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Tese de Doutorado

EFEITOS DA SUPLEMENTAÇÃO DE TAURINA OU FLAVONÓIDES DE CACAU ASSOCIADO À ACHOCOLATADO SOBRE A COMPOSIÇÃO CORPORAL, METABOLISMO DE CARBOIDRATOS E PROTEINAS, DESEMPENHO FISICO, DANO MUSCULAR E ESTRESSE OXIDATIVO EM ATLETAS

Flávia Giolo de Carvalho

ARARAQUARA/SP
2015

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Tese apresentada ao Programa de Pós Graduação em Alimentos e Nutrição da Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista "Julio de Mesquita Filho" – UNESP, como requisito para obtenção do título de Doutor em Ciências Nutricionais.

Orientadora: Profa. Dra. Ellen Cristini de Freitas.

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Muito Obrigada!!!

“If you have a dream,

give it a chance to happen”

Richard M. DeVos

LISTA DE ABREVIATURAS

ACSM	*American College of Sports Medicine*
ANVISA	Agencia Nacional de Vigilância Sanitária
ADA	*American Dietetic Association*
CAPES	Coordenação de Aperfeiçoamento Pessoal de Nível Superior
CDO	Cisteínadioxigenase
CHO	*Carboidrato; carbohydrate*
CHOC	Leite achocolatado; *chocolate milk*
CM	*Chocolate milk*
CK	*Creatine kinase*
CSDA	cisteínasulfinato descarboxilase
CocoaCHOC	Leite achocolatado adicionado de flavonóide de cacau; *chocolate milk with additional cocoa*
DOMS	Dor muscular tardia
DRI	*Dietary Reference Intakes*
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
GSH	Glutationa reduzida; *reduced glutatione*
GLUT 4	*Glucose transporter type 4*
HOMA-IR	*Homeostatic model assessment – insuline resistance*
HR	*Heart rate*
HSRC	*Human Subjects Review Committee*
LEFS	*Lower extremity functional scale*
LT	*Lactate threshold*
IOM	*Institute of medicine*
iNOS	Óxido nítrico sintase induzida
IRS	Substratos de insulina
ISSN	*International Society of Sports Nutrition*
MS	*Muscle soreness*
MDA	Malondialdeído; *malondiahdeide*
mTOR	*Mammalian Target of Rapamycin*

N ur	*24 hours nitrogen excretion*
NB	*Nitrogen Balance*
OH^-	Radical hidroxila
PKA	Proteína quinase A
PKC	Proteína quinase C
POST	*After treatment*
PRE	*Before treatment*
PTN	Proteína; *Protein*
PGC-1α	Proteínas co-ativadoras alfa
PPAR	Proliferadores de peroxissomos
RDA	Recomendações de Ingestão Diária
RNA	Ácido ribonucleico
RPE	*Rate of perceived exertion*
SBME	Sociedade Brasileira de Medicina Esportiva
TAUchoc	Suplementação de taurina e achocolatado
SD	*Standar deviation*
Tau-Cl	Taurina-cloramina
TAUT	*Taurine transporter*
TBARS	Substâncias reativas ao ácido tiobarbitúrico
TNF- α	Fator de Necrose Tumoral –α
VAS	*Visual analog scale*
Vmax	*Maximum aerobic velocity*
VO2 máx	Volume máximo de oxigênio
3-KTT	*3-km time trial*

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RESUMO

INTRODUÇÃO: A taurina é um composto nitrogenado que apresenta diversos efeitos fisiológicos benéficos podendo-se destacar ação antioxidante, aumento da força de contração muscular e da sensibilidade à insulina. A prática de triatlo, esporte de intensidade média a alta, provoca a elevação da taxa de consumo de oxigênio durante o exercício intenso e o consequentemente aumento da produção de radicais livres e o estresse oxidativo, podendo comprometer o desempenho do atleta. Devido à necessidade de treinamento semanal intenso, os triatletas necessitam de cuidados nutricionais que vão desde a adequação de calorias e macronutrientes até a utilização de nutrientes específicos que possam auxiliar para manutenção da saúde. Assim, acredita-se que a utilização de taurina associada ao achocolatado no pós-exercício, possa favorecer a ação metabólica geral da insulina de modo à regular o metabolismo dos carboidratos, auxiliar na recuperação muscular, na glicogênese e prevenir danos oxidativos decorrentes da atividade física intensa, e desta forma favorecer o desempenho atlético e o ganho de massa muscular de triatletas. **OBJETIVO:** Avaliar os efeitos da suplementação de taurina e leite com achocolatado sobre a composição corporal, desempenho atlético, metabolismo proteico e estresse oxidativo em triatletas de elite. **MÉTODOS:** Foi realizado um estudo duplo-cego, crossover, com *wash out* de 2 semanas, com 10 triatletas, do sexo masculino, com idade entre 25 a 35 anos, no qual foi oferecido três gramas de taurina ou placebo, e 400 ml de achocolatado pronto para beber durante o período de 8 semanas. Foi realizada coleta de amostras de sangue para a quantificação de insulina, glicose e de marcadores de estresse oxidativo (MDA, GSH e vitamina E) plasmáticos. Foi realizada a coleta de urina de 24 horas a fim de avaliar os efeitos da suplementação sobre o metabolismo protéico (concentração de nitrogênio urinário, creatinina e uréia). A composição corporal dos atletas foi avaliada pelo método de água marcada com Deutério. As avaliações ocorreram em quatro momentos: 1º- antes de iniciar a temporada de treinamento; 2º- após 8 semanas de treino; 3º- o no início da segunda temporada de treino e, 4º- após 8 semanas de treino e encerramento da suplementação. **RESULTADOS:** A suplementação de taurina associada à achocolatado durante o período de 8 semanas não resultou em alterações significativas na composição corporal, na concentração de glicose, insulina e de marcadores anti-oxidantes séricos, e também não interferiu na concentração de creatinina e uréia urinaria. Não foram constatadas alterações nos parâmetros aeróbios após o período de suplementação. No entanto foram constatadas reduções na excreção de nitrogênio urinário e na produção de marcadores de indicativos de peroxidação lipídica (MDA). **CONCLUSÃO:** A suplementação de taurina pode ser considerada como um possível recurso ergôgenico com a finalidade de prevenir o catabolismo protéico e favorecer a manutenção da massa magra, além de reduzir danos oxidativos decorrentes da atividade física intensa, e desta forma pode prevenir lesões musculares e beneficiar o desempenho de triatletas.

Palavras-chaves: taurina, achocolatado, triatlo, estresse oxidativo e composição corporal.

ABSTRACT

BACKGROUND: Taurine is a nitrogenous compound, which has several beneficial physiological effects like antioxidant action, and could increase muscle force contraction and insulin sensibility. Triathlon is a sport of medium to high intensity, that can increase oxygen consumption rate during intense exercise and consequently raise free radicals production and oxidative stress, which may compromise an athlete's performance. Due to the weekly intense training, triathletes need nutritional care ranging from calories and nutrients adequacy to the use of specific nutrients that can help to maintain health. Thus, it is believed that the use of taurine associated with chocolate milk after exercise can promote general metabolic insulin action in order to favor carbohydrates metabolism regulation, assist muscle recovery, improve glycogenesis, prevent oxidative damage and thus promote triathletes performance and muscle mass gain. **OBJECTIVE:** Evaluate the effects of taurine and chocolate milk supplementation in body composition, athletic performance, protein metabolism and oxidative stress in triathletes. **METHODS:** A double-blind, crossover, 2-week washout study was conducted with 10 male triathletes, aged 25 to 35 years, which capsules containing three grams of taurine or placebo and 400 ml of chocolate milk ready to drink was offered during eight weeks. In order to assess the effects of supplementation urinary nitrogen, creatinine and urea, and plasmatic glucose, insuline and oxidative stress markers (MDA, GSH and vitamin E) were quantified. Body composition was measured by the method of deuterium labeled water. The evaluations occurred in four stages: 1º - before starting the training season; 2º - after 8 weeks of training; 3º- before starting the second training season and 4º- after 8 weeks of training and supplementation completion. Data were organized according to the study groups on average and standard deviation with application of analysis of variance test for verifying significant differences ($p<0.05$). **RESULTS:** Taurine supplementation associated with chocolate milk during 8 weeks did not resulted in significant changes in body composition, glucose, insuline and antioxidant markers seric levels, also no changes were found in regards to urinary creatinine and urea. No significant changes were observed in the aerobic parameters post treatment. However, there were observed reductions in urinary nitrogen excretion by 24 hours day and in markers of lipid peroxidation (MDA). **CONCLUSION:** Taurine may be considered as a potential ergogenic aid in order to prevent protein catabolism and keep lean body mass and decrease oxidative damage produced by intense physical activity, and thus prevent muscle damage and benefit triathletes' performance.

