. <sup>++</sup> Very likely large effect compared with pre. <sup>+</sup> Likely small effect compared with pre. <sup>\*</sup> Possible small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

Effects in % $\Delta$ SJ were *very likely* higher in CWI at 24 h ([95/4/1]; ES=0.75) and *likely* higher in % $\Delta$ PPO\_SJ at 48 h ([86/13/1]; ES= 0.51) than in CON group (Fig 12B and 12D). However, no clear and substantial differences were evident in % $\Delta$ CMJ and % $\Delta$ PPO\_CMJ (Fig 12A and C).



Figure 12.

Percentage delta change between interventions throughout the 72-h recovery period for (A) height countermovement jump, (B) height squat jump, (C) peak power output for countermovement jump, and (D) peak power output for squat jump. <sup>a</sup> *Very likely* large effect compared with control group. <sup>b</sup> *Very likely* moderate effect compared with control group. <sup>#</sup> *Very likely* moderate effect compared with pre. <sup>+</sup>*Likely* small effect compared with pre. <sup>\*</sup>*Possible* small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

Values reported for the CWI in  $\Delta RFD_{30}$  CMJ were *likely* higher than in CON group at post ([92/6/2]; ES=0.83) and 24 h ([93/5/2]; ES=0.93) and *very likely* higher at 30 min ([95/3/2]; ES=0.97) (Fig 13A), while no clear and substantial analysis were reported for other measures (Fig 13B, C and D).



Figure 13.

Percentage delta change between interventions throughout the 72-h recovery period for (A) rate of force development in 30 N·m·s<sup>-1</sup> for countermovement jump, (B) rate of force development in 30 N·m·s<sup>-1</sup> for squat jump, (C) rate of force development in 50 N·m·s<sup>-1</sup> for countermovement jump, and (D) rate of force development in 50 N·m·s<sup>-1</sup> for squat jump. <sup>a</sup> *Very likely* large effect compared with control group. <sup>b</sup> *Very likely* moderate effect compared with control group. <sup>t+</sup> *Very likely* moderate effect compared with pre. <sup>#</sup> *Very likely* moderate effect compared with pre. <sup>\*</sup> *Likely* small effect compared with pre. <sup>\*</sup> *Possible* small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

Changes in  $\Delta$ stiffness in SJ were *likely* lower in CWI than in CON group at 30 min ([4/12/84]; ES=-0.67) and *very likely* at 48 h ([1/4/95]; ES=-0.97) (Fig 14B). However, Magnitude-based inference analysis showed unclear for  $\Delta$ stiffness in CMJ (Fig 14A), % $\Delta$ sprints (10 m and 30 m) (Fig 14C and D), DOMS (Fig 15A, B and C) and PRS (Fig 15D) between groups.



#### Figure 14.

Delta change between interventions throughout the 72-h recovery period for (A) stiffness in countermovement jump, (B) stiffness in squat jump, percentage delta for (C) sprint 10-m, and (D) sprint 30-m. <sup>a</sup> *Very likely* large effect compared with control group. <sup>b</sup> *Very likely* moderate effect compared with control group. <sup>++</sup> *Very likely* large effect compared with pre. <sup>#</sup> *Very likely* moderate effect compared with pre. <sup>\*\*</sup> *Likely* large effect compared with pre. <sup>\*\*</sup> *Likely* moderate effect compared with pre. <sup>\*\*</sup> *Likely* small effect compared with pre. <sup>\*</sup> *Possible* small effect compared with pre. <sup>\*</sup> *Dostible* small effect presents Control (CON).



Figure 15.

