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EFEITOS DA INGESTÃO DE ÁGUA E BEBIDA ESPORTIVA EM PARÂMETROS DE ESTRESSE OXIDATIVO E EXPRESSÃO DE PROTEÍNAS DE CHOQUE TÉRMICO EM JOGADORES DE FUTEBOL

PORTO ALEGRE

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Dissertação de mestrado apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica como requisito à obtenção do grau de Mestre em Bioquímica.

Orientador: Prof. Dr. José Cláudio Fonseca Moreira

PORTO ALEGRE
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"Reparta o seu conhecimento.

É uma forma de alcançar a imortalidade"

Dalai Lama

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PARTE I

RESUMO

O aumento do consumo de oxigênio durante o exercício pode causar uma maior produção de espécies reativas do oxigênio (ERO), por outro lado, as defesas antioxidantes (AO) podem adaptar-se e aumentar sua atividade através da exposição ao exercício regular. A desidratação e a depleção de carboidratos podem ser as causas da fadiga durante o exercício. É possível que a expressão de HSPs possa complementar a defesa enzimática antioxidante. Este estudo tem como objetivo comparar os efeitos de hidratação com água (WAT) ou bebida esportiva (CHO-E) sobre parâmetros de estresse oxidativo em atletas futebolistas submetidos a um protocolo de exercício intermitente. A amostra foi composta por 30 atletas futebolistas de 2 times de futebol em 2 protocolos de experimentais, 18 atletas foram analisados em um protocolo sem reposição de fluídos e 12 atletas foram analisados com reposição de WAT e CHO-E. As coletas de sangue foram realizadas antes, depois e 6 horas após a realização de um protocolo de exercício intermitente. A reposição de líquidos (200 ml) foi efetuada a cada 20 minutos. O exercício físico intermitente por si só foi capaz de gerar aumento nos parâmetros de estresse oxidativo. A utilização de água (WAT) não alterou atividade das enzimas antioxidantes SOD e CAT e os níveis de dano oxidativo a proteínas, porem aumentou os níveis de peroxidação lipídica e expressão de HSP70. A Reposição com CHO-E aumentou significativamente a atividade da enzima SOD, o dano oxidativo a proteínas e não alterou outros parâmetros. Os grupos WAT e CHO-E não apresentaram diferenças significativas na sua taxa de sudorese, percentual de desidratação, perda de suor e osmolalidade plasmática quando comparados. No entanto o grupo CHO-E apresentou uma maior glicemia após o exercício. Concluímos que a reposição de carboidratos deve ser realizada com cautela, e mais estudos, com quantidades diferentes de carboidratos devem ser efetuados para uma prescrição nutricional segura durante o exercício.

Palavras-Chave: Estresse Oxidativo, Futebol. Suplementação de Carboidratos, HSP70.

ABSTRACT

The increase of oxygen consumption during exercise can result a higher production of reactive oxygen species (ERO),on the other hand, the antioxidant defenses (AO) can be adapted and increase its activity through the exposition to regular exercise. The dehydration and the carbohydration depletion can be the factor of fatigue during the exercise. Its Possible that the expression of HSPs can complement the antioxidant enzymatic defense. This study objective compare the effect of exercise and fluid repleacemet with water (WAT) or sport drink (CHO-E) on parameters of oxidative stress in soccers players submitted a protocol of intermittent exercise. The sample was composed for 30 athletes of 2 teamses of soccer in 2 experimental protocols , 18 athletes had been analyzed in a protocol without fluid replacement and 12 athletes had been analyzed with replacement of WAT and CHO-E. The blood samples had been carried through before, later and 6 hours after intermittent exercise.The fluid replacement (200 ml) was realized to each 20 minutes. The intermittent exercise by itself was capable to generate increase in the ofoxidative stress parameters. The WAT did not modify SOD and CAT enzymes activities, carbonyl proteins, but increased the levels of lipid peroxidation and HSP70 expression. The Replacement with CHO-E significantly increased the SOD enzyme activity, the oxidative damage of proteins and it did not modify other parameters. Groups WAT and CHO-E had not presented significant differences in sweat rate, dehydration, sweat loss and osmolality when compared. However group CHO-E presented a increase in blood glucose ater after the exercise. We conclude that carbohydrate replacement must be carried through with caution, and more studies, with different amounts of carbohydrate must be made for a secure nutritional reposition during the exercise.

Key-Words: Oxidative Stress, Soccer, Carbohydration Replacement, HSP70.

LISTA DE ABREVIATURAS

ANOVA: análise de variância

AO: antioxidantes

CAT: catalase

CK: creatina quinase

DNA: ácido desoxirribonucléico

EO: estresse oxidativo

ERN: espécies reativas de nitrogênio

ERO: espécies reativas de oxigênio

EERON: espécies reativas de oxigênio e nitrogênio

GSH: glutationa reduzida

H_2O_2: peróxido de hidrogênio

HOCl: ácido hipocloroso

HSL: lipase hormônio sensitiva

HSP: Heat shock protein

MDA: malondialdeído

$(NO)^2$: monóxido de nitrogênio

$^{\bullet}NO$: óxido nítrico

NOS: *nitric oxide synthase*, óxido nítrico sintase

$^{\bullet}OH$: radical hidroxil

$O_2^{\bullet-}$: ânion superóxido

$ONOO^-$: peroxinitrito

PBS: Tampão fosfato tamponado com salina

SOD: superóxido dismutase

TBARS: substâncias reativas ao ácido tiobarbitúrico

TRAP: *total radical-trapping antioxidant potential*, potencial total antioxidante de captura de radical

VO2: consumo máximo de oxigênio

1. INTRODUÇÃO

1.1 Futebol – Características Fisiológicas

O futebol pode ser considerado um esporte no qual jogadores apresentam características fisiológicas diferentes entre si. Para manter a performance e o alto rendimento, necessitam de habilidades físicas, fatores fisiológicos, técnica e tática. Lesões e seqüelas podem afetar a habilidade física e o desempenho do atleta e do time (Arnason, 2005). É um esporte que implica a prática de exercícios intermitentes, de intensidade variável. Aproximadamente 88% de uma partida de futebol envolvem atividades aeróbias e os 12% restantes atividades anaeróbias de alta intensidade (Barros, 2004). O futebol é caracterizado por pequenos “sprints”, rápida aceleração e desaceleração, giros, pulos, chutes e marcação (Arnason, 2005).

A principal via metabólica durante o futebol competitivo é a aeróbia e as respostas metabólicas são em geral análogas às encontradas nos exercícios de endurance (Reilly et al., 2000). Constata-se que o sistema anaeróbio alático é o principal sistema anaeróbio da modalidade, no entanto, é um esporte com componentes anaeróbios aláticos e láticos (Bangsbo et al., 1991; Bangsbo, 1994; Bangsbo, 1994; Bangsbo et al., 2006). As competições, apesar de serem o ápice da prática esportiva, representam apenas uma fração do tempo dedicado a essa prática. As sessões de treinamento são extremamente importantes e influenciam uma série de hábitos e possibilidades que irão refletir durante a competição. Os treinos são freqüentes e com um período de recuperação curto entre eles, assim é fundamental estabelecer práticas que permitam um ótimo desempenho (Aragón-Vargas, 2004).

1.2 - Estresse Oxidativo

As principais espécies reativas de oxigênio (ERO) produzidas pela redução de um elétron do O_2 são: o superóxido (O_2^-) e o radical livre hidroxil ($OH^\bullet$), existe também, a forma parcialmente reduzida, o peróxido de hidrogênio (H_2O_2). O radical hidroxil é o mais potente das ROS, e provavelmente o iniciador da cadeia de reações que formam lipoperóxidos e radicais orgânicos. O peróxido de hidrogênio, entretanto não atua como um radical, mas sim como um agente oxidante e na presença de Fe^{+2} ou outro metal de transição, e gera o radical hidroxil pela reação de Fenton (Halliwell & Gutteridge, 2007).

A geração de espécies reativas de oxigênio e nitrogênio (ERON) ocorre regularmente como parte normal do metabolismo celular e aumentam potencialmente em algumas situações de estresse (Bloomer et al., 2005). As ERON podem reagir potencialmente com uma variedade de componentes químicos, sendo estreitamente relacionadas com a fisiopatologia de diversas doenças (Halliwell & Gutteridge, 2007).

No organismo as ERON são neutralizadas por um elaborado sistema de defesa antioxidante, que consiste em enzimas como a catalase, superóxido dismutase, glutationa peroxidase e de antioxidantes não-enzimáticos, incluindo as vitaminas C e E, glutationa, ubiquinona e flavonóides (Urso and Clarkson, 2003).

O desequilíbrio entre a produção de ERON e sistemas de defesa antioxidante, pode ocasionar dano oxidativo em lipídios celulares, proteínas e DNA. Por outro lado, as ERON possuem um papel fundamental na sinalização molecular em várias vias metabólicas (Close et al., 2005).

Os níveis elevados de consumo de oxigênio mitocondrial, o aumento na circulação de catecolaminas, a resposta inflamatória, as ações intermitentes e de sprints repetitivos – causando uma isquemia-reperfusão temporária, são eventos musculares que podem estar influenciando a produção de ERON durante e após um treino ou jogo de futebol (Ascensão et al., 2008).

Em treinos de “sprints”, em que predomina o exercício anaeróbico, outras fontes de radicais livres (RL) podem ser geradas através de outros processos: 1) Produção de Xantina e NADPH oxidase; 2) Isquemia-Reperfusão; 3) alterações na homeostase do cálcio; entre outros (Fehrenbach and Northoff, 2001). A produção de RL durante o exercício é aumentada pelo aumento da temperatura central, de catecolaminas e de ácido lático (Clarkson and Thompson, 2000; Cooper et al., 2002; Ji, 1996).

O Estresse oxidativo (EO) é caracterizado por um desequilibro entre os sistemas pró-oxidantes e antioxidantes, onde os agentes pró-oxidantes encontram-se em uma proporção maior causando dano celular ou liberando produtos tóxicos nocivos ao organismo (Cazzola et al., 2003). O exercício físico é caracterizado por um aumento no consumo de oxigênio por todo o organismo, principalmente pelos músculos. Esse aumento nas taxas de consumo de oxigênio acompanha uma elevação na produção de espécies reativas de oxigênio (ERO). Exercícios muito intensos e extenuantes podem diminuir as defesas antioxidantes do organismo e aumentar os marcadores de peroxidação lipídica e carbonilação protéica (Cazzola et al., 2003). Por outro lado, as defesas antioxidantes podem se adaptar e aumentar sua atividade através da exposição ao exercício regular.

Esportes como futebol, podem produzir um desequilíbrio entre a produção de ERON e sistemas de defesa antioxidantes, quando isto acontece ocorre o chamado “estresse oxidativo”.

A resposta celular para o estresse térmico envolve a síntese de proteínas de choque térmico, as “heat shock proteins” (HSP). A Família HSP70 vem sendo bem caracterizada por possuir um papel protetor como chaperona. A Indução na expressão de HSP vem sendo observada em situações como hipertermia, privação de glicose, aumento de cálcio intracelular, estresse oxidativo, hipóxia, entre outros (Rao et al., 1999; Welch, 1992). É possível que a expressão de HSPs possa complementar a defesa enzimática antioxidante (Essig and Nosek, 1997). Os mecanismos celulares de proteção que estão associados ao aumento das HSP ainda não estão claros.

1.3 – Termorregulação

O exercício eleva a taxa metabólica. Quando a demanda de energia é alta, como durante o exercício, são encontradas altas taxas de produção de calor (Maughan et al., 2004; Sawka et al., 2007). Para limitar o aumento potencialmente prejudicial da temperatura central, a taxa de perda de calor (e consequentemente a taxa de sudorese) tem de ser aumentada proporcionalmente.

O volume sangüíneo adequado (quantidade total de água no corpo) deve promover a remoção de calor durante o exercício. Durante a contração muscular entre 75 a 85% da energia é convertida em calor, e sem sistema de resfriamento a temperatura corporal aumenta (hipertermia). A termorregulação depende do suprimento adequado de água (hidratação) para a produção de suor (Wolinsky & Driskell, 2000).

A sudorese é uma resposta fisiológica que se empenha em limitar o aumento da temperatura central através da secreção de água na pele e pela evaporação, mas esta perda de líquido nem sempre é compensada pela ingestão. O desempenho e a saúde possivelmente serão prejudicados. O fluído perdido está ligado na necessidade de manter a temperatura corporal dentro dos valores normais (37ºC). É preciso dissipar eficientemente o excesso de calor para o ambiente e evitar a desidratação (Maughan et al., 2004; Sawka et al., 2007; Casa et al., 2000; Maughan et al., 1996; Maughan and Leiper, 1994).