Key words: taurine, chocolate milk, triathlon, oxidative stress, body composition.

SUMÁRIO

INTRODUÇÃO GERAL

A taurina é um composto nitrogenado intracelular livre, encontrado principalmente no coração, leucócitos, retina, sistema nervoso central, e principalmente no músculo (KIM et al, 2007). Este composto é considerado um aminoácido "semi-essencial" em humanos, uma vez que pode sersintetizada a partir deoutros aminoácidossulfurados, como metionina e cisteína, associados à vitamina B6. No entanto, a produçãoendógena é insuficiente, ea taurina deve ser também obtida através da dieta, principalmente em alimentos de origem animal e marinha (SZYMANSKI e WINIARSKA, 2008).

Pesquisadores têm relacionado à utilização de taurina como um potente antioxidante devido à presença de uma molécula de ácido sulfônico em sua estrutura química, a qual promove a conversão de íon cloro e ácido hipocloroso, substâncias altamente citotóxicas, em cloramina relativamente estável (TAPPAZ, 2004; ZHANG et al, 2004; SUN et al, 2012). Além disso, a hipotaurina, um precursor de taurina, pode inibir a peroxidação por atuar como carreador de radical hidroxila (OH-) e inibir a auto-oxidação de íons ferro (Fe_2^+) (TADOLINI et al, 1995).

Outro fato referenciado na literatura quanto à taurina é o aumento da sensibilidade à insulina. De acordo com Carneiro et al (2009), a taurina controla a homeostase da glicose através da regulação da expressão de genes necessários para estimular a secreção de insulina pelas células β, e assim melhora a sensibilidade periférica à insulina. Considerando a ação da taurina sobre a insulina, e que este hormônio apresenta um importante papel para os atletas por regular o metabolismo dos carboidratos, a utilização de taurina poderia favorecer o aumento da disponibilidade de glicose e consequentemente resultar em maior produção de energia, maior estímulo à síntese proteica e à ressíntese de glicogênio e possivelmente possa favorecer o desempenho físico de atletas.

O fornecimento de nutrientes específicos, como taurina, associado a nutrientes em qualidade e quantidade adequadas pode beneficiar o desempenho de atletas. Especificamente o triatlo é um esporte composto por três modalidades sendo a natação, corrida e ciclismo (BALIKIAN JR e DENADAI, 1999), e os praticantes desta modalidade realizam treinos de 20 horas semanais, e mais de uma vez ao dia, a fim de aperfeiçoar o desempenho nas três modalidades esportivas. Este fato implica no aumento das necessidades nutricionais, sendo

que uma alimentação adequada não garantirá por si o sucesso do triatleta, mas a sua ausência comprometerá a expressão de seu potencial máximo (BENTLEY et al, 2008). A falta de alimentos e líquidos adequados ao consumo após estas sessões de treino dificultam o suprimento da energia adicional necessária para a recuperação e ressíntese de glicogênio (NOGUEIRA e DA COSTA, 2004).

Além dos fatores relacionados aos cuidados nutricionais do atleta, é importante ressaltar algumas características fisiológicas relacionadas a esta modalidade esportiva. Os esportes intensos como triatlon e ultra-maratonas apresentam metabolismo predominantemente aeróbio (FRY, 2004). O treinamento aeróbio promove a elevação da taxa de consumo de oxigênio, podendo aumentar a produção de radicais livres durante o exercício intenso e promover um desequilíbrio oxidativo, e consequentemente comprometer o desempenho do atleta (BENTLEY et al, 2008; SUN et al, 2011; PINGITORE et al, 2015).

Em razão a todas as alterações fisiológicas decorrentes do exercício físico, a ingestão de nutrientes após o treino é essencial para o desempenho do atleta. Atualmente, são encontradas no mercado bebidas que auxiliam na recuperação no pós-exercício (PRICHETT et al, 2009). Autores relatam também a utilização de leites aromatizados, como leite com chocolate, para a suplementação de atletas devido a sua composição nutricional ser semelhantes à algumas bebidas repositoras, e, desta forma, possibilitando a obtenção de nutrientes e o reabastecimento do glicogênio depletado nos músculos durante o exercício, favorecendo assim a recuperação após o exercício (KARP et al, 2006; PRITCHETT et al, 2009).

Visto a importância de um aporte nutricional adequado considerando calorias, dentre carboidratos e proteínas, afim de maximizar a recuperação de glicogênio e síntese proteica (BERARDI et al, 2006) etambém as alterações metabólicas decorrente do esforço intenso, a utilização de taurina associada a uma fonte de proteínas de alto valor biológico pode auxiliar na prevenção de danos oxidativos e também favorecer a captação de glicose pelas células hepáticas e musculares, e consequentemente otimizar a síntese de proteínas e o desempenho dos triatletas.

OBJETIVOS

O presente projeto de pesquisa teve como objetivo avaliar os efeitos da suplementação de taurina e leite com achocolatado sobre a composição corporal, metabolismo de carboidratos e proteínas, estresse oxidativo e desempenho físico em triatletas nos seguintes parâmetros:

- Avaliação da ingestão alimentar habitual de triatletas;
- Determinação da composição corporal utilizando o método da água marcada com deutério dos triatletas antes e após os períodos de suplementação;
- Quantificação de níveis séricos de glicose, insulina e a sensibilidade à insulina antes e após os períodos de suplementação;
- Determinação de marcadores de metabolismo proteico (nitrogênio urinário, creatinina e ureia) antes e após os períodos de suplementação;
- Quantificação de marcadores de estresse oxidativo indicativos de peroxidação lipídica: malondialdeído (MDA), marcadores antioxidantes: glutationa reduzida (GSH) e vitamina E antes e após os períodos de suplementação;
- Análise do desempenho físico através da avaliação da capacidade aeróbia dos triatletas antes e após os períodos de suplementação.

A presente Tese foi elaborada em formato de capítulos, sendo que o primeiro capítulo aborda a revisão de literatura à repeito de taurina e triatletas, o segundo e terceiro capítulos abordam os resultados obtidos na forma de dois artigos científicos. No Capítulo II o artigo teve como objetivo de avaliar o efeito da suplementação de taurina e achocolatado sobre marcadores de estresse oxidativo e desempenho físico de triatletas, e no Capítulo III o artigo abordou o efeito da suplementação de taurina e achocolatado sobre a composição corporal e metabolismo de carboidratos e proteínas em triatletas.

O Capítulo IV contém uma breve introdução e um artigo científico que foi elaborado com os resultados do projeto de pesquisa intitulado "*Effects of cocoa flavonols supplementation on oxidative stress and muscle recovery markers in rugby players*" desenvolvido durante o Doutorado Sanduíche – PDSE CAPES, realizado no *Department of Nutrition, Exercise, and Health Sciences na Central Washington University*, Ellensburg-WA/EUA, sob orientação da Profa Dra Kelly Pritchett no período de Fevereiro à Julho de 2015.

Capítulo I

Revisão Bibliográfica

Revisão Bibliográfica

1. TAURINA: Aspectos gerais e metabolismo

A taurina (ácido 2-aminoetanosulfônico) é um composto nitrogenado intracelular livre, encontrado no coração, leucócitos, retina, sistema nervoso central e no músculo (KIM et al, 2007). De acordo com Gaull (1989) 60% do pool de aminoácidos livres presentes no coração correspondem à taurina, já a musculatura esquelética apresenta a maior concentração de taurina humana, que corresponde a 75% do total de aminoácidos livres. Foi primeiramente descoberta e isolada na bile do bovinos pelos pesquisadores austriacos Friedrich Tiedemanne e Leopold Gmelin em 1827, e sua denominação taurina originou-se do nome em latim da espécie *Bos taurus* (BIRDSALL, 1998).

Apesar de ter sido descoberta em 1827, a taurina foi considerada relevante somente em 1975 quando se observou que bebês prematuros alimentados por nutrição parenteral total não apresentavam níveis plasmáticos e urinários de taurina adequados, diferentemente de bebês alimentados com leite materno (CHESNEY, 1987). De acordo com Ferreira (2005), o leite materno contem em média 55 μg de taurina a cada litro de leite. A deficiência de taurina em recém nascidos pode resultar em diversos processos patológicos como cardiomiopatias, degeneração da retina, risco de má-formação cerebral em recém-nascidos e retardo no crescimento, principalmente se esta carência de taurina ocorrer na fase de desenvolvimento infantil (HEIRD, 2004).

A taurina é considerada um aminoácido "semi-essencial" em humanos, uma vez que pode ser sintetizado a partir de outros aminoácidos sulfurados, como metionina e cisteína através de reações de oxidação e transulfuração reguladas por enzimas, entre elas a enzima a cisteína dioxigenase (CDO), a qual promove a oxidação de cisteína a cisteína ácido sulfínico, a qual posteriormente será descarboxilada pela enzima cisteína sulfinato descarboxilase (CSAD) e convertida a taurina (Figura 1), sendo estas reações dependentes da presença de vitamina B6 (LOURENÇO e CAMILO, 2002).