Change between interventions throughout the 72-h recovery period for (A) for (A) DOMS quadriceps, (B) DOMS hamstrings, (C) DOMS trunk, (D) and perceived recovery scale. ^ *Almost Certain* large effect compared with pre. <sup>++</sup> *Very likely* large effect compared with pre. <sup>#</sup> *Likely* moderate effect compared with pre. <sup>\*\*</sup> *Likely* large effect compared with pre. <sup>&</sup> *Likely* moderate effect compared with pre. <sup>+</sup> *Likely* small effect compared with pre. <sup>\*</sup> *Possible* small effect compared with pre. <sup>\*</sup> *Double* small effect compared with pre. <sup>\*</sup> *Likely* small effect compared with pre. <sup>\*</sup> *Double* 

# 4.1.5 Discussion

The present study verified the effects of CWI on post-match recovery relating markers of inflammation, neuromuscular fatigue, and perceptual markers in Brazilian Rugby Union players. As expected, significant impairments were evident with increased inflammation, neuromuscular fatigue, soreness and reduced perceived recovery in both groups within 72 h post-match. Furthermore, CWI positively affected inflammation markers (i.e., TNF- $\alpha$ ) and neuromuscular fatigue (i.e., JH, PPO and RFD from CMJ), though no effects were observed in sprint speed or perceptual recovery. Our results were in general agreement with our main hypothesis in which CWI would result in a decrease in inflammatory markers and improved neuromuscular fatigue responses during the 72 h post-match.

To date, the effects of CWI on physiological recovery in Rugby players remain debatable. CWI is suggested to ameliorate EIMD via several mechanisms associated with cooling, hydrostatic pressures and redistribution of blood flow (COCHRANE, 2004; IHSAN; WATSON; ABBISS, 2016; WILCOCK; CRONIN; HING, 2006). Cooling therapy induced vasoconstriction is suggested to aid the maintenance of cellular integrity by decreasing circulatory and lymphatic permeability, facilitating interstitial fluid gain and blunting pro-inflammatory events (WILCOCK: CRONIN: HING, 2006). Although IL-6 concentration peaked 24 h post-match in both groups, no treatment effect was observed. Conversely, a reduced peak in TNF- $\alpha$  was evident 24 h-post following CWI. Previous studies reporting the effects of CWI after EIMD on inflammatory markers are equivocal. For example, Tseng et al. (2013) showed that ice treatment did not influence plasma cytokine concentrations 1 h after eccentric exercise, despite reductions in plasma IL-6 and TNF- $\alpha$  24 h post-exercise. In addition, CWI has been shown to decrease total leukocyte count and attenuate pro-inflammatory cytokines following EIMD (POURNOT et al., 2011). Previous results indicated that CWI induced greater increases in pro- and anti-inflammatory markers after high-volume bouts of resistance exercise (JAJTNER et al., 2015). However, other studies show no difference from using CWI on TNF- $\alpha$  (or TNFR1 expression) (TOWNSEND et al., 2013), IL-6 (GONZALEZ et al., 2014; ROBERTS et al., 2014) in Rugby players (LINDSAY et al., 2015; TAKEDA et al., 2014). Whilst speculative, within the scope of the current literature the decrease evident in TNF- $\alpha$  in this study may suggest a role for inflammatory recovery following CWI (BAUMERT et al., 2016).