Como o suor é hipotônico em relação aos fluídos do corpo, o efeito da transpiração prolongada é o aumento da osmolalidade do plasma, que exerce efeito significativo sobre a habilidade de manter a temperatura do corpo. Foi observada relação direta entre a osmolalidade do plasma e a temperatura do corpo durante o exercício. A hiperosmolalidade do plasma, induzida antes do exercício, parece influenciar a diminuição da habilidade de regular a temperatura corporal; registra-se elevação no limiar da transpiração e queda do fluxo de sangue quente para a pele (Maughan & Burke, 2002).

1.4 – Hidratação: reposição de fluídos e carboidratos

A manutenção do organismo com níveis adequados de água é de extrema importância para o bom funcionamento do sistema cardiovascular, termorregulação eficiente e desempenho físico durante a prática de exercícios. Cerca de 60 % da massa corporal é constituída por água, ou seja, 42 litros para um indivíduo pesando cerca de 70 kg. O sangue tem por função levar o oxigênio e outros nutrientes para execução do trabalho muscular, e transportar o calor produzido pelos músculos para a pele, onde ocorre a evaporação do suor, que auxilia na dissipação do calor para o meio ambiente. Em competições

esportivas, a irrigação sangüínea inadequada em nível muscular ou o acúmulo excessivo de calor devido a uma dissipação insuficiente, ocasiona uma queda no desempenho atlético e uma sensação de mal-estar (Lamb, 1999).

Quando o organismo está com níveis adequados de água, dizemos que ele está num estado de euhidratação (normoidratado). A hipohidratação se caracteriza como uma situação na qual o organismo apresenta uma redução do conteúdo de fluídos do corpo e a hiper-hidratação se caracteriza por um volume de água no corpo acima do normal. O termo desidratação define uma redução rápida da água corporal, levando o organismo de um estado de euhidratado para hipohidratado. Por exemplo, um jogador de futebol, que não pode repor os líquidos perdidos durante uma partida, gradualmente vai entrando no estágio de desidratação (Lamb, 1999). Um nível adequado de hidratação só é mantido em pessoas que praticam atividades físicas se estas ingerirem quantidades suficientes de líquidos antes, durante e depois dos exercícios. A dificuldade de se manter um balanço entre a perda e o consumo de líquidos se dá devido a limitações na freqüência da ingestão de líquidos, esvaziamento gástrico e absorção intestinal (Maughan & Shirrefs, 1998).

Hidratação pré-exercício

Os jogadores devem iniciar um jogo ou um treino euhidratados. Para isso recomenda-se uma ingestão de cerca de 500 ml de uma bebida esportiva aproximadamente 2h antes da partida, o que permite completar as reservas de líquidos corporais, qualquer excesso será eliminado pela urina no percorrer desse tempo e não causaria problemas durante o jogo. Imediatamente antes do jogo é sugerido ingerir um adicional de 250 ml de bebida esportiva (Sawka et al., 2007).

As pessoas que começam a exercitar-se quando estão hipohidratadas com hipovolemia concomitante e hipertonicidade exibem uma menor capacidade de dissipar o calor corporal durante o exercício subseqüente. Elas demonstram uma elevação mais rápida da temperatura corporal central e maior sobrecarga cardiovascular (Lamb, 1999).

Hidratação durante o exercício

Há evidências de que a ingestão de água ou de bebidas esportivas com carboidratos e eletrólitos durante o exercício ajuda a reduzir a queda que normalmente ocorre no volume do plasma: isso ajuda a manter a potência cardíaca, por meio da conservação do volume de batidas, aumenta o fluxo de sangue pela pele, e por sua vez, promove a perda de calor, que limita o aumento da temperatura central. Embora a ingestão de água pura melhore o desempenho no exercício, são observados benefícios posteriores quando se acrescenta glicose ou glicose + eletrólitos (Maughan & Burke, 2002). Durante os exercícios, os atletas bebem tipicamente volumes insuficientes de líquido para contrabalançar as perdas nas formas de suor. Esta observação foi denominada “desidratação voluntária”. São recomendados 150 a 200 mL de água ou repositores de energia conforme a necessidade, a cada 15 a 20 min. a partir do início do exercício (Casa et al, 2000; Sawka et al., 2007).

Conforme sugestão de Shi & Gisolfi (1998), a bebida ideal para se ingerir durante esportes coletivos com características intermitentes como futebol, deve ter osmolalidade entre 250 e 370 mOsm/L, uma concentração de carboidratos entre 5 e 7 %, e se deve usar combinação de vários carboidratos que são transportados ativamente no intestino. A hidratação durante exercício pode influenciar a função cardiovascular, termorregulatória, muscular, o volume

sangüíneo e a performance. Realça a dissipação do calor, aumenta o fluxo sangüíneo e a taxa de suor, mantém limites de hipertonicidade do plasma, equilibra os batimentos cardíacos, conserva a circulação central de fluídos e intensifica as respostas fisiológicas no exercício extenuante (Casa et al, 2000).

Os benefícios da rehidratação durante o exercício na função termorregulatória é provavelmente a manutenção da volemia, redução da hiperosmolalidade, redução da desidratação celular e a manutenção extravascular de fluídos (Casa et al, 2000).

Hidratação pós-exercício

A rehidratação é parte vital do processo de recuperação pós-exercício. A recomendação usual para garantir a reposição adequada de fluídos após o exercício é repor o quilo de massa corporal perdido durante o exercício com 1L de fluído. Atualmente, indícios demonstram a necessidade de pelo menos ingerir 50 % mais do que o volume de perda pela transpiração para alcançar a rehidratação (Maughan & Burke, 2002).

A reposição do volume de líquidos perdido e a restauração dos estoques de glicogênio muscular são pontos críticos no processo de recuperação após o exercício (Casa et al, 2000). Neste período, na maioria das vezes, o atleta não consome líquidos em quantidades suficientes para repor o que ele perdeu durante o exercício. O atleta deve ingerir uma bebida que contenha carboidrato para repor os estoques de glicogênio muscular, sódio e não contenha nem álcool nem cafeína que são substâncias diuréticas (Maughan & Murray, 2001).

Para que o processo de rehidratação seja eficiente é necessário um plano especial de ingestão de líquidos, uma vez que a sede e a ingestão voluntária irão interferir na restauração das perdas através do suor na fase

aguda (0 – 6 horas) do processo de recuperação. O líquido a ser consumido neste período deve ser palatável e deve conter sódio para maximizar a retenção dos líquidos ingeridos (Maughan & Murray, 2001).

Bebidas Esportivas

A ingestão espontânea de líquidos é influenciada por uma variedade de informações sensoriais como o odor, gosto, temperatura, cor e qualidade subjetiva. Estudos com diferentes líquidos mostram que a ingestão voluntária de líquido é máxima quando os líquidos são refrigerados entre 15º e 20ºC, líquidos com sabores leves são normalmente mais aceitos do que água pura (Lamb, 1999).

Pesquisas evidenciam a necessidade da ingestão de carboidratos e eletrólitos juntamente com o fluído em exercícios físicos. Adequadamente formuladas estas bebidas com água, aromatizante, carboidratos e eletrólitos, incentivam a ingestão de líquidos, promovem rápida absorção de fluídos, repõem eletrólitos perdidos, melhoram o desempenho e intensificam a rehidratação (Maughan & Murray, 2001).

Toda a comunicação entre músculos e nervos depende do fluxo de corrente elétrica dentro de cada uma das células. Os músculos são estimulados por sinais de seus nervos associados. Isto depende da concentração de partículas eletricamente carregadas (eletrólitos). A concentração de eletrólitos deve ser mantida dentro de limites estreitos, para a transmissão nervosa (Wolinsky & Driskell, 2000).

Aumentar o teor de carboidrato de uma bebida para níveis acima do normal pode oferecer energia extra para o desempenho, mas acabará retardando o esvaziamento gástrico e a absorção intestinal de fluídos,

reduzindo, portanto, a rapidez com que os fluídos são repostos no corpo em processo ativo de desidratação (Passe, Horn & Murray, 2000).

O uso de carboidratos tem o objetivo de recuperar e otimizar as reservas de glicogênio muscular. A reposição necessária de carboidratos para manter a glicemia e retardar a fadiga é de 30 a 60 g por hora, com concentração de 4 a 8 g por decilitro (Carvalho et al., 2003).

A ingestão de carboidratos durante o exercício aumenta a glicemia e a concentração de insulina, sustenta a oxidação de carboidratos e, provavelmente traz benefícios ao sistema nervoso central (Davis, Welsh & Alderson, 2000).

Com o exercício extenuante a capacidade oxidativa não supre as necessidades podendo aumentar a produção de lactato, causando uma acidemia, com a diminuição do pH há uma séria de respostas de defesas, como o aumento na produção das HSP70. A ingestão de água serviria como um fator essencial na diminuição da temperatura central e da freqüência cardíaca. Quando a reposição de fluídos é efetuada com carboidratos, há uma diminuição do catabolismo protéico prevenindo os processos de EO e lesão muscular (Barclay et al, 1991; Febbraio et al 2004; Banfi et al, 2006; Krustrup et al 2006).

O presente trabalho fundamenta-se no questionamento de como a hidratação com água e hidratação com carboidratos e eletrólitos afetam os parâmetros de estresse oxidativo e de HSP no sangue de atletas submetidos a exercício intermitente.

2. OBJETIVOS

2.1 – Objetivo Geral

Comparar os efeitos de re-hidratação com água e bebida esportiva sobre parâmetros sanguíneos de estresse oxidativo em atletas futebolistas submetidos a esforço físico repetitivo.

2.2 – Objetivos Específicos

Analisar parâmetros de estresse oxidativo

Analisar percentual de desidratação, taxa de sudorese, osmolalidade do plasma e glicose sanguínea.

PARTE II

CAPÍTULO I

Manuscrito: **Oxidative Stress in young Football (Soccer) Players in Intermittent High Intensity Exercise Protocol**

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Oxidative Stress in young Football (Soccer) Players in Intermittent High Intensity Exercise Protocol

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Abstract

The exercise can be associated with various benefits for the health, but excessive physical activity may be stressful, for example high performance football, leading to oxidative cellular damage. Football training can produce an imbalance between RNOS and antioxidants, which is referred to as oxidative stress. We performed in the present work quantification of lipid peroxidation, carbonylation levels, and antioxidants enzyme activities SOD and CAT in Intermittent High Intensity Exercise Protocol. In the herein presented work, we investigated the effects of high intensity exercise on redox state. Eighteen trained football players perform exercise protocol. Blood samples were obtained before and immediately after exercise and centrifugation was used to separate plasma and cell fraction. The SOD and CAT enzymes activities had been determined by spectrophotometer. Lipid oxidative damage was mensuraded by TBARS and protein damage by carbonyl groups. This study showed that this type of specific Repeated-Sprint Exercise protocol for football players, produce oxidative stress by significant differences in lipid peroxidation and protein carbonyls after exercise, and a increased activity of SOD and CAT enzymes, suggesting an antioxidant adaptation of these athletes to this protocol.

Introduction

Football is a complex sport widely played, in which players need technical, tactical, and physical skills to succeed [1], being considered an intermittent exercise, predominantly aerobic with periods of high-intensity exercise. The observation that players perform 150-250 brief intense actions during a game and have blood lactate values of 2-14 mM indicates that the rate of anaerobic energy turnover is high during periods of a game [2,3]. Recent game analysis has shown that some of these sprints are separated by short rest periods (<30s) [4], which have been shown to negatively affect subsequent sprint performance [5]. For that reason, long sprint training can improve repeated-sprint performance [6]. However, only a few studies utilizing sprint protocols have been undertaken in humans until now.

In football physical preparation, long duration sprints (>10s) are used to train both the resistance to lactate and the ability to conduct long sprints and high-speed, which are required in a counter-attack, for example. Long sprints are not usually seen in official football games, but play an essential role in the physical preparation of athletes, being a widespread practice in Brazilian teams.

The generation of reactive oxygen and nitrogen species (RNOS) occurs regularly as part of normal cellular metabolism and is increased under conditions of physical stress [7]. Thus, football training can produce an imbalance between RNOS and antioxidants, which is referred to as oxidative stress. Physical activity increases oxygen consumption and generation of free radicals in several ways, indeed 2 to 5% of oxygen used in the mitochondria originates free radicals in aerobic exercises [8]. In sprint training, which is a predominantly anaerobic exercise, another source of RNOS may be mediated

through various other pathways: namely xanthine and NADPH oxidase production, prostanoid metabolism, ischemia/reperfusion, phagocyte respiratory burst activity, disruption of iron-containing proteins, and alteration of calcium homeostasis [8,9]. Other processes involved in RNOS production during exercise are increased central temperature, catecholamine and lactic acid, which have the ability to convert superoxide to hydroxyl [10,11] .