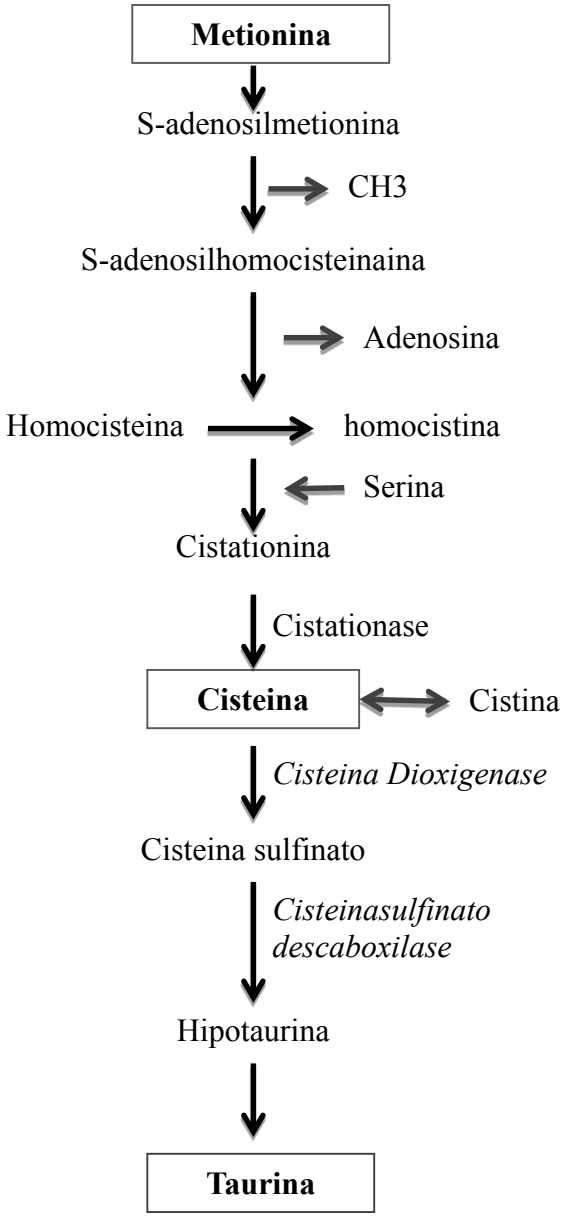

Figura 1. Síntese de taurina. Adaptado de Lourenço e Camilo (2002).

O que difere a taurina dos demais aminoácidos é a presença de um grupamento sulfônico (-SO_3) ao invés do grupamento carboxílico (COOH) (Figura 2). Apesar de apresentar características de um aminoácido, a taurina não participa da síntese proteica, no entanto é essencial para diversos processos biológicos como no desenvolvimento do sistema nervoso central e da retina, modulação de cálcio, estabilização da membrana, reprodução e imunidade (SCHULLER-LEVIS e PARK, 2003).

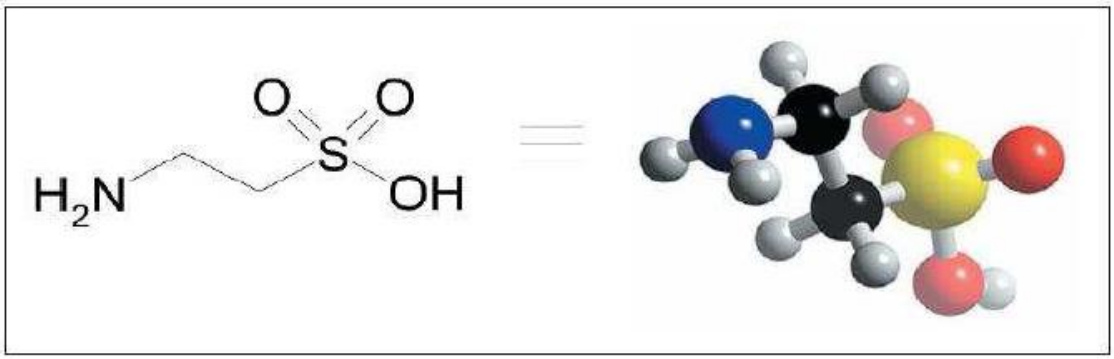

Figura 2: Esquema ilustrativo da estrutura molecular (à esquerda) e estrutura atômica (à direita) da taurina (SZYMANSKI e WINIARSKA, 2008).

Os principais locais de síntese de taurina são fígado e sistema nervoso central, pois nesses tecidos há a maior produção das enzimas CDO e CSAD, contudo, essas enzimas já foram encontradas no tecido adiposo branco, rins e testículos, sugerindo outros possíveis locais de síntese de taurina (BOUCKNOOGHE et al, 2006). Entretanto a produção endógena é insuficiente havendo a necessidade da obtenção de taurina através da ingestão de alimentos de origem animal e marinha (SZYMANSKI e WINIARSKA, 2008).

De acordo com Laidlaw et al (1990) os crustáceos e moluscos contém em média 7000 mg de taurina por quilo de alimento (mg/kg), pescada contem 1720 mg/kg, carne escura de frango contem 2000 mg/kg e de peru 3000 mg/kg, é também encontrada em menores concentrações no leite de vaca, nozes e feijão. Considerando as fontes alimentares de taurina a ingestão dietética estimada de taurina entre onívoros é de 58 mg ao dia, segundo Schuller-Levis e Park (2003). Outras fontes de taurina são as bebidas energéticas, a Agencia Nacional de Vigilância Sanitária (ANVISA) instituiu a resolução RDC 273, a qual determina que as bebidas energéticas podem conter no máximo 400 mg de taurina a cada 100 ml de bebida (BRASIL, 2005).

Ainda não foram estabelecidas as recomendações de ingestão diária (RDA) de taurina, no entanto, estudos sugerem doses de taurina variando entre 2 gramas/dia (GEIB et al, 1994; BAUM e WEISS, 2001), 3 gramas/dia (SAWAMURA, 1992; AZUMA, 1994; AZUMA e YAMORI et al, 1996) e 6 gramas/dia (AZUMA et al,1983; AZUMA et al, 1985; FUJITA et al, 1987; MIZUSHIMA et al, 1996).

A taurina obtida pela ingestão alimentar é absorvida pelo trato gastrointestinal,

especificamente no intestino curto através de seu receptor TAUT (*taurine transporter*), o qual é modulado pelas enzimas proteína quinase C (PKC), sendo essa responsável pela inibição do transporte, e a enzima proteína quinase A (PKA), que irá estimular ou inibir o transporte de taurina, dependendo do tecido, ambas as enzimas são sensíveis à concentração de cálcio intracelular. Após ser absorvida, a taurina será distribuída para diversos órgãos por meio de transporte ativo, sendo que este transporte é regulado pelo gradiente de concentração (HAN et al, 2006). Uma parcela da taurina ingerida será utilizada pelo fígado para a conjugação com ácidos biliares e produção de sais biliares, e o excesso será excretado pelas vias renais (HAN et al, 2006; MERHEB et al, 2007; LAMBERT, 2014).

A concentração plasmática de taurina varia de 10 a 100 μM, sendo que nos tecidos metabolicamente mais ativos a concentração é superior (HUXTABE, 1992). O estudo de Ghandforoush-Sattari et al (2010) avaliou o efeito da administração oral de 4 gramas de taurina em oito adultos jovens saudáveis e constatou pico de concentração plasmática de taurina de 0,69 ± 0,15 mmol (86.1 ± 19.0 mg/L) uma hora e 30 minutos após a administração da dose.

1.1 TAURINA: Funções

1.1.1 Ação anti-inflamatória e anti-oxidante

Estudos têm demonstrado que a taurina apresenta ação anti-inflamatória (SCHULLER-LEVIS e PARK, 2003; RA et al, 2013) e anti-oxidante (TAPPAZ, 2004, SUN et al, 2012; ZHANG et al, 2004). Estas funções estão relacionadas à presença de uma molécula de ácido sulfônico em sua estrutura química, a qual promove a conversão de íon cloro e ácido hipocloroso, que são substâncias altamente citotóxicas e oxidantes, em cloramina, uma substancia relativamente estável (ZHANG et al, 2004; SUN et al, 2012).

A figura 3 (SCHULLER-LEVIS e PARK, 2003) apresenta o fluxo intracelular de taurina e possíveis mecanismos de ação da taurina e mostra sua participação como imunomoduladora e componente de RNA. A taurina encontrada no plasma é transportada para o citoplasma por meio de transportadores de taurina presentes na membrana celular, sendo que a taurina citoplasmática será transportada para o interior da mitocôndria por

"supostos transportadores de taurina" (*putative mitochondrial taurine transporter*) para que seja incorporada no RNA mitocondrial (SUZUKI et al, 2002).