Regarding neuromuscular fatigue, CWI showed an improvement in recovery at 48 h post-match in functional tests (SJ) and in more specific and sensitive indicators of muscle function (i.e., PPO and RFD). In addition, despite smaller delta stiffness from SJ were reported in CWI at 30 min and 48 h between groups, unchanged effects were showed within CON across 48 h post-match. Minett et al. (2014) showed enhanced recovery of MVC following CWI was attributable due to the faster return of central activation achieved via acute reduction in core temperature, by maintenance of contractile force, decreased muscle soreness or/and greater blunted blood-based markers after intermittent-sprint exercise. Webb and colleagues (2013) reported greater effects of CWI on jump performance measured height at 1. 18. and 42 h after professional Rugby league game. In this regard, CWI may have accelerated the rate of neuromuscular recovery (i.e., PPO and RDF) to restore force indices (CORMIE: MCBRIDE: MCCAULLEY, 2009: KENNEDY; DRAKE, 2017; MCLELLAN; LOVELL; GASS, 2011). Furthermore, our results are in agreement with Roberts et al. (2014), who demonstrated that CWI enhances the recovery in muscle function as demonstrated by the capability to perform more volitional work in the squat exercise. Thus, this study shows that functional tests that may be more specific indicators of muscle function are improved by post-match CWI. Nevertheless, others studies have failed to find benefits of the CWI using different neuromuscular function (e.g., JH, sprints, reaction time, maximal pedaling power and agility test) (GARCIA; DA MOTA; MAROCOLO, 2016; HIGGINS; CLIMSTEIN; CAMERON, 2013; TAKEDA et al., 2014). Although speed impairments were reported here, no improvement in recovery with CWI was noted, corroborating previous investigations (POINTON; DUFFIELD, 2012; TAKEDA et al., 2014). Thus, strength tests appear to be more sensitive to a contractile-dependent muscular performance than sprints after CWI therapy (BAILEY et al., 2007). Therefore, it is suggested the use of a more specific and greater indirect marker of eccentric exercise-induced muscle damage (KENNEDY; DRAKE, 2017; PEÑAILILLO et al., 2015) (e.g., RDF and PPO), especially from jumps, may present different outcomes as reported

Despite increased DOMS and reduced PRS, no change was reported between groups. The analgesic effects of cooling has been documented (LEEDER et al., 2012; WHITE; WELLS, 2013), potentially reducing ratings of muscle soreness by mitigating acute tissue edema and ensuing inflammatory responses to muscle damage (BAILEY et al., 2007; VAILE; GILL; BLAZEVICH, 2007). However, no effects were found

in the aforementioned studies.

regarding perceptual markers, controversially noted in studies with Rugby players (GARCIA; DA MOTA; MAROCOLO, 2016; HIGGINS; CLIMSTEIN; CAMERON, 2013; POINTON; DUFFIELD, 2012; TAKEDA et al., 2014; WEBB et al., 2013). In fact, a close examination of the literature suggests that the perceived recovery is positively influenced by CWI (POINTON; DUFFIELD, 2012). This is in line with the suggestion that most of the effects of CWI are mediated by the placebo effect (BROATCH; PETERSEN; BISHOP, 2014). Furthermore, reductions in soreness may depend on the type of exercise performed (LEEDER et al., 2012); whereby endurance-type activity responds more favorably to these recovery modalities than heavy stretch-shortening cycle based activity that is often undertaken by Rugby players. Still, whether and/or how improved perceptual ratings of muscle soreness and recovery may influence subsequent neuromuscular performance is yet to be identified.

This disagreement in aforementioned research findings used to assess the effects of CWI likely relates to the different contraction, physical demands, blood-borne markers and/or psychophysiological stress, the short timeline post-exercise recovery (<48 h) and soreness measures (GARCIA; DA MOTA; MAROCOLO, 2016; LINDSAY et al., 2015; POINTON; DUFFIELD, 2012; TAKEDA et al., 2014; WEBB et al., 2013). Nevertheless, while the efficacy of post-exercise cooling in improving biochemical perturbations and neuromuscular functional incurred during exercise is contentious, the greater positive changes in the jump height, PPO and RDF in the CWI post-match in the current study may indicate recovery in Rugby athletes. Finally, although these findings add novel insight into the effects of post-exercise cooling, especially on neuromuscular recovery, it is prudent that some limitations are acknowledged. These include the lack of control over confounding factors as recall foods or diets, sleep, rest and the measurements of collision during the match. Furthermore, the placebo effect was not controlled in this study.