The exercise can be associated with various benefits for the health, but excessive physical activity may be stressful, for example high performance football, leading to oxidative cellular damage [12,13]. Cellular damage is often characterized by modifications in various macromolecules, including proteins, lipids, and nucleic acids, and can occur as a response to high-intensity exercise such as football, which requires the athlete aerobic and anaerobic skills during a game [14-16]. In the body, RNOS are neutralized by an elaborate antioxidant defense system consisting of enzymes such as CAT, SOD, GPx, and numerous non-enzymatic antioxidants, including vitamins E and C, glutathione, ubiquinone, and flavonoids [8,17]. Regular Exercise training are well-known to be potential factors of SOD, CAT and GPX increase as shown by numerous studies, providing additional "protection" during times of intense physical stress which appears to be true for both aerobic and anaerobic exercise [18-21].

Then, due to a limited of information regarding the redox effects of physical exercise in football players, we performed in the present work quantification of lipid peroxidation, carbonylation levels, and antioxidants enzyme activities SOD and CAT in intermittent high intensity exercise protocol.

Material and Methods

Subjects

Eighteen trained young football players, volunteers, from Grêmio Football Porto Alegrense (Brazil). Mean age, height, body mass and percentage body fat: 17 ± 0.5 years, 174 ± 5 cm, 74.02 ± 6.52 kg and 9.7% ± 1.6% respectively. Body fat percentage was estimated from sum of skin folds according to Jackson & Pollock [22]. All players gave written informed consent to participate after the details of the study had been explained to them. The study was approved by the Research Ethics Committee of Universidade Federal do Rio Grande do Sul (UFRGS) Protocol 2006658**.** No Subject reported antioxidant suplement compounds intake, including vitamins and/or medications. The athletes could not drink alcoholic beverages or with caffeine for at least 24 hours before exercise testing day. All subjects were informed about the possible risks and discomforts involved in the study, and all signed a consent form. The athletes were fed 4 hours before the training, and consumed 500ml of fluid (sport drink) 2 hours before the training, to ensure a state of euhydration. The athletes had been guided not to consume fluid during the exercise.

Experimental protocol

The tests were performed in the grass training field of Grêmio Football Porto Alegrense Stadium, in the morning with ambient temperature of 24 degrees Celsius and relative humidity of 54 percent. Being characterized as a day of fine temperature, not offering an additional heat stress.The samples were collected on a routine training of the team, which consisted of 15 minutes of warming and ten series of two 200 meters sprints totaling 20 sprints from 200 meters. Each

sprints was followed up by a 30 seconds recovery and every 2 sprints, 90 seconds.The athletes traveled a route of 200 meters in 40 seconds, thus, an average speed of 18 kilometers per hour. Characterizing a repeated sprint training. This exercise is classified as long duration repetead-sprint (>10s) training [23]. Blood samples (10ml) were obtained from antecubital region vein, before and immediately after exercise and centrifugation was used to separate plasma and cell fraction.

Dehydration and Osmolality

Before and after training, all players were weighed wearing only underpants, using a digital balance (Plena, model MS-601). The relatively small changes in mass due to substrate oxidation and other sources of water loss were ignored. Players were asked to micturate and defecate if necessary immediately before the measurement of body mass. The dehydration was calculated by the percentage of difference of the initial and final weight. The plasma osmolality (mOsm/kg) was measured with Vapro® Vapor Pressure Osmometer (Wescor., Logan, USA).

Blood Lactate and Glucose

The concentration of lactate and Glucose were obtained through the blood of digital pulp and immediately measured. For determined blood glucose (mg/dl), it was used glucose analyzer (Accu Check Active, Roche, Germany), and concentration of blood lactate (mmol/l) by Lactate analyzer (AccuSport, Roche, Germany.

Antioxidant enzyme activities

The superoxide dismutase (SOD, E.C. 1.15.1.1) activity in plasma samples was measured spectrophotometrically by the inhibition rate of auto-catalytic

adrenochrome formation in a reaction buffer containing 1 mM adrenaline/50 mM glycine–NaOH (pH10.2)/1 mM catalase, as previously described (Klamt et al., 2002). Catalase (CAT, E.C. 1.11.1.6) activity was assayed by measuring the hydrogen peroxide (H2O2) decreasing rate[24]; erythrocytes samples (300 µL) were centrifuged (3000 ×g; 10 min) and 100 µL of supernatant was mixed with 1 mL of phosphate buffer with 14 mM H2O2 (40 µL). Absorbance was followed for 60 s at 240 nm.

Proteins carbonyl content

As an index of protein oxidative damage, the carbonyl groups were determined accordingly [25]. Proteins from 300 µl of plasma were precipitated by the incubation with thiobarbituric acid (TCA) 20% (100 µl) for 5 min on ice, and then centrifuged at 4000 ×g for 5 min. The pellet was dissolved in 100 µl of NaOH 0.2 M, and 100 µl of HCl 2 M or 10 mM of 2,4-dinitrophenylhydrazine (DNPH) in HCl 2 M was added to duplicate aliquots for blanks or for carbonyl groups derivatizing, respectively. Samples were maintained for 30 min at room temperature. Proteins were precipitated with TCA, and washed three times with 500 µl 1:1 ethanol: ethyl acetate with 15 min standing periods to remove excess of DNPH. Samples were dissolved in 200 µl 6 M guanidine in 20mM KH_2PO_4, pH 2.3 and the absorbance was read at 370 nm. The carbonyl content (nmol/mg protein) was calculated using a molar extinction coefficient of 22,000 M/cm at 370 nm after subtraction of the blank absorbance.

Lipid oxidative damage

Formation of malondialdehyde (MDA), an index of lipid peroxidation, was assessed by the quantification of thiobarbituric acid-reactive species (TBARS) as previously described [26]. Briefly, samples were mixed with 600 µL of TCA

10%, centrifuged (10,000 ×g; 10 min) and 500 µL of the supernatant mixed with 500 µL of thiobarbituric acid 0.67% (TBA). Then, the sample was heated in a boiling water bath for 15 min. TBARS were determined by absorbance at 532 nm.

Statistical Analyses

All data are expressed as means ± SEM. Data were analyzed using SPSS (Version 12.0) statistical software. Paired *t*-test was used for comparisons relationship variables before and after exercise. Significance was indicated by p values < 0.05.

Results

Dehydration, osmolality, Blood glucose and lactate

Athletes dehydrate 1.806 ± 0.18% of their body mass at the end of the training. Plasma osmolality was 252.6 ± 4.79 mOsmol/kg at rest and 272.8 ± 4.38 mOsmol/kg after exercise. Data suggest that exercise did not alter dehydration. In addition, after exercise a significant increase in blood glucose and lactate levels was observed. Blood glucose in rest was 82.72 ± 2.75 mg/dl and 99.22 ± 3.36 mg/dl after exercise (Figure 1A). Blood lactate in rest was 1.57 ± 0.10 mg/dl and 4.15 ± 0.39 mg/dl after exercise (Figure 1B).

Oxidative stress parameters

Plasma SOD activity increased significantly revealing values of 9.10 ± 0.71 U SOD / mg protein at rest and 12.0 ± 0.77U SOD/mg protein after exercise (Figure 1C). Catalase activity was observed increased, form 51.91 ± 2.66 U CAT/mg protein at rest and 62.53 ± 2.74 U CAT/mg protein after exercise (Figure 1D) The relation between enzymes (SOD/CAT) did not change significantly being 0.18 ± 0.015 at rest and 0.20 ± 0.018 after exercise (Figure

2A). Lipid peroxidation, which was measured by TBARS assay, increased significantly from 0.32 ± 0.02 nmol/mg protein at rest to 0.57 ± 0.06 nmol/mg protein after exercise (Figure 2B) Plasma protein carbonyl groups increased significantly in after exercise, being 0.59 ± 0.049 nmol/mg protein at rest and 1.13 ± 0.06 nmol/mg protein after exercise (Figure 2C).

Discussion

Dehydration

In the herein presented work, we investigated the effects of high intensity exercise on redox state in soccer players. We observed that the fluid loss of players was about 1,8% of their body weight. Many authors have studied and showed that football players have considerable percentage of dehydration, [27-29] and it was reported that football players dehydrated 1.2% and 1.4% after training sessions and competition [30]. American College of Sports Medicine [31] recommends that a percentage equal to or greater than 2% and plasma osmolality must be higher than 290 mOsmol/kg characterize dehydration can bring damage to performance. In our study, the negative effect of dehydration was not noticed.

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Blood Lactate and Glucose

We found a significant increase in glucose after exercise when compared to the rest group, even though in our protocol no carbohydrate consumption was allowed during exercise. This fact can be perhaps explained by the potential hormonal factors that may have played a role in exercise glucose kinetics. Many authors also found higher values of glucose after exercise when

compared to the moment of rest, linking this fact with concentrations as well as higher levels of catecholamines progressively increasing [32-34].

During exercise, blood glucose levels are maintained by the liver through hepatic glycogenolysis and gluconeogenesis. As a result, circulating glucose levels are maintained at a relatively constant level. This relatively simple and effective relationship between the liver and the skeletal muscle is maintained by a complex interplay of circulating and locally released neuroendocrine controllers [35]. Moderate blood lactate concentrations found in the present study suggest that the rate of glycolysis is high for any periods of time during the exercise.

Gaitanos et al. (1993) [36] report that there was no change in lactate during their protocol test, they suggest that a greater contribution from aerobic matebolism partly counteracted the reduction in anaerobic glycogenolysis, then it appears that while aerobic contibution to a single, short duration sprint is relatively small, there is increasing aerobic contribution to repetead sprints. The energy system contribution during repeated sprints appears to be heavily influenced by the duration of the sprints [23].

In our study, with the completion of 20 sprints repeated it is clear that the rate of moderate lactate and high levels of glucose in the blood can be explained by the reduction of anaerobic glycogenolysis and the increase of aerobic metabolism.

Antioxidant Enzyme Activies

The significant increase in SOD activity in our study can be explained by the increase in the superoxide radical during exercise [37]. Superoxide radical is

produced and is connected to the conversion of hemoglobin to metheinoglobin in the erythrocyte during exercise and is converted into H_2O_2 by SOD [38]. SOD functions in the cell as one of the primary enzymatic antioxidant defenses against superoxide radicals, increases in SOD enzyme activity corresponds with enhanced resistance to oxidative stress [39]. Another study observed a similar relationship, with higher resting plasma SOD activity in football players compared to untrained subjects [40].

In our analysis, we find significant differences in CAT activity, and there is little evidence to suggest that exercise training promotes an increase in CAT activity in skeletal muscle. However, studies report decreases or non alterations in erythrocyte CAT activity immediately after the exercise protocol [41-43]. As an antioxidant enzyme, CAT catalyses the breakdown of H2O2 to form water and O2. CAT is widely distributed in the cell, high concentrations are found in both peroxisomes and mitochondria and this activity is greatest in muscle fibres with high oxidative capacities and lowest in muscle fibres with low oxidative capacities [44,45].

The imbalance between the antioxidant enzyme SOD and CAT can be followed by oxidative damage [23,46,47]. In our study, SOD/CAT balance did not change in this experimental model. Hence, demonstrating a balance in the activity of these two antioxidants enzymes.

Protein and lipid oxidative damage

In our study plasma carbonylation levels were increased post exercise when compared with rest. Other authors also showed an increase in protein carbonyls immediately after exercise [48,49]. Thus, the exercise of long duration or high intensity can increase the concentration of carbonyl significantly after exercise.

Several factors may trigger the production of RNOS and increase the ability for protein oxidation, eg. invasion of phagocytic cells, hemolysis with a consequent increase in free iron and an imbalance in the calcium homeostasis [50]. The increase in RNOS production resulting from any of the these sources could lead to oxidation of amino acid side chains and fragmentation of polypeptides, as all amino acids are susceptible to metal-catalyzed oxidation [21].