Em sequencia, a figura demonstra a participação da taurina na modulação de processos inflamatórios. Durante a resposta inflamatória ocorre inicialmente a migração de leucócitos para a área da lesão, sendo que estes leucócitos irão produzir grandes concentrações de ácido hipocloroso. A presença de taurina irá possibilitarpossibilitar a conversão de ácido hiplocloroso em taurina-cloramina (Tau-Cl), o qual será transportado ativamente para os leucócitos e consequentemente atenuará a produção de mediadores inflamatórios através do bloqueio da produção de iNOS (oxido nítrico sintase induzida) e de fator de necrose tumoral –α (TNF- α) (SCHULLER-LEVIS e PARK, 2003).

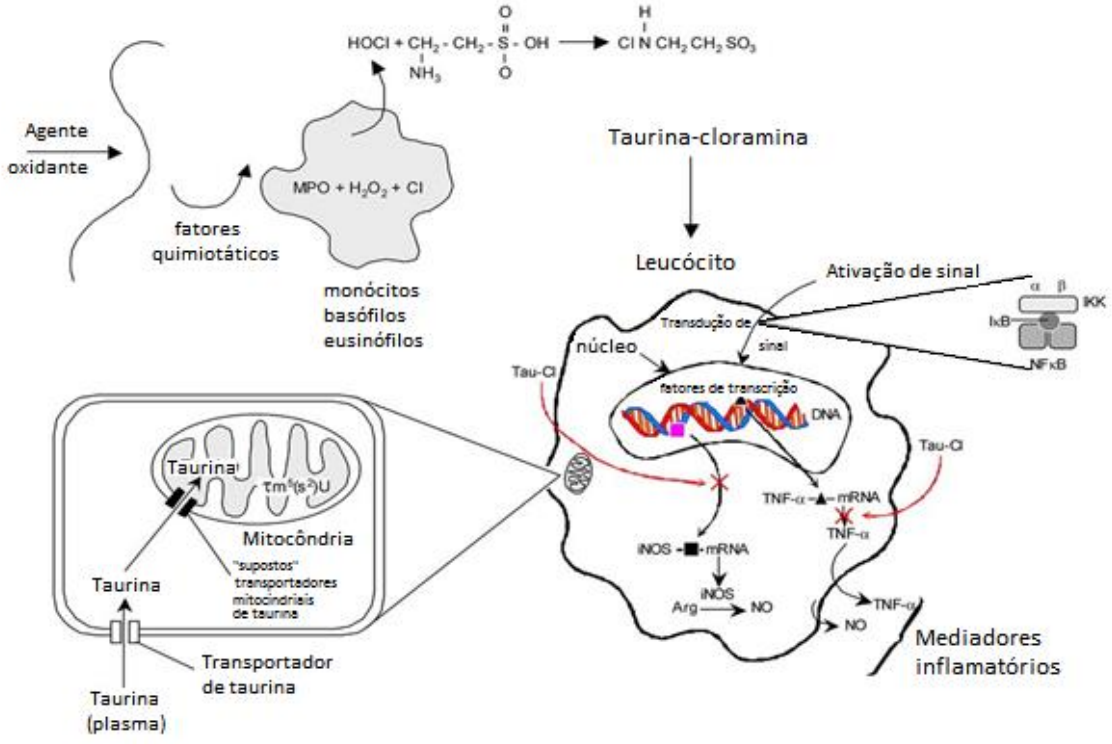

Fig. 3. Representação esquemática da produção de Tau-Cl durante o processo inflamatório, dos mecanismos utilizados pela Tau-Cl para inibir a produção de mediadores inflamatórios nos leucócitos. Adaptado de Suzuki et al (2002) e Schuller-Levis e Park (2003).

Ademais, a hipotaurina, um precursor de taurina, pode atuar como carreador de radicais hidroxila (OH^-) e inibidor da auto-oxidação de íons ferro (Fe_2^+), e consequentemente prevenir a ocorrência de reações de peroxidação lipídica (TADOLINI et al., 1995). Estudos com taurina radioativa evidenciaram que a taurina adicionada ao meio de cultura é captada preferencialmente por mitocôndrias (KLAMT e SHACTER, 2005), assim, pode-se inferir que grande parte da concentração intracelular de taurina esteja no interior da mitocôndria, auxiliando na regulação da produção de radicais livres e prevenindo um desequilíbrio oxidativo.

Zhang et al. (2004) avaliaram o efeito protetor proveniente da suplementação de taurina (6 gramas) sobre o estresse oxidativo induzido pelo exercício físico em jovens ciclistas, no período de sete dias. Foram constatados aumentos significativos no volume máximo de oxigênio utilizado pelo organismo (VO2 máx), no tempo de exaustão em ciclo ergômetro e na carga máxima de trabalho, reduzindo a produção de substâncias reativas ao ácido tiobarbitúrico (TBARS).

Já o estudo de Silva et al (2011) foi investigado o efeito da suplementação de 300 mg/kg de taurina sobre marcadores de estresse oxidativo após a realização de exercício extenuante em ratos. A suplementação foi realizada por gavagem durante o período de 15 dias. Foi observado que a suplementação de taurina reduziu a produção de radicais superóxidos, lipoperoxidação e carbonilação, comprovando o efeito protetor da taurina.

Com relação à ação anti-inflamatória, Kato et al (2015) avaliaram o efeito da administração aguda de taurina 1 hora antes da realização de exercício intenso em ratos durante 10 dias consecutivos. Os autores concluíram que os ratos que receberam taurina apresentaram menores concentrações de marcadores inflamatórios (interleucina-6 e CD 68).

Ra et al (2015) avaliaram o efeito da suplementação de 2 gramas de taurina ou placebo sobre dor muscular tardia (DOMS) e lesão muscular após a realização de exercícios de alta intensidade em adultos. A suplementação foi realizada duas semanas antes da sessão de exercício e novamente três dias após a sessão, e foi constatado que o grupo que recebeu taurina apresentou DOMS menos severa, podendo este fato estar relacionado à capacidade de modulação da resposta inflamatória proveniente da taurina. No entanto não foram encontradas alterações significativas em relação aos marcadores de lesão muscular.

Além de benefícios encontrados na associação de taurina e exercício, o estudo de Rosa et al (2014) mostrou efeito benéficos da utilização de taurina para casos patológicos. Os autores avaliaram o efeito da suplementação de taurina (3 gramas/dia) associado a aconselhamento nutricional durante o período de oito semanas em mulheres obesas e constataram redução na concentração de marcadores inflamatórios (proteína C reativa) e de peroxidação lipídica (TBARS).

1.1.2 Aumento da força de contração muscular

De acordo com Schaffer et al (2010) a taurina está relacionada à regulação da capacidade de depósito de cálcio no retículo sarcoplasmático, além de estimular a taxa de bombeamento do mesmo, podendo favorecer a elevação da concentração de cálcio nas proteínas miofibrilares contráteis, resultando em aumento da força de contração muscular. A atuação da taurina sobre as proteínas miofibrilares contráteis resulta em ações farmacológicas e fisiológicas muito similares às características dos fármacos digitálicos (GEIB et al, 1994). Desta forma, a taurina exerce um efeito protetor quando o coração está sob situações de estresse devido a sua capacidade de regular a homeostasia intracelular de Cálcio (XU et al, 2008).

Baum e Weiss (2001) realizaram uma investigação ecocardiográfica antes e após a realização de protocolos de exaustão e consumo de bebida energética contendo taurina ou placebo em treze atletas de *endurance*. Foi constatado aumento na contratilidade do átrio esquerdo, maior fração de encurtamento cardíaco (*fractional shortening*) e ejeção sanguínea após o consumo de 100 ml de bebida energética comercial contendo 1 grama de taurina.

O estudo realizado por Yatabe et al (2003) avaliou as concentrações de taurina em músculo esquelético de ratos após corrida de resistência e verificou o tempo de exaustão e constatou que o grupo que recebeu taurina (0,5 g/kg/dia) apresentou maior resistência física.

1.1.3 Regulação do metabolismo

Outro fato referenciado na literatura quanto à taurina é a regulação do metabolismo de carboidratos. De acordo com Carneiro et al (2009), a taurina auxilia no controle da homeostase da glicose através da regulação da expressão de genes necessários para estimular a secreção de insulina pelas células β, aumentando a sensibilidade periférica à insulina e a captação de glicose.