# 4.1.6 Conclusion

In conclusion, a Rugby match caused impaired neuromuscular performance, altered blood-borne markers of inflammation and changed perceptual markers of pain/recovery over 72 h post-match in the athletes. However, implementing a single bout of CWI did accelerate recovery, specifically a marker of inflammation and neuromuscular function measured from vertical GRF. In addition, the results suggesting that it is important to use more sensitive and specific markers compared with those usually reported in other studies (e.g., CK, MVC and jump height) to measure the effects of CWI post-match or training. Actually, some recovery markers in the CWI group were greater than CON, supporting the use of either this therapy to accelerate post-match recovery of Rugby players.

# Conflict of interest statement

The authors express that there are no conflicts of interest to report.

# References

The references of the paper are at "references section" page 98.

# 5 CONCLUSION

According to the specific objective of study 1, it was observed that the use of cFIR emitting clothes for two hours over three days was not able to improve the muscle force production behavior, muscle damage and perceptual markers; these findings do not convincingly support the hypothesis of a causal relationship between cFIR recovery method and improved performance.

As for the main goal of study 2, the present work showed that the use of cFIR during sleep recovery over a short chronic period contributed to reduce perceptual DOMS and training load. However, the findings were not conclusive about biochemical markers and neuromuscular performance; whilst it supports in parts the hypothesis of this study of a positive relationship between the use of bioceramics material and perceptual recovery.

Finally, answering the purpose of study 3, CWI post-match showed positive effects on neuromuscular function measured from vertical GRF and inflammation markers. These results confirm a "causeeffect" relationship between CWI and improved performance, but not regarding perceptual markers.

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## 7 ATTACHMENT

## 7.1 A – Parecer consubstanciado do CEP

## UNIVERSIDADE DO SUL DE SANTA CATARINA - UNISUL

Conordia Congra

## PARECER CONSUBSTANCIADO DO CEP

### DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITOS DA IMPREGNAÇÃO EM CALÇA ESPORTIVA DE UM MATERIAL CERÂMICO EMISSOR DE INFRAVERMELHO NO DANO MUSCULAR EM Pesquisador: DANIEL FERNANDES MARTINS Área Temática: Versão: 2 CAAE: 44851915.0.0000.5369 Instituição Proponente: Universidade do Sul de Santa Catarina - UNISUL Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.069.515 Data da Relatoria: 25/06/2015

## Apresentação do Projeto:

Estudos têm demonstrado que exercício de alta intensidade incluindo atividades excêntricas e exercícios de longa duração induzem a uma resposta inflamatória aguda, nesse sentido, uma adequada recuperação torna-se um aspecto importante de todo programa de treinamento, e apesar dos avanços nas pesquisas com os diferentes métodos para atenuar o processo inflamatório após as respostas agudas e crônicas ao treinamento, grande parte utiliza-se de técnicas com coletas sanguíneas e/ou com submersão em temperaturas que possa levar os indivíduos a uma condição de stress/desprazer. Assim, este estudo avalia uma alternativa uma técnica com material biocerâmico impregnado em modelos de calças que podem ser ativados por meio de resistências elétricas, nos quais emitem sinais infravermelho para fibra muscular com o intuito de atenuar o processo inflamatório.

### Objetivo da Pesquisa:

Avaliar o efeito das calças impregnadas com biocerâmicas na recuperação muscular de indivíduos saudáveis submetidos a um protocolo de dano muscular excêntrico.

Endereço: Avenida Pedra branca,25							
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Telefone:	(48)3279-1036	Fax:	(48)3279-1094	E-mail:	cep.contato@unisul.br		

Página 01 de 03

## UNIVERSIDADE DO SUL DE SANTA CATARINA - UNISUL



Continuação do Parecer: 1.069.515

#### Avaliação dos Riscos e Benefícios:

Os benefícios dos estudos superam os possíveis riscos aos participantes da pesquisa.

### Comentários e Considerações sobre a Pesquisa:

Projeto em conformidade com a Resolução CNS nº 466/12.

## Considerações sobre os Termos de apresentação obrigatória:

Termos em conformidade com a Resolução CNS nº 466/12.