As in our study, many authors also found increased lipid peroxidation levels after exercise [51,52] while others showed the opposite [53,54]. Perhaps this discrepancy is due to the fact that each study has different variables, such as intensity, sample nature, type of exercise. The elevations in MDA due to ROS production are more dependent on increased oxygen uptake or transient periods of ischemia/reperfusion, as may be likely during isometric handgrip exercise, rather than infiltration of phagocytic cells and a potential loss in calcium homeostasis secondary to muscle injury, that may be the case for protein carbonyls [55]. In Summary, this study showed that this type of specific Repeated-Sprint Exercise protocol for football players, produce oxidative stress by significant differences in lipid peroxidation and protein carbonyls after exercise, and a increased activity of SOD and CAT enzymes, suggesting an partially antioxidant adaptation of these athletes to this protocol. Viewed the oxidative damage to proteins and lipids, this work can serve to a basis for physical preparation to minimize the oxidative stress in football players.

Figures

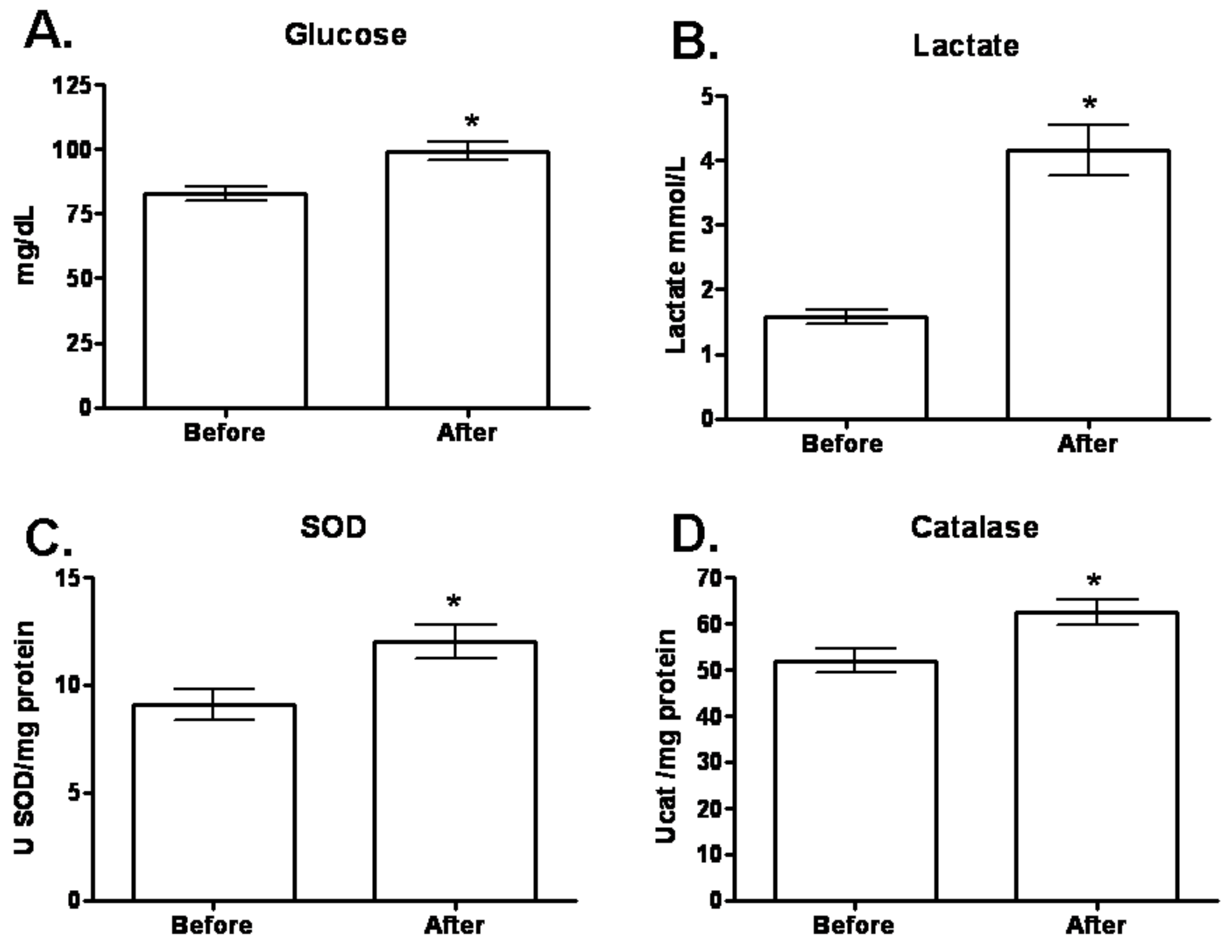

Figure 1. (A) Blood glucose, (B) Lactate, (C) Superoxide Dismutase activity (SOD) and (D) Erythrocyte catalase activity (CAT) (n=18). Results are expressed as means ± SEM. * Different from the rest ($p<0.05$)

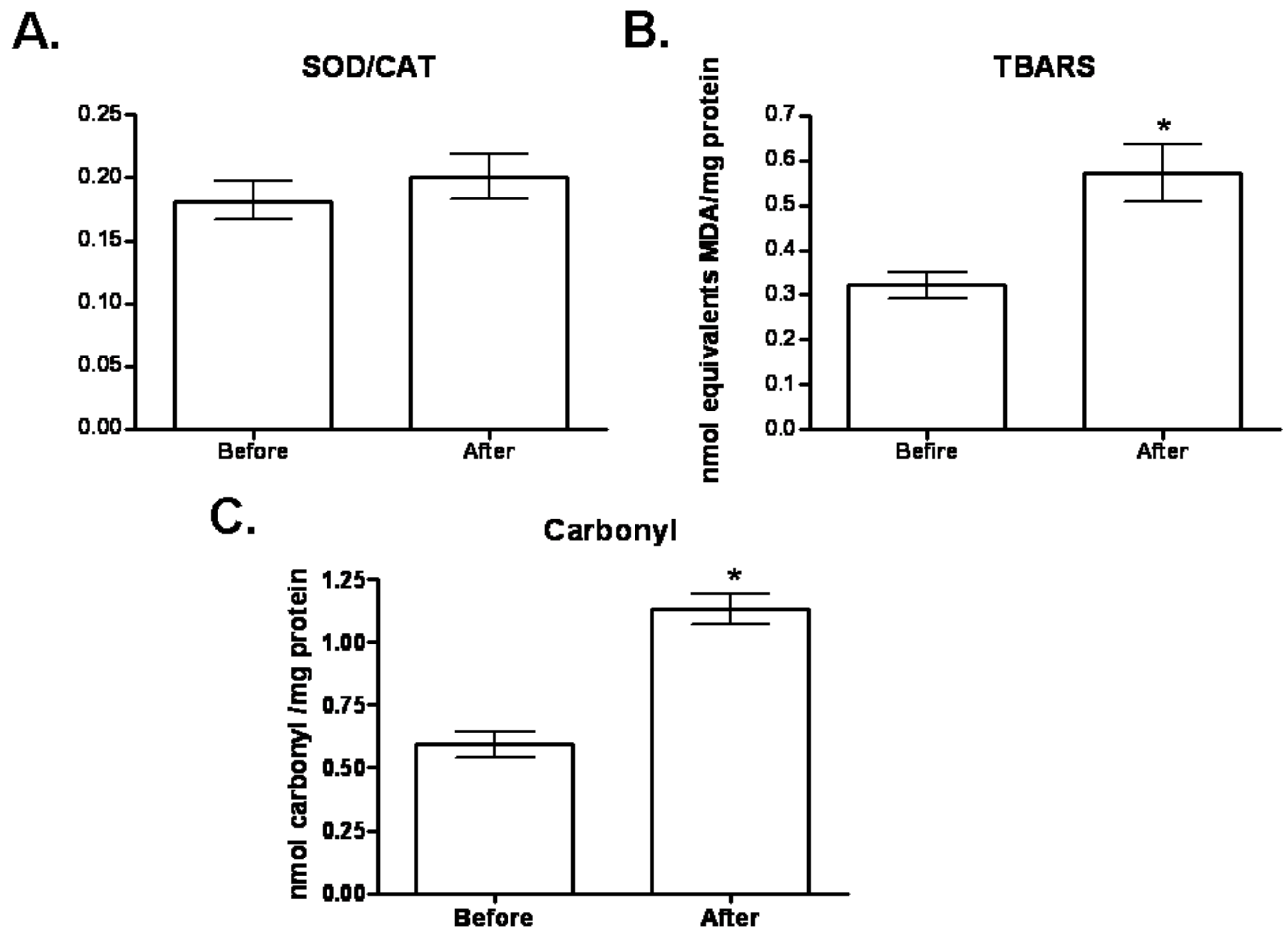

Figure 2: Oxidative stress parameters (A) SOD/CAT Balance (B)Thiobarbituric acid reactive substances (TBARS) in plasma after exercise; (C) Plasma levels of protein carbonyl groups as detected by dinitrophenylhydrazine method. Data were compared to resting levels and groups (n=18). Results are expressed as means ± SEM. * Different from the rest ($p<0.05$)

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CAPÍTULO II

Manuscrito: **Effects of Water and Carbohydrate-Electrolyte Fluid ingestion on Oxidative Stress Parameters and Heat Shock Protein (HSP70) Expression during Football Training**

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Effects of Water and Carbohydrate-Electrolyte Fluid ingestion on Oxidative Stress Parameters and Heat Shock Protein (HSP70) Expression during Football Training

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Abstract

Football players often utilize supplements such as carbohydrate-electrolyte drinks (CHO-E) to delay fatigue during exercise. This study was undertaken in order to compare the effects of CHO-E or water (WAT) reposition on oxidative stress. Eighteen players performed intermittent exercise. The SOD and CAT enzymes activities were spectrophotometrically measured. Lipid oxidative damage was determined by TBARS and protein damage by carbonyl groups and HSP70 by Wester Blotting. In Both groups, sweat loss, sweat rate,dehydration, plasma osmolality and erythrocyte CAT activity did not alter. In WAT group there was an increase in lipoperoxidation after exercise. In CHO-E, SOD and protein carbonyl groups increased after exercise. On the other hand, CHO-E did not alter the exercise-induced Hsp70 expression in leukocytes compared to WAT reposition. This paper may thus contribute to the understanding of beneficial or deleterious effects of CHO-E reposition on stress parameters in elite athletes.

Keywords: Oxidative Stress, Exercise, Football, Hsp70

Introduction

Football is considered as a high intensity intermittent sport being characterized by periods of medium or high intensity exercise separated by resting periods (Krustrup et al. 2006). A common theme across intermittent sports as football is the decrease in performance at the latter stages of a game, with less distance being covered, a lower fractional work intensity, and reduced blood glucose levels (Helgerud et al. 2001; Patterson and Gray 2007). In this context, elite football players need to have high aerobic endurance fitness as prerequisite to success in the modern football games. An increasing number of football players are involved in high training loads which may generate muscular fatigue and losses in performance.

The causes of fatigue are multifactorial and can occur as a result of either central or peripheral factors. Oxidative stress, dehydration, hypoglycemia and muscle glycogen depletion have been considered important factors associated with muscle fatigue in exercise (Patterson and Gray 2007). It has been demonstrated that a loss in body mass of only 2 % contributes to an elevation in core temperature as well in cardiovascular strain (Sawka et al. 2007).

In several sports, individuals are encouraged to consume drinks before and during participation in order to supplement body carbohydrate stores and replace the water lost in sweat thus delaying the fatigue process (Leiper et al. 2001). It has been demonstrated that adequate pre-match and half-time carbohydrate intake can substantially improve endurance capacity, as well as intermittent sprint power in the performance (Hespel et al. 2006; Williams and Serratosa 2006). Football players often utilize supplements such as

carbohydrate-electrolyte sports drinks to delay fatigue during submaximal endurance exercise. The potential mechanisms by which sports drinks reduce muscular glycogen depletion are probably the maintaining of blood glucose as an important energy source for both muscle and brain, and/or the alteration of the neurotransmitter activity that could influence cognition, motivation, and motor skill performance (Patterson and Gray 2007).

The elevated metabolic rate associated with physical exercise can increase mitochondrial O2 consumption in muscle tissue and, consequently, mitochondrial reactive oxygen species generation, xanthine oxidase activity, and immune system activation (Groussard et al 2003; McAnulty et al. 2003).