Vettorazzi et al (2014) suplementaram taurina à 5%, durante 8 semanas, em ratos e constataram que a taurina potencializou a ação da insulina no fígado e no músculo esquelético de camundongos. Foi constata maior fosforilação de substratos de insulina (IRS) e ativação da cascata de insulina, consequentemente houve aumento da translocação de transportadores de glicose do tipo GLUT 4 para membrana citoplasmática, promovendo a entrada de glicose para o meio intracelular.

Considerando a ação da taurina sobre a insulina, e que este hormônio apresenta um importante papel para os atletas por regular o metabolismo dos carboidratos, a utilização de taurina poderia favorecer o aumento da disponibilidade de glicose e consequentemente resultar em maior produção de energia, maior estímulo à síntese proteica e à ressíntese de glicogênio e possivelmente pode favorecer o desempenho físico de atletas.

Além do metabolismo de carboidratos, a taurina atua também sobre o metabolismo lipídico (TSUBOYAMA-KASAOKA et al, 2006; MURAKAMI, 2015). A taurina estimula a produção de RNA para expressão de proteínas PGC-1α e PPAR no tecido adiposo branco e promovendo aumento do gasto energético (TSUBOYAMA-KASAOKA et al, 2006). Ademais, a taurina aumenta a expressão de genes relacionados a produção das enzimas lipase lipoproteica, acil-Coa oxidase, acil-CoA syntetase, e acil-CoA desidrogenase, as quais estão relacionadas ao metabolismo de substratos lipídicos. O PGC-1 α é um potente regulador do gasto energético e o aumento na sua expressão favorece a maior utilização de lipídios. E é por este motivo que a taurina pode ser considerada um "*fat burner*" e uma possível ferramenta para o tratamento de obesidade (MURAKAMI, 2015).

Martiniano et al (2015) avaliaram o efeito da suplementação de taurina (2%) associada a treinamento físico durante o período de 11 semanas em ratos obesos alimentados com dieta hiperlipídica. Os autores constataram que os animais que receberam taurina

apresentaram menor quantidade de gordura visceral e menor peso de gordura epididimal, mostrando que a taurina beneficiou a composição corporal dos ratos.

A maior utilização de lipídios pode também ser interessante para atletas, considerando não só a questão da composição corporal, mas também uma maior disponibilidade de substratos energéticos, especificamente lipídios. O estudo de Dato (2014) avaliou o efeito da suplementação aguda de taurina (6g) ou placebo 120 minutos antes da realização de um teste de desempenho máximo de natação e constatou que a taurina induziu a um aumento significativo de 24 % no nível de lipólise plasmática, porém não foram observados benefícios no desempenho físico dos atletas.

Diversos estudos abordaram a suplementação de diferentes quantidades de taurina e ressaltaram efeitos benéficos, no entanto, ainda não foram estabelecidas as doses adequadas para a suplementação de atletas e/ou tratamento de doenças. Visto as diversas ações metabólicas descritas na literatura, são necessários mais estudos para avaliar os benefícios da utilização de taurina para atletas a fim de prevenir de danos oxidativos, e favorecer o desempenho físico.

2. Triatlo

O triatlo ou "*triathlon"* é um esporte composto por três modalidades esportivas: natação, ciclismo e corrida. Existem quatro tipos de triatlo, sendo estas denominadas de acordo com as distancias percorridas, curta ou longa distancia. O triatlo de curta distancia são o "*Short"*, o qual é composto por 750 metros de natação, 20 km de ciclismo e 5 km de corrida; e "*Olímpico"*, composto por 1.5 km de natação, 40 km de ciclismo e 10 km de corrida. E os dois tipos de triatlo de longa distancia são "*Meio-Ironman"*, composto por 1.9 km de natação, 90 km de ciclismo e 21 km de corrida; e "*Ironman"*, composto por 3,8 km de natação, 180 km de ciclismo e 42,2 km de corrida (BENTLEY et al, 2008).

A primeira competição oficial foi realizada no Hawaí em 1978 e foi denominada *"Ironman"*, sendo esta composta por 3,9 Km de natação, 180 km de ciclismo e 42,2 Km de corrida, contendo apenas 14 competidores (BALIKIAN JR e DENADAI, 1999). Devido à extrema complexidade da prova, o "*Ironman*" transformou-se na mais importante

competição de triatlo, sendo que os participantes são reconhecidos como verdadeiros "homens de ferro". No Brasil atualmente são realizadas provas de "Ironman" em Brasília, Florianópolis, Foz do Iguaçu, Rio de Janeiro e Fortaleza em diferentes períodos do ano, e a prova realizada em Florianópolis é a mais tradicional.

Em geral, os triatletas de elite realizam treinos de 20 horas semanais, sendo que regularmente treinammais de uma vez ao dia a fim de otimizar o desempenhonas três modalidades esportivas. Este fato implica em um aumento nas necessidades nutricionais e hídricas como consequência direta do volume de treinamento diário (BENTLEY et al, 2008).

Entre os fatores que determinam um bom desempenho no triatlo pode-se destacar a genética, dedicação e adaptação muscular aos treinos, preparo psicológico, motivação e principalmente a nutrição. Uma alimentação adequada não garantirá por si o sucesso do triatleta, mas a sua ausência comprometerá a expressão de seu potencial máximo (BENTLEY et al, 2008). A seleção apropriada de alimentos, tanto quanto à composição, quanto à quantidade e momento de ingestão poderá promover melhores adaptações aos estímulos dos treinos, além de reduzir o risco de lesões, manter a função imunológica, preservar a massa muscular e óssea, e contribuir para uma melhor recuperação após o treino ou competição (NOGUEIRA e DA COSTA, 2004).

Além dos fatores relacionados ao atleta, é importante ressaltar algumas características fisiológicas relacionadas a esta modalidade esportiva. Os esportes intensos como triatlon e ultra-maratonas apresentam metabolismo predominantemente aeróbio. O intenso treinamento aeróbio promove adaptações específicas que incluem o predomínio de fibras musculares do tipo I, aumento na quantidade de capilares e de mitocôndrias, o que consequentemente ocasiona um acréscimo nas enzimas envolvidas no metabolismo oxidativo, resultando em uma maior capacidade de produção de energia no músculo (FRY, 2004).

Estas adaptações metabólicas podem afetar a função mitocondrial e resultam em elevação da taxa de consumo de oxigênio durante o exercício intenso. O aumento do consumo de oxigênio é uma resposta fisiológica do exercício e necessária para promover a adaptação do atleta à pratica desta modalidade esportiva (POWERS e JACKSON, 2008). No entanto, esse aumento pode induzir a produção exacerbada de radicais livres e promover um

desequilíbrio oxidativo, e como consequência pode ocasionar danos celulares e comprometer o desempenho do atletla (WU et al, 2004; BENTLEY et al, 2008; SUN et al, 2011).

Desta forma, é necessário atentar-se ao aporte nutricional de triatletas visando primeiramente atender a alta demanda energética, mas também a utilização de estratégias nutricionais que previnam possíveis danos consequentes do intenso metabolismo oxidativo.

2.1 Necessidades nutricionais de triatletas

As necessidades de calorias diárias de um triatleta podem ultrapassar 4000 kcal/dia devido à realização de treinos longos (NOGUEIRA e DA COSTA, 2004). De acordo com Nogueira e Da Costa (2004), o gasto calórico diário de triatletas do sexo masculino, com idade de 26 anos e peso médio de 70 kg é de aproximadamente 3250 kcal/dia. No entanto, a recomendação de macronutrientes é semelhante para os demais atletas e difere-se apenas no computo total de calorias necessárias diariamente (HERNADEZ e NAHAS, 2009).

Segundo a Sociedade Brasileira de Medicina Esportiva (SBME), a recomendação diária de carboidratos para atletas varia entre 60 a 70% do valor calórico total, sendo que para a otimização e recuperação muscular recomenda-se que a ingestão de carboidratos corresponda a 5 e 8 gramas/kg de peso/dia. Já em atividades de longa duração, bem como treinos intensos de triatletas, a necessidade de carboidratos pode chegar até 10g/kg de peso/dia visando recuperação do glicogênio muscular (HERNADEZ e NAHAS, 2009; RODRIGUEZ et al, 2009).

Quanto à ingestão de proteínas, a recomendação das *Dietary Reference Intakes* (DRI) é de 0,8 gramas de proteína por kg/dia para indivíduos adultos sedentários, enquanto que para indivíduos ativos a ingestão recomendada é de 1,2 a 1,4 g/kg de peso/dia (IOM, 2005; BACURAU, 2007). Já para atletas que praticam esportes de resistência, a Sociedade Brasileira de Medicina do Esporte recomenda a ingestão de 1,2 a 1,6 g/kg de peso, por dia. A ingestão de proteína associada à ingestão de carboidratos pode acelerar a taxa de síntese de glicogênio e melhorar a capacidade de resistência. De acordo com Ivy et al (2003), a razão 4:1 (carboidrato: proteína) é a mais adequada para exercícios de resistência.