## Recomendações:

## Conclusões ou Pendências e Lista de Inadequações:

Projeto em conformidade com a Resolução CNS nº 466/12.

## Situação do Parecer:

Aprovado

### Necessita Apreciação da CONEP:

Não

## Considerações Finais a critério do CEP:

Protocolo de pesquisa em consonância com a Resolução 466/12 do Conselho Nacional de Saúde.

Cabe ressaltar que compete ao pesquisador responsável: desenvolver o projeto conforme delineado; elaborar e apresentar os relatórios parciais e final; apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento; manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa; encaminhar os resultados da pesquisa para publicação, com os devidos créditos aos pesquisadores associados e ao pessoal técnico integrante do projeto; e justificar fundamentadamente, perante o CEP ou a CONEP, interrupção do projeto ou a não publicação dos resultados.

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Página 02 de 03

# UNIVERSIDADE DO SUL DE SANTA CATARINA - UNISUL



Continuação do Parecer: 1.069.515

PALHOCA, 19 de Maio de 2015

Assinado por: Fernando Hellmann (Coordenador)

## 7.2 B – Parecer consubstanciado do CEP

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

## PARECER CONSUBSTANCIADO DO CEP

### DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DE DIFERENTES TÉCNICAS DE RECUPERAÇÃO APÓS DOIS MODELOS DE DANO MUSCULAR EM ATLETAS DE RUGBY DE ELITE

Pesquisador: Luiz Guilherme Antonacci Guglielmo Área Temática: Versão: 2 CAAE: 74509417.0.0000.0121 Instituição Proponente: Universidade Federal de Santa Catarina Patrocinador Principal: Financiamento Próprio

### DADOS DO PARECER

Número do Parecer: 2.448.708

#### Apresentação do Projeto:

Pesquisa experimental, quantitativa, a qual selecionará 50 atletas de elite de rugby do sexo masculino, randomizados por posição em 5 diferentes grupos: grupo controle (GC), grupo que vestirá calça impregnada com biocerâmica durante o sono ao longo de 72 horas após o dano muscular (GB), grupo biocerâmica placebo (GBPL), grupo que será submetido Imersão de membros inferiores em posição vertical em água fria por 10min a uma temperatura de 10C° pós dano muscular GCr), grupo crioterapia placebo (GCrPL), separados por dois momentos distintos de indução de dano muscular com intervalo de aproximadamente 30 dias. Na primeira fase do projeto os sujeitos irão realizar um protocolo de indução de dano miofibrilar através de um jogo oficial de rugby, e após aproximadamente 4semanas a segunda etapa do projeto será realizada através do protocolo excêntrico de dano muscular no dinamômetro isocinético.Os atletas realizarão um total de 6 visitas ao laboratório, sendo que na primeira visita os participantes realizarão uma avaliação antropométrica (massa corporal, estatura, circunferências e percentual de gordura), seguido de familiarização nos testes de saltos na plataforma de força, de velocidade (30m) e das escalas perceptuais (dor muscular de início tardio [DMIT], escala de recuperação, e percepção subjetiva de esforco (PSE), seguido do teste de Carminatti (T-CAR) para a caracterização da amostra. Na segunda visita os atletas realizarão a coleta dos marcadores sanguíneos (fator de necrose tumoral alfa [TNF-], Fator de crescimento semelhante à insulina tipo-1 [IGF1], interleucina 6 [IL-6], interleucina 10, substâncias reativas ao ácido tiobarbitúrico [TBARS],proteína carbonil, Superoxido

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Página 01 de .04

## UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC



Continuação do Parecer: 2.448.708

dismutase [SOD] e catalase [CAT] desempenho muscular (saltos e sprint). Antes do início da partida os atletas farão uso de um sistema de posicionamento global (GPS) para controle da carga interna do jogo. Imediatamente após a partida os marcadores perceptuais (PSE e DMIT), sanguíneos, circunferências, desempenho muscular serão obtidos seguido da respectiva técnica de recuperação.