Physiologically, ROS are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and numerous non-enzymatic antioxidants, including vitamins E, C, and glutathione (Halliwell and Gutteridge 2007; Urso and Clarkson 2003). Strenuous exercise may lead to an imbalance between ROS production and antioxidant defenses, resulting in ROS accumulation and oxidative stress (Ji 1999; Alessio et al. 2000). Oxidant molecules generally induce oxidative modifications in proteins, lipids, and DNA (Halliwell and Gutteridge 2007), and can occur during high-intensity exercises such as football, which requires the athlete's both aerobic and anaerobic answers during a game (Bloomer and Goldfarb 2004; Krustrup et al. 2006; Mastaloudis et al. 2001; Sjodin et al. 1990). Oxidative damage to biomolecules has been associated with muscle fatigue after exercise (Essig and Nosek 1997; Finaud et al. 2006). The cellular responses to oxidative stress involve the increase in antioxidant enzymes (SOD, CAT and GPx) activity, and the

synthesis of heat shock proteins (HSP) (Leeuwenburgh and Heinecke 2001; Radak et al. 2001). The induction of the synthesis of HSP has been observed after exposing cells, including lymphocytes, to a variety of stressful conditions such as hyperthermia, glucose deprivation, increased intracellular calcium ion concentration, oxidative stress and hypoxia, which also occur in exercise(Welch 1992; Rao et al. 1999; Febbraio et al. 2004). HSP induction after exercise has also been reported in humans (Puntschart et al. 1996).

In spite of the well-known beneficial effects of carbohydrate-electrolyte fluids intake on muscle fatigue prevention, there are few studies reporting whether those sports drinks effects could be related to alterations on redox parameters during exercise (McAnulty et al. 2003; McAnulty et al. 2007; McAnulty et al. 2007; Nieman et al. 2005). Particularly, we haven't found in the literature any study testing this effect in football. Thus, the aim of this study was to evaluate the oxidative stress parameters, and the expression of heat shock proteins (HSP70) in two different situations: with carbohydrate-electrolyte fluid (CHO-E) and with only water (WAT) in Brazilian young football players.

Material and Methods

Subjects

In this study, we analyzed blood samples obtained from twelve volunteer trained football players from Tamoio Football Clube (Brazil). Mean age, height, body mass, percentage body fat, and VO_2 max were, respectively, 17 ± 0.5 years, 177.32 ± 5 cm, 68.55 ± 6.71 kg, 9.75% ± 1.9% and 54.1 ± 4.7 ml $\cdot kg^{-1} \cdot min^{-1}$. Body fat percentage was estimated from sum of skinfolds according to Jackson & Pollock(Jackson and Pollock 1978). All players gave written informed consent to participate after the details of the study had been explained to them. The

study was approved by the Research Ethics Committee of Universidade Federal do Rio Grande do Sul (UFRGS) Protocol 2006658 . Subjects agreed to avoid the use of large-dose vitamin or mineral supplements (above 100% of recommended dietary allowances), nutritional supplements, ergogenic aids, herbs, and medications known to possibly affect oxidative stress for 1 week before test sessions. The athletes should not drink alcoholic beverages or caffeine drinks for at least 24 hours before exercise day. All subjects were informed about the possible risks and discomforts during the exercise protocol, and all signed a consent form. In order to ensure a state of euhydration, the athletes were fed 4 hours before training, and consumed 500 ml of water 2 hours before test sessions.

Experimental Design

All participants completed 2 training sessions separated by a period of at least 7 days, under similar environmental conditions (18–20 °C) and relative humidity of 54-65 percent. The athletes did not practice physical activity 48 hours before the test. The order of these trials was randomized. On each occasion, participants consumed either a carbohydrate-electrolyte fluid or commercial water.

Experimental protocol

All players performed a typical training of their routine, a repeated sprint protocol. The repeated sprint test consisted of five 600 m sprints, separated by a 2.5 min period of recovery, repeated 3 times (3X5x600m). Tests were performed in a grass field. Blood samples (10 ml) were collected from antecubital region vein, before, immediately after and 6 hours after exercise.

Fluid Consumption

During the training, all players had access to commercial water or carbohydrate-electrolyte solution in individually identified drink bottles. The chosen commercial sports drink presents 6 % of carbohydrate and lemon flavor. This product contains, in 100 ml, 6g of carbohydrate (6 %), 45mg of sodium, 12.5mg of potassium, 42mg of chloride, and osmolality 260-290 mOsmol/g. Fluid was replaced (200 ml) before training and every 20 minutes of training (totaling 1000ml and 60g of carbohydrate per hour) such as standardizes the ACSM (2007) recommendation.

Sweat loss, Sweat Rate and Dehydration

Before and after training, all players were weighed wearing only underpants, using a digital balance (Plena, model MS-601). Players were asked to micturate and defecate if necessary immediately before the measurement of body mass. Sweat loss was calculated from the change in body mass after correction for fluid intake. The relatively small changes in mass due to substrate oxidation and other sources of water loss were ignored. Sweat rate in ml $\times$ h^{-1} was calculated using the following formula: sweat rate = preexercise body mass – postexercise body mass + fluid intake – urine volume/exercise time in hours(Casa et al. 2000). The dehydration was calculated by the percentage of difference between the initial and final weight.

Blood Glucose and osmolality

The concentration of Glucose (mg/dl) was obtained through the blood of digital pulp and immediately measured with glucose analyzer (Accu Check Active, Roche, Germany), before and after exercise. The plasma osmolality (mOsm/kg) was measured with Vapro® Vapor Pressure Osmometer (Wescor., Logan, USA).

Antioxidant enzyme activities

The superoxide dismutase (SOD, E.C. 1.15.1.1) activity in plasma samples was spectrophotometrically measured by the inhibition of the rate of auto-catalytic adrenochrome formation in a reaction buffer containing 50 mM glycine–NaOH buffer (pH10.2), 1 mM adrenaline, and 1 mM catalase(Klamt et al. 2002). (CAT, E.C. 1.11.1.6) activity in erythrocytes was assessed by measuring the rate of hydrogen peroxide (H_2O_2) consumption at 240 nm as previously described (Aebi 1984).

Protein carbonyl groups

The carbonyl groups were determined (Levine et al. 1990) as an index of protein oxidative damage. Plasma proteins (2 mg) were precipitated by incubating with trichloroacetic acid (TCA) 20% for 5 min on ice, and then centrifuged at 4000 ×g for 5 min. The pellet was dissolved in 100 µl of NaOH 0.2 M, and 100 µl of HCl 2 M or 10 mM of 2,4-dinitrophenylhydrazine (DNPH) in HCl 2 M was added to duplicate aliquots for blanks or for carbonyl groups derivatizing, respectively. Samples were maintained for 30 min at room temperature. Proteins were precipitated with TCA, and washed three times with 500 µl 1:1 ethanol: ethyl acetate to remove excess of DNPH. Samples were dissolved in 200 µl 6 M guanidine in 20 mM KH_2PO_4, pH 2.3, and the absorbance was read at 370 nm. The carbonyl content (nmol/mg protein) was calculated using a molar extinction coefficient of 22,000 M/cm at 370 nm after subtraction of the blank absorbance.

Lipid oxidative damage

Lipoperoxidation was assessed by the quantification of thiobarbituric acid-reactive species (TBARS) as previously described (Esterbauer and Cheeseman

1990). Briefly, plasma samples were mixed with 600 µL of TCA 10%, centrifuged (10,000 ×g; 10 min). After, 500 µL of the supernatant was mixed with 500 µL of thiobarbituric acid 0.67% (TBA), and heated in a boiling water bath for 15 min. TBARS were determined at 532 nm.

Hsp70 levels

The expression of HSP 70 was analyzed as previously described (Antunes-Neto el al. 2006). Whole blood was layered carefully 1:1 onto Histopaque-1077 (Sigma Diagnostics, St Louis, USA) and centrifuged at 400 g for exactly 30 min .The total leukocytes was aspirated with a Pasteur pipette and washed by suspending in 3 volumes of isotonic phosphate buffered saline solution and centrifuged at 250 x g for 10 min (3 times). The final leukocyte pellet was diluted in sample buffer (pH 6.8) containing 180 mM Tris –HCl, 30% glycerol, 6.9% SDS and 200 mM dithiothreitol. These samples were stored at -80 ºC until SDS/PAGE separation using 12.5% acrylamide gels, in which 60 ug of total leukocyte protein was loaded. Proteins were electrotransfered to polyvinylidene difluoride (PVDF) membranes using a transfer system (Amersham Pharmacia, Uppsala, Sweden). After protein transfer, PVDF membranes were blocked with 5% nonfat dried milk powder in T-TBS (TBS; 150mM NaCl and 20mM Tris–HCl, pH 7.5 plus 0.1% Tween 20) for 1 h. Blots were washed for 1 min in T-TBS and incubated overnight at 4ºC with a monoclonal antibody specific for HSP70 (SPA-810, StressGen, Canada) diluted 1:500 with T-TBS. The membranes were then washed three times (10 min each) with T-TBS and incubated for 1 h with the secondary antibody (goat anti-mouse IgG HRP-conjugated) diluted 1:5000 in T-TBS. After washing, HRP substrate was incubated, and bands were detected using X-ray films. Bands were quantified using Image-J software.

Statistical Analyses

The data had been structuralized and analyzed using statistical package SPSS (Statistical Package will be Social Sciences), version 12.0 for Windows. The tests of Shapiro-Wilk and Levene had been used to verify estimated normality and the homogeneity of the variances, respectively. ANOVA one way was used and post hoc of Tukey. The results had been expressed on average ± error-standard and the accepted level of significance was $p < 0,05$.

Results

Sweat loss, sweat rate, dehydration, osmolality and glucose

The sweat loss was 1.45 ± 0.16 and 1.68±0.22, for the WAT and CHO-E groups, respectively, and no significant differences was showed (Figure 1A).Similary, Sweat Rate is 14.50±1,6 and 16.83±2.27 ml/min for the WAT and CHO-E groups, respectively, and also no significant differences were showed (Figure 1B).Athletes dehydration 0.67 ± 0.22% and 1.08 ± 0.36% of their body mass at the end of the training, for the WAT and CHO-E groups, respectively, and no significant differences was demostrated (Figure 1C)..Plasma osmolality was 269.3 ± 4.62 mOsmol/kg at rest and 267.3 ± 1.65 mOsmol/kg after exercise in WAT-Group and 277.5 ± 6,21 mOsmol/kg after exercise in CHO-E group (Figure 1D). Data suggest that reposition with CHO-E fluid do not alter sweat loss, sweat rate, dehydration and plasma osmolality when compared with WAT – Group. In addition, CHO-E reposition promoted a significant increase in blood glucose levels after exercise and compared to WAT - group. Blood glucose in rest was 82.16 ± 2.72 mg/dl and 86.92 ± 2.55 mg/dl after exercise in WAT group. In CHO-E group, glucose levels significantly increased to 110.7 ± 5.96 mg/dl after exercise (Figure 2A).

Antioxidant Enzyme Activies

Plasma SOD activity increased significantly in CHO-E group from 2.83 ± 0.075 U SOD/mg protein at rest to 3.379 ± 0.186 U SOD / mg protein after exercise, but did not change significantly in WAT group, being 2.66 ± 0.09 U SOD/mg protein after exercise(figure 2B). Catalase activity did not alter significantly in both situations after exercise, being 26.84 ± 0.97 at rest and 24.21 ± 0.82 U CAT/mg protein after exercise in WAT group and 28.52 ± 2.81 U CAT/mg protein after exercise in CHO-E group (Figure 2C). The relation between enzymes SOD/CAT did not alter significantly in both groups and between groups (Figure 2D).

Lipid and protein oxidative damage

Lipid peroxidation, which was measured by TBARS assay, increased significantly from 0.33 ± 0.015 nmol/mg protein at rest to 0.51 ± 0.015 nmol/mg protein after exercise in WAT group. In CHO-E reposition group, lipoperoxidation did not increase after exercise, being 0.39 ± 0.03 nmol/ mg protein after exercise(Figure 3A). On the other hand, plasma protein carbonyl groups increased significantly in CHO-E group after exercise, being 0.89 ± 0.069 nmol/mg protein at rest and 1.48 ± 0.26 nmol/mg protein after exercise, but did not alter significantly in WAT – group, being 0.93 ± 0.045 nmol/mg protein after exercise(Figure 3B).

Hsp70 expression

Finally, we performed western blot analysis in protein leukocyte homogenates isolated 6 h hours after exercise protocol in order to detect heat-shock protein 70 (Hsp70) levels. As shown in figure 3C, HSP 70 level were increased in

WAT- group compared to basal levels (resting). On the other hand CHO-E group did not alters significantly.