As recomendações de lipídios para atletas são semelhantes à população adulta em geral, que corresponde a cerca de 1 grama de gordura por Kg/peso corporal, devendo variar

entre 20 a 30% do valor calórico total da dieta (ADA, 2009). Deve-se atentar-se a prescrição de 8 a 10 gramas ao dia de ácidos graxos essenciais, sendo que 10% devem corresponder a ácidos graxos saturados, 10% de poli-insaturados e 10% de monoinsaturados.

De acordo com a ADA (2009) o consumo de lipídios não deve ser inferior a 15% do valor calórico total ingerido, pois pode comprometer a manutenção da saúde. Se acaso houver necessidade de restringir a ingestão de lipídios, deverão ser utilizadas as seguintes proporções: 8% de gorduras saturadas, 8% de monoinsaturadas e de 7 a 10% de poli-insaturadas (HERNADEZ e NAHAS, 2009).

A SBME e *International Society of Sports Nutrition* (ISSN) ressaltam que as necessidades de macronutrientes podem ser contempladas perfeitamente em uma alimentação equilibrada, adequada ao gasto calórico total, sem haver necessidade de suplementação (HERNADEZ e NAHAS, 2009; KREIDER et al, 2010).

3. Intervenção nutricional e *performance*: Suplementação de taurina e achocolatado para triatletas

A fim de manter ou melhorar a qualidade do treino sequencial, os atletas buscam práticas nutricionais para maximização de todos os aspectos relacionados à recuperação pós-treino, incluindo redução de danos e dores musculares, e aumento de ressíntese de glicogênio após o exercício. As estratégias de recuperação no pós-exercício envolvem tempo, tipo de bebida, volume e frequência a ser ingerido, e também sua composição em calorias, carboidratos e proteínas, visando otimizar a recuperação de glicogênio (BERARDI et al, 2006).

De acordo com Karp et al (2006), a ressíntese de glicogênio entre as sessões de treinamento ocorre mais rapidamente quando a reposição de carboidratos é realizada dentro de 30 minutos a 1 hora após o exercício. E para maximizar a ressíntese de glicogênio, a ACSM (2000) recomenda a ingestão de 50 a 75 g de carboidratos dentro de 30 a 45 min após o exercício, com a ingestão de 1,2 a 1,5 g de CHO / kg de peso corporal / hora para as próximas horas (JEUKENDRUP et al, 2005).

O desempenho do exercício de resistência, como por exemplo triatloe ultra-maratonas, é influenciado pela quantidade de glicogênio armazenado nos músculos esqueléticos. Desta forma, o fornecimento de nutrientes específicos, como a taurina, associado à macro nutrientes em qualidade e quantidade adequadas pode beneficiar o desempenho de triatletas.

O período pós-treino é o principal momento em que se deve realizar a reposição de nutrientes, pois neste momento ocorre uma priorização do fluxo sanguíneo para os músculos e os receptores de insulina nas células musculares estão mais sensíveis. Desta forma a captação de nutrientes ocorre de forma mais eficiente, tanto por ação fisiológica da insulina em resposta à disponibilidade de glicose, mas também pelo efeito do próprio exercício, por potencializar o efeito da insulina na fosforilação dos substratos receptores de insulina (IRS-2), tendo como consequência o aumento da atividade da fosfoinsusitol-3-quinase ou cinase (PI (3) K) e maior fosforilação da Akt em serina. Tais reações irão favorecer a translocação do GLUT 4 para a membrana citoplasmática, potencializando a captação e utilização de glicose pelas células (CARVALHEIRA et al, 2002; ROPELLE et al, 2005).

A disponibilidade de glicose no meio intracelular irá promover a fosforilação de mTOR (*mammalian Target of Rapamycin*), uma proteína relacionada à ativação da cascata de síntese proteica no núcleo celular, resultando em aumento da atividade anabólica, crescimento e reparo celular (WULLSCHLEGER et al, 2006). Além da ativação da mTOR, o aumento da disponibilidade de nutrientes e a consequente fosforilação da AKT induzirão também na ativação da enzima glicogênio sintase e resultarão na síntese e reposição dos estoques de glicogênio no fígado e no músculo (AVRUCH et al, 2009; CARVALHEIRA et al, 2002).

Tem-se observado que a atuação da insulina também pode ser influenciada pela ação da taurina. De acordo com Ribeiro et al (2010), a taurina é encontrada em altas concentrações nas ilhotas pancreáticas e regula a secreção de insulina através da mobilização de íons de cálcio para as células β-pancreáticas quando há glicose disponível, resultando na liberação de insulina. Além de favorecer a secreção de insulina, a taurina potencializa a ação da insulina no fígado e no músculo esquelético de camundongos, aumentando a fosforilação de substratos de insulina e consequentemente ativa a cascata de insulina, promovendo a disponibilização de glicose para as células (VETTORAZZI et al, 2014).

Desta forma, a utilização de taurina associada a uma fonte de nutrientes após a pratica esportiva poderia favorecer a ação da insulina, ativação da mTOR e da enzima glicogênio sintase, conforme mecanismo descritos previamente, favorecendo a reposição dos estoques de glicogênio e podem resultar em benefícios no desempenho de atletas.

Atualmente são encontradas no mercado diversas bebidas esportivas que tem a finalidade de fornecer nutrientes no pós-exercício como, por exemplo, bebidas de reposição de carboidratos, que contém carboidrato adicional para reabastecer o glicogênio muscular depletado após o exercício exaustivo, e também, bebidas ricas em eletrólitos, utilizada para reposição de fluídos (PRICHETT et al, 2009).

Autores relatam também a utilização de leites aromatizados, como leite com chocolate, para a suplementação de atletas. Estes alimentos contém teor de carboidratos e proteínas semelhantes à algumas bebidas repositoras, e, desta forma, torna-se um meio eficaz para o reabastecimento de glicogênio depletado nos músculos, favorecendo assim a recuperação após o exercício de alta intensidade. Além disso, os leites achocolatados são de fácil acesso, palátaveis e de boa aceitabilidade (KARP et al, 2006; PRITCHETT et al, 2009). O fornecimento de achocolatado após o treino tem a finalidade de aumentar a disponibilidade de nutrientes, tanto de carboidratos quanto de proteínas, em um momento em que as células musculares estão mais sensibilizadas à captação de nutrientes (PRITCHETT et al, 2009).

O estudo realizado por Karp et al (2006) comparou o efeito da utilização de leite com achocolatado, bebida rica em carboidratos ou bebida rica em eletrólitos logo após o treino e duas horas após a recuperação, no treino de nove ciclistas. Os autores constataram que a bebida achocolatada resultou em maior tempo para atingir o estado de exaustão em relação às demais bebidas.

O estudo de Pritchett et al (2009) comparou o efeito da utilização de uma bebida achocolatada e uma bebida de reposição de carboidratos, sendo estas isocalóricas, sobre a recuperação após o exercício intermitente de alta intensidade em ciclistas e triatletas treinados. Os voluntários consumiram uma das bebidas após a primeira sessão exercício, e após uma semana, o mesmo protocolo foi repetido consumindo-se a outra bebida após o treino. Os autores concluíram que a utilização de bebida achocolatada promoveu resultados semelhantes a bebida de reposição de carboidratos, o que permitiu concluir que a bebida achocolatada pode também ser utilizada para auxiliar na recuperação de treinos intensos.

Visto a importância de um aporte nutricional adequado considerando calorias, dentre carboidratos e proteínas, visando maximizar a recuperação de glicogênio e síntese proteica (BERARDI et al, 2006) mas também as alterações metabólicas decorrente do esforço intenso, a utilização de taurina associada a uma fonte de proteínas de alto valor biológico possivelmente possa auxiliar na prevenção de danos oxidativos e também favorecer a captação de glicose pelas células hepáticas e musculares, e consequentemente otimizar a síntese de proteínas e o desempenho dos triatletas.

4. REFERENCIAS

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Capítulo II

Artigo I

Taurine: a potential ergogenic aid for preventing muscle damage and decreasing oxidative stress produced by intense physical activity

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ABSTRACT

INTRODUCTION: Triathlon is a sport of high intensity, that can increase oxygen consumption rate during exercise and consequently increase free radical production and oxidative stress, which may compromise an athlete's performance. Taurine is a nitrogenous compound which has several proposed physiological benefits including antioxidant action and possible increases in muscle force contraction and insulin sensitivity. Therefore, research examining effectiveness of the supplementation of taurine associated to low fat chocolate milk post exercise on the prevention of oxidative damage and triathlete's performance is warranted.