Aproximadamente 30 minutos, 24 horas (visita 3), 48 horas (visita 4) e 72 horas (visita 5) pós jogo, os atletas serão submetidos as coletas sanguíneas, circunferências, testes de desempenho e dos marcadores perceptuais. Após aproximadamente 4 semanas a segunda etapa do projeto será realizada.

#### Objetivo da Pesquisa:

Analisar os efeitos da calça impregnada com biocerâmica e crioterapia na recuperação muscular após dois modelos de indução de dano muscular em atletas de Rugby de elite.

## Avaliação dos Riscos e Benefícios:

Riscos:

Para participar deste estudo o atleta deve estar apto a realizar exercícios físicos de alta intensidade. Da mesma forma, o mesmo deve estar ciente da possibilidade de ocorrência de desconforto gerado pelo esforço máximo dos testes e modelos experimentais, ou pelas coletas de sangue venosa,que serão realizadas na veia cubital do braço por um profissional habilitado e capacitado. O desconforto refere-se à picada da agulha, não requerendo nenhum cuidado especial posterior, além de existir a possibilidade de náuseas, vômito e dores musculares em decorrência do esforço intenso realizado nos testes e experimentos. No entanto, menos de 1% da população americana apresenta desconforto durante ente tipo de teste (American College os Sports Medicine). Será assegurada assistência à você tanto de forma imediata quanto integral em caso de danos decorrentes,direta ou indiretamente, pela pesquisa. Benefícios:

Quanto aos benefícios e vantagens em participar deste estudo, os atletas contribuirão para o desenvolvimento da ciência, principalmente sobre esta modalidade em expansão no Brasil, dando possibilidade a novas descobertas e ao avanço das pesquisas. Além disso, todos serão informados sobre sua composição corporal, marcadores sanguíneos e de desempenho muscular, a partir do repasse do relatório individual de sua avaliação.

## Comentários e Considerações sobre a Pesquisa:

O problema de pesquisa está bem justificado, com objetivos claros e método bem definido. Uma

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Página 02 de 04

## UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC



Continuação do Parecer: 2.448.708

vez obtidos os dados conclusivos proporcionará aos pesquisadores meios para contribuir para futuros estudos na área.

## Considerações sobre os Termos de apresentação obrigatória:

Documentos de acordo com as exigências do CEP.

### Recomendações:

Não se aplica.

## Conclusões ou Pendências e Lista de Inadequações:

Todas as pendências e inadequações listadas no parecer anterior foram atendidas, não havendo impedimento ético para que esta pesquisa inicie.

#### Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas	PB_INFORMAÇÕES_BÁSICAS_DO_P	22/11/2017		Aceito
do Projeto	ROJETO 945604.pdf	03:53:53		
Outros	RESPOSTA_AS_PENDENCIAS.pdf	22/11/2017	Renan Felipe	Aceito
		03:52:36	Hartmann Nunes	
TCLE / Termos de	TCLE.docx	22/11/2017	Renan Felipe	Aceito
Assentimento /		03:49:15	Hartmann Nunes	
Justificativa de				
Ausência				
Projeto Detalhado /	Projeto.docx	25/08/2017	Renan Felipe	Aceito
Brochura		15:23:06	Hartmann Nunes	
Investigador				
Declaração de	Declaracao_Renan.pdf	25/08/2017	Renan Felipe	Aceito
Instituição e		15:22:51	Hartmann Nunes	
Infraestrutura				
Folha de Rosto	FolhaDeRosto.pdf	25/08/2017	Renan Felipe	Aceito
		15:21:07	Hartmann Nunes	

### Situação do Parecer: Aprovado

Necessita Apreciação da CONEP: Não

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Página 03 de 04

# UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

Continuação do Parecer: 2.448.708

FLORIANOPOLIS, 19 de Dezembro de 2017

Assinado por: Ylmar Correa Neto (Coordenador) Plataforma