Discussion

We haven't found significant differences in sweat loss and sweat rate between the groups, demonstrating that the CHO-E does not modify these termoregulation parameters. Factors such as dehydration and hyperthermia are important contributors to fatigue in football players (Sawka et al. 2007; Maughan et al. 2005; Krustrup et al. 2006). Several studies have shown that carbohydrate reposition delayed fatigue and improved endurance-running compared with placebo or water intake (Davis et al. 2000; Nicholas et al. 1995; Welsh et al. 2002)

In the current study, athletes lost 0.67 ± 0.22% and 1.08 ± 0.36% of their body mass at the end of the training, in the WAT and CHO-E groups, respectively. Many authors have reported that football players have considerable percentage of dehydration(Barr 1999; Da Silva and Fernandez 2003; Maughan et al. 2005; Maughan et al. 2004), which could reach 1.2 % and 1.4% after training sessions and competition (Broad et al. 1996). The American College of Sports Medicine (Sawka et al. 2007) reports that a percentage equal to or greater than 2% on fluid loss is related to loss in performance efficiency. Thus, it appears that the fluid loss in our model was not an important component since we obtained values lower than 2 % after the exercise protocol. There wasn't significant difference in the plasma osmolality before and after the exercise in both groups, the values show that the individuals were not dehydrated using this technique as a reference. American College of Sports

Medicine postulates that the plasma osmolality must be higher than 290 mOsmol/kg to characterize dehydration.

Data presented here show a higher blood glucose level in CHO-E group than WAT group after the intermittent exercise, probably, for the carbohydrate consumption during exercise. Besides dehydration and hyperthermia, glucose deprivation has been associated with oxidative stress, cell damage and muscle fatigue during exercise. Evidence suggests that glucose deprivation may induce oxidative stress through the generation of superoxide and hydrogen peroxide during mitochondrial respiration (Lord-Fontaine and verill-Bates 2002), and by increasing the plasma levels of stress-response hormones as adrenaline which can undergo autooxidation to generate ROS, mainly superoxide anion (McAnulty et al. 2003; Nieman et al. 2005). Thus, carbohydrate ingestion during exercise might diminish oxidative stress by decreasing the stress-hormone levels and mitochondrial ROS production.

It may be explained that high-intensity physical exercise disrupts the fragile balance between oxidants and antioxidant defenses. Cellular defense against superoxide radicals is provided by SOD. SOD dismutates superoxide radicals to form H2O2 and O2 (Inal et al. 2001; Halliwell 2007). Plasma SOD increases activities in CHO-E group compared to WAT group after exercise. These increases in SOD activity may suggest a response to anion superoxide production induced by exhaustive exercise(Powers and Lennon 1999).

Brites et al (1999) observed a similar relationship, with higher resting plasma SOD activity in football players compared to untrained subjects. On the other hand research shows that the CHO-E intake during exercise would prevent oxidative stress by increasing blood glucose levels, consequently

decreasing stress-hormone levels secretion and thus ROS formation from its autooxidation (McAnulty et al. 2003; McAnulty et al. 2007; Nieman et al. 2005).

As an antioxidant enzyme, CAT catalyses the breakdown of H_2O_2 to form water and O_2. To maintain catalytic activity, CAT requires Fe^{3+} as a cofactor (Powers and Lennon 1999).There were no alterations in the activity of the CAT enzyme after exercise in both CHO-E and WAT group. Similar to our study, Rokitzki et al. (1994) found no difference in erythrocyte CAT activity following a marathon running. In this context, studies report decreases or non alterations in erythrocyte CAT activity immediately after the exercise protocol (Aguilo et al. 2000; Tauler et al. 2007; Lekhi et al. 2007) followed by increases in the enzyme expression and activity at later time suggests adaptative responses. As example, Marzatico et al. (1997) reported that sprinters who performed a sprint-type exercise did not present alterations in erythrocyte catalase activity, but distance runners who performed an endurance exercise showed increases in CAT activity at 24 and 48 h post-exercise.

The imbalance between the antioxidant enzyme catalase (CAT) and superoxide dismutase (SOD) can be followed by oxidative damage (Ritter et al. 2003; Andrades et al. 2005). This is accompanied by a differential modulation of antioxidant defenses that could result in hydrogen peroxide accumulation and potential for oxidative damage. In our study the sod/cat balance in both groups did not have difference when compared with the rest and between groups. Thus showing a balance in the activity of these two antioxidants enzymes.

Lipid and protein oxidative damage

In this study, CHO-E reposition did not alter the exercise-induced TBARS levels but water intake (WAT group) presented significantly higher

lipoperoxidation levels in plasma. Differently, McAnulty et al (2003) showed that despite an attenuation in the cortisol response, carbohydrate alone compared to placebo ingestion does not attenuate the increase in oxidative stress or modulate plasma antioxidant potential in athletes running 3 h at 70% VO2(max).

Karolkiewicz et al. (2001) studied the effect of a carbohydrate and protein supplement on TBARS in 19 teenage track and field athletes divided into supplement and placebo groups and carbohydrate and protein supplementation have no effect on plasma TBARS. Carbohydrate supplementation can also serve to decrease free fat acids delivery and lower whole-body fat-oxidation rates during exercise, It is probable that, in the WAT group more lipid mobilization has occurred, which can have favored the lipoperoxidation.. In addition, Wojtaszewski et al. (1999) showed a inhibitory effect more lipid mobilization of glucose ingestion on hormone sensitive lipase activity (HSL). Watt et al. (2004) demonstrated that HSL is blunted during exercise when glucose is ingested, most likely mediated by increased insulin and decreased epinephrine.

In spite of no increase in lipoperoxidation, the data shows a significant increase of plasma protein carbonyl groups following CHO-E reposition, that did not occur in WAT group after exercise. In a pro-oxidant environment, as occurs in exercise, carbohydrates can be oxidized to form ROS and reactive carbonyl derivatives. In addition, proteins may be modified with carbohydrates through non-enzymatic mechanisms of carbonyl formation (Miyata et al. 1998). However, to determine whether the increase in plasma protein carbonylation reflects a systemic effect of CHO-E reposition, or if it is only a consequence of the increase in plasma carbohydrate levels leading to plasma

compartmentalized effects, more investigation is needed. The hypothesis that exercise increases oxidative stress is supported by the observation that blood levels of protein carbonyl groups increased significantly after exercise. The oxidative protein damage in this group could indicate that CHO-E replacement induces an imbalance between ROS production and antioxidant defenses, which leads to the development of oxidative stress.

Interesting data was obtained from Hsp70 determination in leukocytes. The levels of the chaperone Hsp70 have been considered an important biomarker of the diverse systemic stress, particularly thermal, metabolic and oxidative stress, being useful to evaluate physical stress after exercise (Febbraio et al. 2004; Suzuki et a.l 2006). In our model, we found that CHO-E intake did not induce Hsp70 expression compared to WAT group. This effect on Hsp70 expression could be associated with the increase of the protein carbonylation in CHO-E group. Therefore, chaperonas plays an important role in the protection of cellular proteins, and its little expression in group CHO-E, can´t have had a beneficial effect; therefore in this particular group there was a very significant increase protein oxidative damage.

Febbraio et al. (2004) showed that glucose deprivation and reduced glycogen availability – which occurs during exercise – increase Hsp70 expression. During CHO reposition, the rise in hepatic glucose production is suppressed by glucose ingestion because the demand for glucose is met by the ingested carbohydrate. Hence, the metabolic stress placed on the liver may be reduced under such circumstances, ultimately resulting in a decreased Hsp70 expression (Jeukendrup et al. 1999).

In our study we also found higher levels of the expression of HSP70 in the group that did not consume carbohydrate (WAT), however, we questioned if this fact is really beneficial, since we found other markers of oxidative stress in the group that received glucose. The increase in the activity of SOD enzyme could have been an antioxidant (beneficial) adaptation as well as a result of the increase in the superoxide production (deleterious effect). In contrast to some studies that showed that carbohydrate consumption attenuates production of free radicals (Davis et al. 1997; Davis et al. 2000; McAnulty et al. 2005; McAnulty et al. 2003; McAnulty et al. 2007), in our study, in this protocol of training, the results don't show benefits regarding the carbohydrate consumption. The beneficial effects of the CHO-E reposition on fatigue and systemic stress have been well accepted among elite athletes. However, this is the first study showing a comparison between CHO-E and WAT reposition with oxidative stress parameters on football players.

Conclusions

Data presented here showed results on antioxidant enzymes modulation, lipoperoxidation, carbonylation, Hsp70 expression, and thermal regulation following CHO-E supplementation during an intermittent exercise protocol. This work may thus contribute to the understanding of beneficial or deleterious effects of CHO-E reposition on stress parameters in elite athletes. In this case, we have to be careful when preconizing the exaggerated use of carbohydrates during exercise, because oxidation rate of exogenous glucose can reach about 1.2 g/min (Jeukendrup et al. 1999; Yan Burelle 2006). More studies with different and lesser amounts of carbohydrates per hour during the exercise

must be carried through for a safe lapsing of this nutrient for intense exercises such football training.

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FIGURES

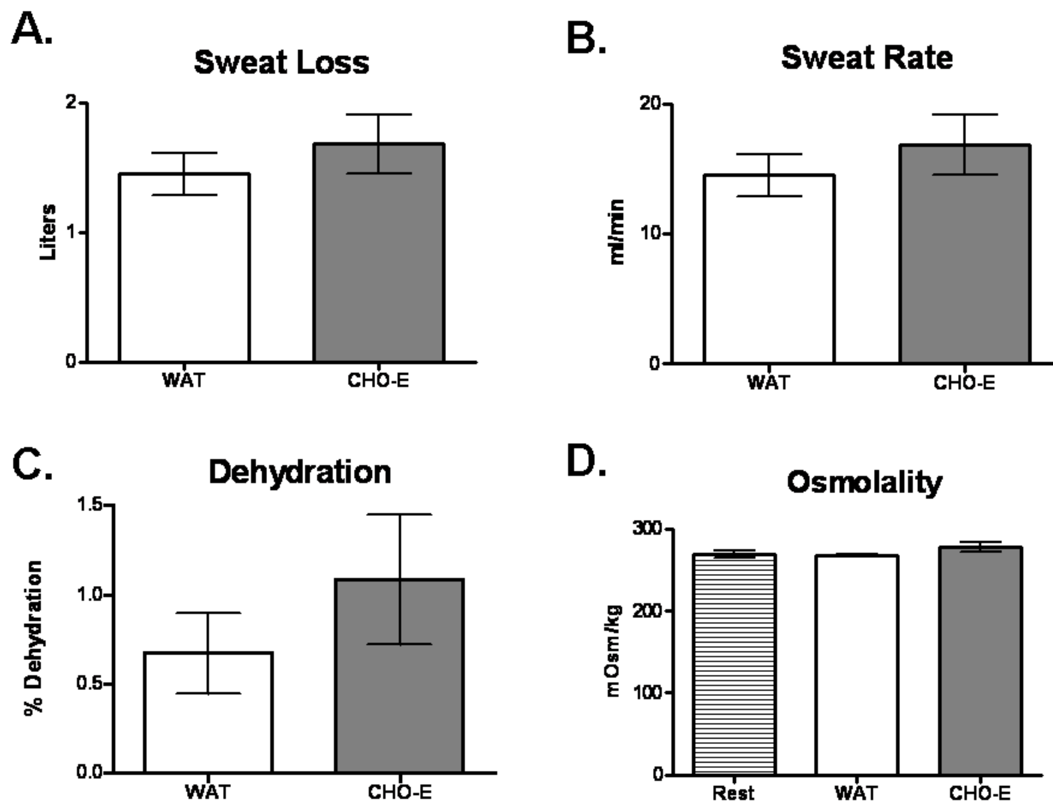

Figure 1: (A) Sweat Loss, (B) Sweat Rate, (C) Dehydratation, and (D) Osmolality after exercise (n=12). Results are expressed as means ± SEM.