POURPOSE: The aim of this study was to evaluate the effects of taurine and chocolate milk supplementation on oxidative stress markers and aerobic parameters in triathletes. **METHODS:** A double-blind, crossover study was conducted with 10 male triathletes, aged 25 to 35 years. 3 grams of taurine and 400ml of chocolate milk (TAUchoc), or a placebo (400 ml of chocolate milk) (CHOC) was ingested post exercise for eight weeks. A two-week washout period was implemented in between trials. Plasmatic oxidative stress markers (MDA, GSH and vitamin E) were examined and a maximal incremental running test was performed to determine aerobic parameters before and after 8 weeks of training and supplementation. **RESULTS**: TAUchoc during the 8 weeks did not resulted significant changes in GSH and vitamin E seric levels, and no changes were found in regards to aerobic parameters. However, a significant decrease (19,4%) was observed in MDA levels after TAUchoc treatment (p= 0,03). **CONCLUSION**: Taurine may be considered a potential ergogenic aid for preventing muscle damage and decreasing oxidative stressproduced by intense physical activity.

Key-word: triathlon, chocolate milk, oxidative stress, aerobic parameters.

INTRODUCTION

Triathlon is a sport of high intensity, which can increase energy expenditure and oxygen consumption rate during exercise and consequently increase free radical production and oxidative stress, which may compromise an athlete's performance (Bentley et al., 2008). This increase occurs naturally and it's well established in the literature that low-to-moderate levels of oxidants play multiple regulatory roles in cells such as cell signaling pathways regulation, on the other hand overproduction of free radicals can damage cellular components then the use of the antioxidant

compounds post exercise aims to prevent oxidative stress but not to limit free radicals production (Powers and Jackson, 2008).

In order to minimize the effects of exercise, researchers are looking for nutrients that could help to prevent oxidative damage and also to attend athlete's higher energy requirements. In regards to oxidative stress, researchers have reported taurine as a potent antioxidant due to the presence of a sulfonic acid molecule in its chemical structure which promotes the conversion of highly cytotoxic substances as chloride ion and hypochlorous acid in relatively stable chloramine (Tappaz, 2004; Sun et al, 2012; Zhang et al, 2004). Furthermore hipotaurine, taurine precursor, can act as hydroxyl radicals (OH-) scavenger and inhibit lipid peroxidation, and prevent iron (Fe_2^+) self-oxidation (Tadolini et al, 1995).

And also, taurine is known for several proposed physiological effects including increase muscle force contraction (Schaffer et al, 2010), regulation of lipid metabolism (Murakami, 2015) and increase in insulin sensitivity (Vettorazzi et al, 2014), by this way, taurine can improve carbohydrates metabolism and may favor glycogen resynthesize and increase glycongen stores (Ribeiro et al, 2010). Beyond that, some reserachers has observed that taurine supplementation improved time to exhaustion in runners (Lee et al. 2003) and cyclists (Zhang et al. 2004, Rutherford et al. 2010), however the precise mechanisms that elucidate how taurine may affect human endurance performance are still unclear (Galloway et al, 2008).

In regards to nutrients, the use of flavored milks, such as chocolate milk, have been found to be an effective post exercise recovery aid for athletes (Karp et al, 2006; Pritchett et al, 2009; Gilson et al, 2010), since it carbohydrate and proteins content is similar to some sports recovery drinks, and thus become effective for replenishing depleted glycogen in the muscles, and may favor recovery after high intensity exercise. In addition, chocolate milk have good taste and good acceptability between athletes (Karp et al, 2006; Pritchett et al, 2009). The intake of chocolate milk after practice has the purpose of increase carbohydrate and protein availability in the moment that muscle cells are more sensitive to the nutrients uptake (Pritchett et al, 2009).

By this way, the provision of specific nutrients such as taurine, associated with nutrients in appropriate quality and quantity can prevent oxidative stress and benefit the athlete's performance. Therefore, this study has two purposes, 1) to examine the effectiveness of taurine to low fat chocolate milk post exercise on markers of oxidative stress and 2) to examine the efficacy of taurine associated to low fat chocolate milk post exercise on triathletes aerobic parameters.

METHODS:

Subjects:

Ten well-trained male long distance triathletes (age =30.9 ±1.3 yr, stature = 1.79 ± 0.01 m, mass = 77.45 ± 2.4 kg; mean ± SD) who competed in semiprofessional triathlon volunteered to take part in the study. They focus their training in the Brazilian Ironman Championships, that is long-distance triathlon category.

After institutional ethical approval, the participants were invited to go to the lab and all the experimental procedure, the associated risks and benefits were explained; the participants then assigned informed consent. Participants were excluded if they had experienced a muscle injury in the past six months and/or were currently taking chronic or daily doses of anti-inflammatory medication or nutritional supplements. Participants who had history of cardiovascular diseases were excluded from the study (McBrier et al, 2010).

Participants were instructed to maintain their habitual diet throughout the study. They were asked to record their diet 1 day before performing the data collection and record three days during the trials.

Eighteen triathletes were recruited to attend the research however only ten triathletes completed the treatment because some of them presented muscle injuries or health problems during the protocol.

Experimental Design

A double blind, crossover with 2-week washout study design was conducted. Participants were assigned to one of two independent groups: (i) Taurine + Chocolate milk (TAUchoc) and (ii) Placebo+Chocolate milk (CHOC). The study consisted of 8-week supplementation period.

Subjects were required to attend the laboratory for 8 times. On the first visit (PRE), they were required to arrive at the laboratory in the morning after 12 hours fast, anthropometric measures including height and weight and blood samples were taken in order to quantify oxidative stress markers.And in the day after data collection, a maximal incremental running test was performed to determine aerobic parameters.After these all procedures the first period of treatment was started. Following the 8 weeks of treatment, the subjects were required to come back to the lab for blood collection and to perform a maximal incremental running test (POST). A 2-week wash out was done and then the protocol was repeated with the other treatment (PRE and POST second trial).

Nutritional treatment

The treatment consisted of capsules containing 3 grams of pure taurine (*Aminoethylsulfonic Acid*, Ajinomoto®) (Shao and Hathcock, 2008), or placebo. The placebo consisted of a similar capsule containing starch. Subjects received daily three capsules (taurine or placebo) and 200 ml of provided low fat chocolate milk (CM) immediately after exercise and more 200 ml of CM one-hour post exercise for a total of 60 days (Karp et al, 2006). And after a 2-week washout period, the protocol was repeated with the other treatment (taurine or placebo capsules). Taurine and placebo capsules were manipulated by the Department of Industrial Pharmacy of the School of Medicine of Ribeirão Preto, University of São Paulo.

The CM used in this study was 200 ml packages of low fat chocolate milk (LowFat Chocolate milk Pepsico, Sao Paulo, SP) that contained 92 kcal, 16 grams of carbohydrate, 4 grams of protein and 1,2 grams of lipids. The CM contains the optimal ratio of carbohydrate to protein (4:1), which is recommended to nutrients replenishment after exercise in order to favor glycogen resynthesizes (IVY et al, 2003). Also, CM presents good taste, high acceptability by the athletes and the package was convenient for them to carry CM to their daily practice (Karp et al, 2006; Pritchett et al, 2009).

Dietary assessment

Subjects were instructed on the protocol for completing three-day food records and were asked to record dietary intake on the day before data collection and three non-consecutive days during each trial, in order to control for dietary intake. ESHA software (Esha Research Inc, Salem, OR) was used to examine kcal, carbohydrate, protein and fat content.

MEASUREMENTS

Oxidative stress markers

Blood was collected into 5 ml tubes containing separator and clot activating gel at PRE and POST treatments after a 12-hour fast. The samples were then stored in a freezer at –80°C until the time for analysis. The following oxidative stress markers indicative of lipid peroxidation were determined: reduced glutathione (GSH) by the method of Sedlak and Lindsay (1968), malondialdehyde (MDA) by the method proposed by Gerard-Monnier et al (1998), with some adaptations. Blood vitamin E (total α-tocopherol) was determined by the method of α-tocoferol (Fabianek et al, 1968).

Aerobic Parameters

All subjects performed a maximal incremental running test with 1% gradient on a treadmill (Super ATL, Inbramed, Brazil) inside a controlled environment. The test began at 8 $km \cdot h^{-1}$ and speed was increased by 1 $km \cdot h^{-1}$ every three minutes until volitional exhaustion. Immediately after each stage, capillary blood samplesfrom the ear lobe (25µl) were assessed and blood lactate concentration with a lactate analyzer (YSI 2300 – Yellow Springs, Ohio, USA). Heart rate (HR) monitor (Polar, RS400, Finland) was used to measure heart rateand a 0-10 Foster's scale (Foster et al, 2001) was used to determine rate of perceived exertion for each stage (RPE).