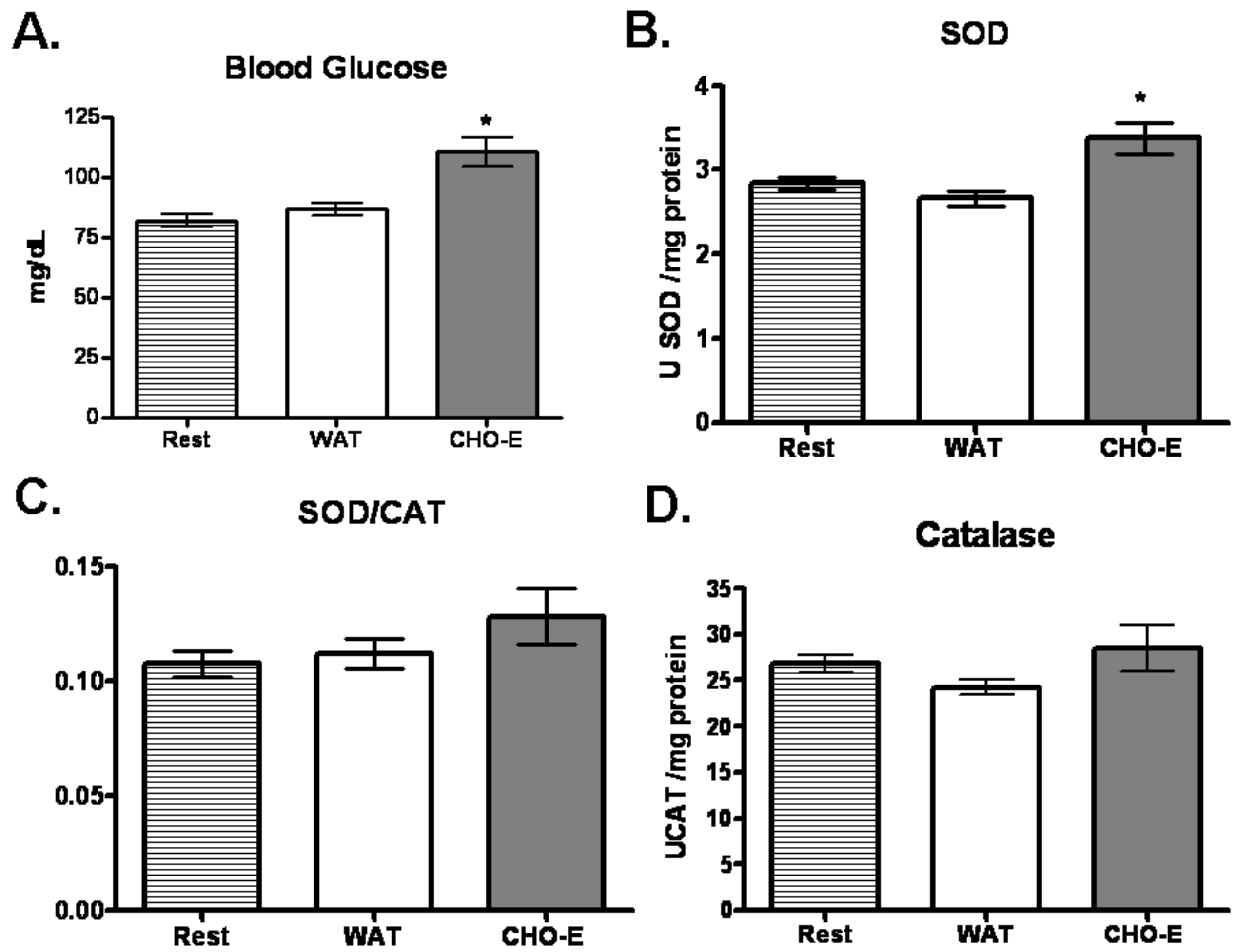

Figure 2. (A) Blood glucose, (B) Superoxide Dismutase activity (SOD); (C) Erythrocyte catalase activity (CAT) and (D) SOD/CAT Balance (n=12).. Results are expressed as means ± SEM. * Different from WAT group (p<0.05)

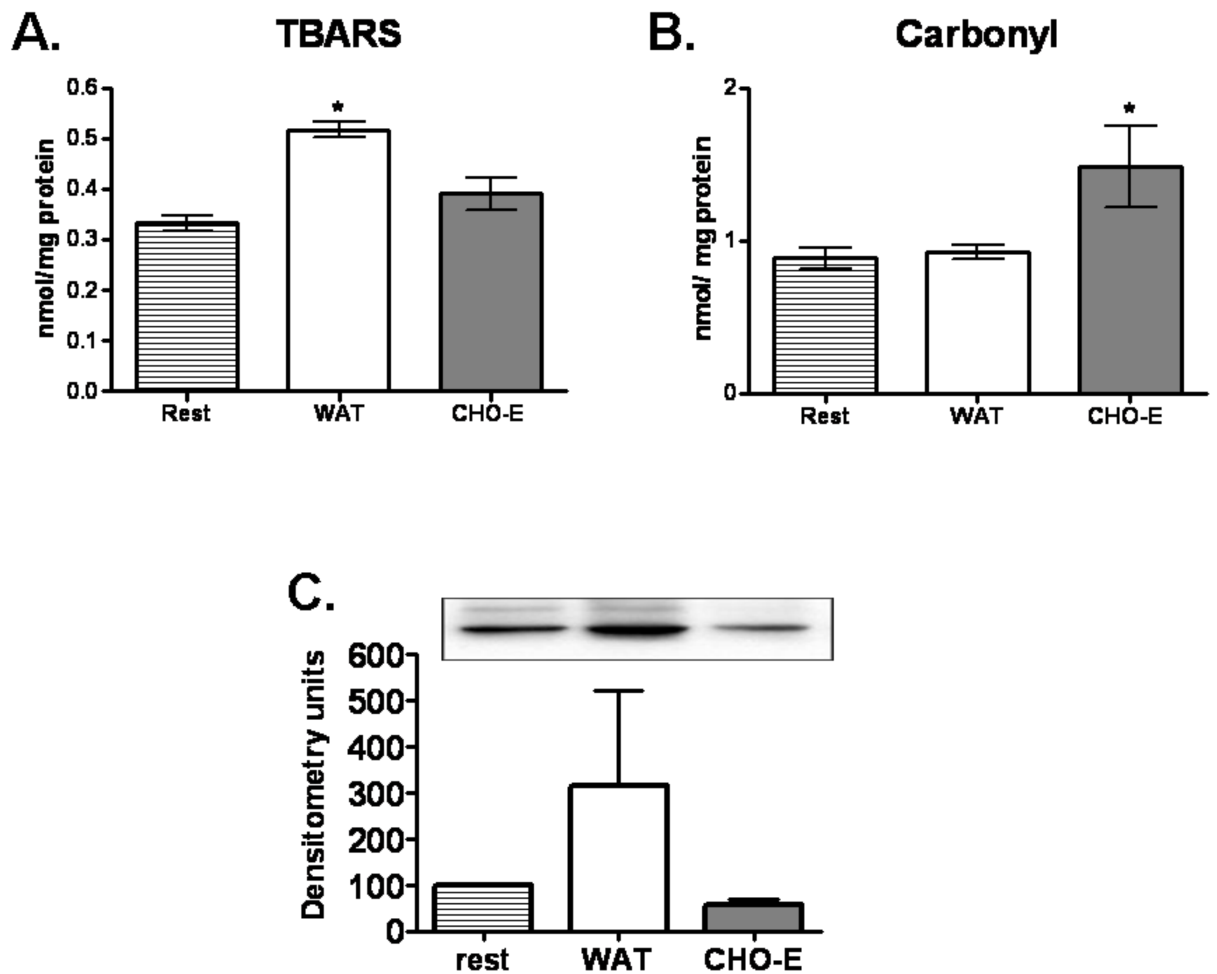

Figure 3: Oxidative stress parameters in WAT and CHO-E supplemented athletes. (A) Thiobarbituric acid reactive substances (TBARS) in plasma after exercise; (B) Plasma levels of protein carbonyl groups as detected by dinitrophenylhydrazine method. (C) Hsp70 immunocontent in leukocytes isolated before and 6 hours after exercise protocol. Representative immunobloting with respective densitometry. Data were compared to resting levels and groups (n=12). Results are expressed as means ± SEM. * Different from WAT group ($p<0.05$)

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PARTE III

4. DISCUSSÃO

Fatores como desidratação e hipertermia podem contribuir para o desenvolvimento de fadiga durante treinos e jogos de futebol (Sawka et al., 2007; Maughan et al., 2005; Krustrup et al., 2006). No entanto, não encontramos diferenças significativas no suor perdido, taxa de sudorese, desidratação e osmolidade do plasma, demonstrando que a reposição com CHO-E não modificou estes parâmetros termorregulatórios. No experimento sem intervenção nutricional (DES) nós observamos que o percentual de suor perdido dos atletas foi de 1,8% da massa corporal. No experimento WAT e CHO-E os atletas desidrataram 0.67 ± 0.22% e 1.08 ± 0.36%, respectivamente, após a sessão de exercício.

A American College of Sports Medicine (Sawka et al., 2007) preconiza que percentuais de desidratação maiores que 2% e osmolidade plasmática maior que 290 mOsmol/kg podem acarretar prejuízos na performance e na saúde. Entretanto, em nosso estudo o fator desidratação não pôde ser relacionado com o aumento de alguns parâmetros de estresse oxidativo ou de prejuízos para a performance e saúde, pois o percentual de desidratação foi menor de 2% nas três sessões experimentais e a osmolalidade do plasma menor que 290 mOsmol/kg.

Encontramos um aumento significativo da glicemia no protocolo DES. Este fato pode ser explicado talvez, por fatores hormonais e fisiológicos que regulam a glicemia. Durante o exercício, os níveis de glicose sanguínea são mantidos pela glicogenólise hepática ou gliconeogênese e em exercício de alta intensidade, a glicogenólise muscular é a principal via metabólica fornecedora de energia. A glicogenólise hepática, tem como principal objetivo a liberação

de glicose para a corrente sanguínea, durante o exercício, nos primeiros 40 minutos, pode ocorrer um aumento da glicemia, por ativação desta rota. O aumento dos níveis de lactato sanguíneo nesta sessão experimental sugere que a glicose anaeróbica pode estar ativada, sendo também fornecedora de energia nesses “sprints”.

Em nosso estudo experimental com intervenção nutricional (WAT e CHO-E) encontramos níveis significativamente maiores de glicose no grupo CHO-E quando comparados ao grupo WAT, após o exercício. Provavelmente pelo consumo de carboidratos que ocorreu neste grupo.

Diversos estudos mostram que a reposição de carboidratos pode diminuir a fadiga e promover a performance quando comparados à reposição somente com água (Nicholas et al., 1995, Davis, Welsh e Alderson, 2000, Welsh et al., 2002).

Além da desidratação e hipertermia, a privação de glicose vem sendo associada com o estresse oxidativo, dano celular e fadiga muscular durante o exercício. Evidências sugerem que a privação de glicose pode induzir o estresse oxidativo através da geração do radical superóxido e peróxido de hidrogênio durante a respiração mitocondrial (Lord-Fontaine & Verill-Bates, 2002), e pelo aumento dos níveis plasmáticos de hormônios do estresse como a adrenalina, a qual, pode ser auto-oxidada e gerar ERO, principalmente o ânion superóxido (McAnulty et al., 2003; Nieman et al., 2005). Assim o carboidrato ingerido durante o exercício pode diminuir os níveis de hormônios do estresse e a respiração mitocondrial durante o exercício. No entanto em nosso estudo, encontramos resultados bem interessantes, mostrando aumento

de alguns parâmetros de estresse oxidativo no grupo que recebeu reposição de carboidratos.

Na primeira sessão experimental (DES) verificamos um aumento na atividade tanto da enzima SOD como da enzima CAT. Por tratar-se de um exercício da alta intensidade, a produção de ERO pode estar ocorrendo por diferentes vias (p.e.isquemia-reperfusão/ xantina oxidase), e o aumento na produção de determinadas espécies reativas pode desencadear também um aumento na atividade das enzimas antioxidantes (Ji et al 1993). No entanto no segundo experimento houve um aumento significativo após o exercício apenas na atividade da enzima SOD no grupo CHO-E, não ocorrendo alterações na atividade da atividade da SOD no grupo WAT e também da enzima CAT em ambas as situações (WAT e CHO-E). Estudos reportam que o exercício agudo contribui para a produção de espécies reativas de oxigênio, especialmente em exercício de alta intensidade (Alessio et al., 2000; Goldfarb, 1993) e um aumento na produção de ERO pode estar associado com o aumento das enzimas antioxidantes correspondentes. Foi observada uma relação similar, mostrando a atividade da enzima SOD aumentou após um exercício de futebol (Brites et al., 1999).

Pode-se assumir que o exercício físico de alta intensidade pode alterar o frágil balanço entre agentes oxidantes e defesas antioxidantes. A defesa celular para o anion superóxido é realizada pela enzima SOD, que transforma o superóxido em peróxido de hidrogênio e água (Halliwell, 2007). A enzima SOD aumentou sua atividade no grupo CHO-E quando comparada ao grupo WAT podendo sugerir uma resposta adaptativa para a produção de superóxido

induzida pelo exercício físico exaustivo (efeitos benéficos) ou podendo estar associada à produção exagerada de ERO neste grupo que recebeu reposição de carboidratos (efeitos deletérios).

O mais interessante é que neste mesmo grupo (CHO-E) não houve alterações nos parâmetros de lipoperoxidação antes e após o exercício, entretanto houve um aumento dos parâmetros de carbonilação protéica, o que pode ser justificado por um aumento na produção de radicais livres.

Por outro lado, pesquisas mostram que a ingestão de carboidratos durante o exercício pode prevenir o aumento nos parâmetros de estresse oxidativo pelo aumento da glicemia, conseqüentemente pala diminuição da secreção de cortisol e catecolaminas (McAnulty et al., 2003; McAnulty et al., 2007; Nieman et al., 2005).

Encontramos um aumento significativo da CAT após o exercício no grupo (DES). Não houve diferença significativa em ambos os grupos com intervenção nutricional (WAT e CHO-E) na atividade da enzima CAT. Como enzima antioxidante a CAT catalisa a "quebra" do peróxido de hidrogênio em água e oxigênio e para manter a atividade catalítica a CAT requer Fe3+ como cofator (Halliwell & Gutteridge, 2007).

Corroborando com nosso segundo experimento (com intervenção nutricional), Marzatico et al. (1997) mostrou que corredores de "sprints" não alteraram a atividade da CAT após o exercício. Rokitzki et al (1994), também não encontrou diferença na atividade eritrocitica da CAT após um exercício de maratona. Alguns autores descrevem um aumento na expressão e na atividade da CAT em momentos posteriores a prática da atividade física, sugerindo uma

resposta de adaptação. Como por exemplo, uma alteração na atividade da enzima CAT 24h e 48h após o exercício (Marzatico et al., 1997).

Um dos radicais formados durante o exercício é o superóxido, que é convertido em peróxido de hidrogênio pela SOD, e este peróxido é transformado em água e oxigênio pela CAT. Uma ralação importante é o balanço entre estas duas enzimas, o equilíbrio entre a SOD e a CAT. Com uma maior atividade da SOD a concentração de peróxido irá aumentar na corrente sanguínea, podendo as ERO reagir com outras moléculas, aumentando o estresse oxidativo. A relação SOD/CAT não sofreu alterações significativas em ambos os experimentos (com e sem intervenção nutricional), mostrando que neste estudo não houve um desequilíbrio entre estas enzimas. Estudos recentes do nosso grupo (Ritter et al., 2003; Andrades et al., 2005) mostram que o desbalanço entre estas enzimas pode gerar dano oxidativo.

A reposição com CHO-E não alterou os níveis de TBARS, no entanto o grupo WAT apresentou níveis significativamente maiores de liperoxidação após o exercício quando comparado ao repouso e ao grupo CHO-E. Diferentemente, foi demonstrado que mesmo com uma atenuação da concentração do cortisol, o carboidrato quando comparado ao placebo não foi eficiente em prevenir aumentos em parâmetros de estresse oxidativo (McAnulty et al., 2003).

Em um protocolo similar, mas com menos repetições, (Marzatico et al., 1997), estudaram atletas que percorreram 6 sprints de 150m e encontraram elevados níveis de MDA plasmáticos após o exercício. A primeira sessão experimental demonstrou que este tipo de exercício de alta intensidade, aumenta os níveis de MDA e carbonilação protéica, que são marcadores de

estresse oxidativo e também aumentam a atividade das enzimas antioxidantes CAT e SOD.

A peroxidação lipídica muda a fluidez da membrana e reduz a capacidade de manter em equilíbrio os gradientes de concentração e também pode aumentar a permeabilidade da membrana e a inflamação (Jenkins and Goldfarb, 1993; Radak et al., 2001).

Karolkiewicz (2001) estudou o efeito da suplementação de carboidratos e proteínas nos níveis de TBARS em 19 atletas adolescentes, e quando comparado ao placebo, nem o carboidrato nem a proteína alteraram significativamente os níveis de TBARS,

Durante o exercício ocorre mobilização de ácidos graxos livres para formação de energia, é provável que no grupo WAT tenha ocorrido uma maior mobilização de lipídios e isto possa ter favorecido a lipoperoxidação. Somando aos nossos resultados, Wojtaszewski (1999) mostrou um efeito inibitório da ingestão de glicose na atividade da lípase hormônio sensitiva (HSL). Watt et al (2003) demonstrou que a atividade HSL foi anulada durante o exercício quando houve uma ingestão de glicose, mostrando que estreita relação entre o aumento da insulina e a diminuição da epinefrina.

Por outro lado, o grupo CHO-E apresentou um aumento significativo da carbonilação protéica plasmática quando comparado ao repouso e ao grupo WAT. Em um estado pró-oxidante, como ocorre no exercício, carboidratos podem ser oxidados para formar ERO e produtos da carbonilação protéica e as proteínas podem ser modificadas por carboidratos através de mecanismos não-enzimaticos para formação de grupamentos carbonil (Miyata et al 1998).

Muitos estudos reportam o aumento ao dano oxidativo em proteínas e lipídeos após um protocolo de exercício (Alessio et., al. 2000; Blommer et al. 2007), Mostrando que imediatamente após o exercício há um aumento em grupamentos carbonil e sugerem que tanto o exercício de longa duração, como os de alta intensidade aumentam a concentração de grupamentos carbonil após o exercício.

Entretanto, para determinar se o aumento da carbonilação protéica plasmática reflete em um efeito deletério sistêmico com a reposição de carboidratos, mais pesquisas são necessárias. Um interessante dado foi obtido analisando a expressão das proteínas de choque que térmico (HSP70) nas duas sessões do protocolo com intervenção nutricional. Os níveis de HSP70 vêm sendo considerados bons marcadores de diversos estresses, particularmente hipertermia, estresse oxidativo, e estresse ocasionado pelo exercício.

Em nosso modelo, encontramos que a ingestão de CHO-E não alterou significativamente a expressão de HSP70 quando comparada ao repouso, e que o grupo WAT aumentou significativamente sua expressão. Esse efeito da não-indução das HSP70 pode estar relacionado com o aumento da carbonilação protéica neste mesmo grupo, pois as chaperonas possuem papel essencial na proteção de proteínas celulares, e esta não indução no grupo CHO-E pode não estar tendo um efeito benéfico.

Febbraio et al. (2004), por outro lado, mostraram que a privação de glicose e a redução do glicogênio muscular, que ocorre durante o exercício, pode estar associadas com um aumento na expressão de HSP70. Jeukendrup et al (1999) sugerem que durante a reposição com CHO, a taxa de produção

de glicose hepática é suprimida pela ingestão de carboidrato. Assim, o estresse metabólico no fígado é reduzido, resultando uma menor expressão de HSP70.

Em nosso estudo encontramos níveis elevados na expressão de HSP70 no grupo WAT, no entanto não encontramos níveis significativos de dano oxidativo a proteínas. Questionamos se é realmente benéfica a diminuição ou a não-expressão de HSP70 em determinadas situações. Em contraste existem estudos (Febbraio et al., 2004; Jeukendrup et al., 1999) que demonstram que o consumo de glicose ou outros tipos de carboidrato atenuam a expressão de HSP70 e também de dano oxidativo.

5. CONCLUSÕES

A partir dos dados obtidos na presente dissertação, podemos concluir que:

1. O exercício físico intermitente por si só gera aumento nos parâmetros de estresse oxidativo.

2. A utilização de água (WAT) não alterou atividade das enzimas antioxidantes SOD e CAT e os níveis de dano oxidativo a proteínas, porém aumentou os níveis de peroxidação lipídica e expressão de HSP70. A Reposição com CHO-E aumentou significativamente a atividade da enzima SOD, o dano oxidativo a proteínas e não alterou outros parâmetros.

3. Os grupos WAT e CHO-E não apresentaram diferenças significativas na sua taxa de sudorese, percentual de desidratação, perda de suor e osmolalidade plasmática quando comparados. No entanto o grupo CHO-E apresentou uma maior glicemia após o exercício.

4. Não é possível afirmar que o carboidrato traz benefícios e diminuiu estresse oxidativo em atletas jogadores de futebol, mais estudos são necessários com diferentes quantidades de carboidratos para preconizar a utilização deste nutriente. Com os resultados encontrados podemos sugerir que a utilização de carboidratos deve ser realizada com cautela. Fatores hormonais e inflamatórios futuramente devem ser analisados para completar os dados de parâmetros do estresse oxidativo.

PERSPECTIVAS

Este é um estudo pioneiro na análise do estresse oxidativo e suplementação de carboidratos. Servirá de base para criação de projetos que possam verificar

não só parâmetros de estresse oxidativo, mas também de performance, termorregulação, inflamação e catabolismo muscular.

Como perspectiva temos como objetivo próximo analisar tais parâmetros e avaliar o efeito do carboidrato em todo contexto bioquímico-fisiológico, bem como adequar os resultados experimentais à prática do futebol como um alicerce para a preparação física de alto rendimento.

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ANEXOS

Universidade Federal do Rio Grande do Sul - UFRGS
Instituto de Ciências Básicas de Saúde - ICBS
Departamento de Bioquímica

TERMO DE CONSENTIMENTO INFORMADO

Ao assinar este documento, estou consentindo formalmente em participar da pesquisa da nutricionista Mariana Escobar, orientada pelo Prof. Dr.José Cláudio Fonseca Moreira, do Programa de Pós Graduação em Bioquímica - UFRGS. O estudo da pesquisadora Mariana Escobar tem o objetivo de verificar o efeito da Hidratação com água e hidratação com carboidratos e eletrólitos sobre parâmetros de estresse oxidativo e de lesão muscular em jogadores de futebol em um exercício físico intermitente. As informações coletadas neste estudo serão utilizadas para colaborar na definição de novas estratégias de intervenção de hidratação para manter e otimizar o desempenho do jogador de futebol durante uma partida, prevenindo ou amenizando o estresse oxidativo e/ou lesão muscular decorrente.

Recebi da pesquisadora as seguintes orientações:

1. A minha participação na pesquisa iniciará após a leitura, o esclarecimento de possíveis dúvidas e do meu consentimento livre e esclarecido por escrito. A assinatura do Termo de Consentimento Informado deverá ser em duas vias, permanecendo uma delas comigo.
2. Serei esclarecido sobre todos os procedimentos metodológicos.
3. Terei garantido a confidencialidade e o sigilo referente à minha pessoa, vinculados às informações do estudo.
4. Durante a minha participação na pesquisa, receberei acompanhamento e assistência da nutricionista Mariana Escobar.
5. A minha participação na pesquisa envolverá as seguintes fases: reunião para esclarecimento sobre a pesquisa e os testes que serão realizados, duas sessões de treino com duração em média de noventa minutos cada em meu local de treinamento. Cada sessão será realizada com, no mínimo uma semana de intervalo.
6. As sessões incluem a realização de um protocolo de exercícios (treinamento físico) elaborado pelo preparador físico responsável. No inicio e no final de cada sessão serão realizadas coletas de sangue por profissional habilitado (enfermeiro), totalizando duas coletas em cada sessão.
7. As sessões serão realizadas no campo do clube ao qual me encontro vinculado e o treinamento coordenado pelo preparador físico responsável: Bruno Follmer
8. Serei monitorado em relação ao peso corporal, ingestão de fluídos e percepção do esforço durante as atividades.
9. Terei consumo de líquido controlado e receberei líquido com carboidratos e eletrólitos em uma das sessões, conforme recomendação da pesquisadora.
10. Receberei orientações nutricionais básicas quanto à hidratação e alimentação no dia da sessão e no dia que antecede as sessões.

11. As sessões serão marcadas com antecedência de uma semana, respeitando o calendário de jogos e comunicando ao dirigente do clube de futebol para dispensa do trabalho físico nas 24 horas que antecedem às sessões.
12. No transcorrer ou após o exercício, poderão ocorrer alguns desconfortos como cansaço e câimbras.
13. A minha participação na pesquisa será voluntária. Concordando ou recusando em participar, não obterei vantagens ou serei prejudicado em meu clube de origem. A minha participação em todos os procedimentos da pesquisa, não implicará no pagamento ou recebimento de qualquer taxa, exceto se houver algum gasto adicional decorrente da minha participação.
14. No caso de ser menor de idade, meus pais ou responsável é que devem concordar ou não com a minha participação na pesquisa.
15. Necessitando quaisquer esclarecimentos sobre a pesquisa, entrarei em contato pessoal com a pesquisadora ou pelo telefone (51- 84287188).

Data:

Nome do Participante | Assinatura do Participante

Nome do Responsável | Assinatura do Responsável

Mariana Escobar
Pesquisadora responsável

Bruno Follmer
Preparador Físico responsável