Lactate threshold intensity (LT) was determined using D-máx method, in which the points obtained through the speed, lactate concentration relationship were adjusted linearly and exponentially, the major distance between these two adjustments corresponded to anaerobic threshold intensity (Cheng et al, 1992). And the maximum aerobic velocity (Vmax) was determined as the last complete stage during the incremental protocol.

STATISTICAL ANALYSIS:

A t-test was used to compare percentages of change (Δ% Post-Pre, taurine or placebo) of oxidative stress markers and aerobic parameters between PRE and POST trials. Dietary assessment data from the two treatments was compared with a t-test for independent samples.

Statistical Package for the Social Sciences for Windows software version 15.0 was used for all statistical analyses. All data was reported as means $\pm$ standard deviation. Statistical significance was set at $p < 0.05$ for all analyses.

RESULTS

The subjects characteristics reported as means ± SD for each participant are as follows: age (years): 30.9 ± 1.3, height (cm): 179.0 ± 0.01, weight (kg): 77.45 ± 2.4. According to the dietary analysis, there were no significant differences in macronutrient intake. TAUcho group consumed, in average, 2243.7±770.7 kcal, 4.29±1.35 g/kg of body weight (BW) of carbohydrate, 1.78±0.57 g/kg BW of protein and 0.89±0.28 g/kg BW of lipids. While CHOC group consumed, in average, 2122.6±702.4 kcal, 4.17 ± 1.31 g/kg BW of of carbohydrate, 1.61±0.51 g/kg BW of protein and 0.91±0.23 g/kg BW of lipids.

In regards to oxidative stress markers (table 1), no significant changes were found in GSH and vitamin E levels. However, a significant decrease (-19,4%) was observed in MDA levels after TAUchoc treatment (p= 0,03). This result shows that TAUchoc treatment after practice prevented lipid peroxidation.

Table 1. Oxidative stress markers levels PRE and POST treatment (TAUchoc and CHOC)

Measurements	TAUchoc			CHOC		
	PRE	POST	Δ % (POST-PRE)	PRE	POST	Δ % (POST-PRE)
GSH (mmol/L)	0.72±0.08	0.83±0.08	16.94±11.44	0.69±0.08	0.81±0.06	24.24±10.63
Vitamin E (μmol/L)	33.99±2.52	35.95±2.80	6.54±7.80	31.48±2.12	33.77±3.64	6.54±3.90
MDA (μMl)	3.62±0.64	2.86±0.05	-17.97±11.86*	4.38±0.60	4.30±0.64	-0.46±6.39

Note: GSH, reduced glutathione; MDA, malondialdehyde. TAUchoc, taurine associated to chocolate milk supplementation; CHOC, chocolate milk and placebo supplementation. *Significant difference was observed in MDA levels after TAUchoc treatment. Data reported are means ± SD.

Aerobic parameters were measured by an incremental running test performed before and after treatments. In the Vmax (TAUchoc PRE 13±1.4 km/h and POST 13.22±1.34 km/h; CHOC PRE 13.11±2.34 km/h and POST 13.11±2.72 km/h), the HR was TAUchoc PRE 181.89±24.18 bpm and POST 168.89±46.56 bpm; CHOC PRE 181.56±2.14 bpm and POST 179.78±3.4 bpm, RPE was TAUchoc PRE 8.33±2.4 AU and POST 9.1±2.1 AU; CHOC PRE 8.11±4.94 AU and POST 8.78±2.78 AU, no significant changes were observed post treatment. Figure 1 shows a relative percentage calculated considering the variable values at LT compared to values at Vmax, but no significant changes were found after treatment in both groups.

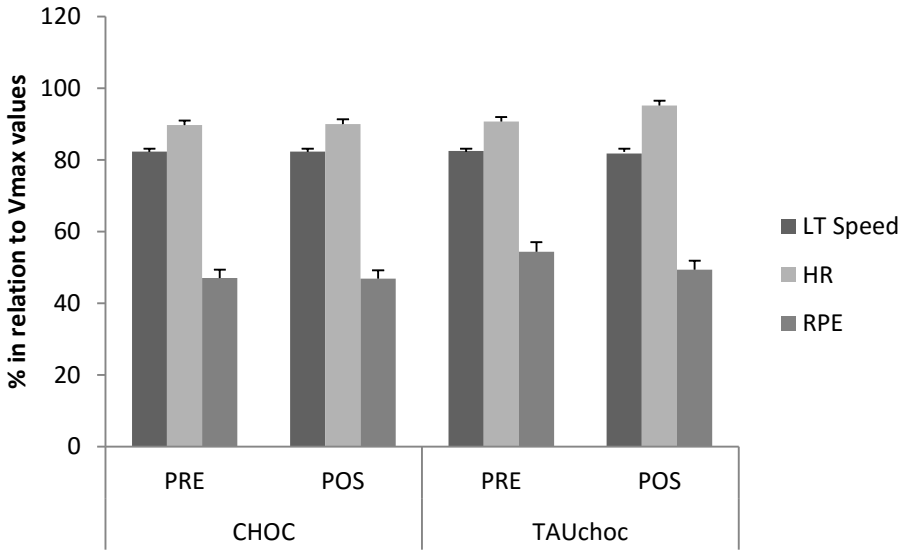

Fig. 1 Relative percentage calculated considering the variable values at LT compared to values at Vmax. CHOC: chocolate milk and placebo treatment. TAUchoc: Taurine and chocolate milk treatment. LT Speed: Speed at lactate threshold. HR: heart rate. RPE: rate of perceived exertion. Vmax: maximum aerobic velocity. No significant changes were found after TAUchoc ans CHOC treatment.

DISCUSSION

The main purpose of this study was to examine the effects of TauCHOC vs. CHOC on indices of oxidative stress and aerobic parameters on triathletes. Exercise performance was assessed using aincremental running test. Our results suggest that the supplementation of taurine or placebo associated to low fat chocolate milk for eight week period provide benefits on oxidative stress markers and but did not improved aerobic parameters.

In regards to oxidative stress, moderate training appears to benefit health and oxidative stress since that exercise stimulus is necessary to promote an up-regulation in endogenous antioxidant defenses (PINGITORE et al, 2015), however, strenuous aerobic exercise can induce reactive oxygen species overproduction and may causes oxidative damage if the endogenous defense is not effective and may compromise athletes performance (SILVEIRA et al, 2008; HAWLEY, 2009). Triathlon has all the characteristics that may favors to oxidative imbalance, seeing that it is a low intense and long duration sport, with predominantly aerobic metabolism (BENTLEY et al, 2008), by this way an overproduction of oxygen reactive species may induces oxidative stress and negatively impacting

performance (MCLEAY et al, 2012; PINGITORE et al, 2015). Therefore, the utilization of antioxidant components post exercise aimed to prevent oxidative stress and possibly enhance performance (KIM et al, 2014, PINGITORE et al, 2015).

The results of the present study indicated that taurine supplementation was effective in decreasing lipid peroxidation, since that was observed a reduction of 17% on the MDA levels after TauCHOC treatment, however no changes were found in regards to antioxidant enzymes (GSH e α-tocoferol). Even though the results were not significant, it's important to note that the average levels of GSH and α-tocoferol post treatment were higher in the TauCHOC treatment than the CHOC treatment, and it suggests that TauCHOC treatment provided a metabolic economy of GSH and α-tocopherol and improved the activities of anti-oxidant defense system.

Similar results were found by Zhang et al (2004) that evaluated the effect of 7-day taurine supplementation (6 g grams/day) on oxidative stress induced by exercise in cyclists, and concluded that taurine decreased levels of thiobarbituric acid reactive substances (TBARS), which are also indicators of lipid peroxidation. Silva et al (2011) investigated the effect of 15-day taurine supplementation (300 mg/kg) or saline on oxidative stress biomarkers after 90-min downhill run session in rats. The authors concluded that taurine affected skeletal muscle contraction by decreasing oxidative stress (superoxide radical production, creatine kinase, lipid peroxidation and carbonylation levels), but no changes were found in the antioxidant enzyme activity after excise protocol, as were observed in the present study.

It was expected that taurine may have improved athletes performance since it can regulate carbohydrate metabolism (CARNEIRO et al, 2009) and when associated to a source of carbohydrate and proteins (chocolate milk), may futher favor athletes post exercise recovery and increase glucogen stores (KARP et al, 2006; PRITCHETT et al, 2009). However, no significant changes were observed in aerobic parameters post treatment. It´s important to highlight that, even though not signicant, there was observed a short increase about 0,15 Km/h in the Vmax and a decrease about 13 bpm in HR at the test performed post TAUchoc treatment, and this results indicates that taurine may have improved cardiorespiratory system since that a higher velocity was supported with lower HR in the last stage of the performance test. Therefore, TAUchoc treatment may offer some practical performance benefits.

Other researchers that investigated time trial performance with taurine supplementation in cyclists did not demonstrate improvement in performance (RUTHERFORD et al. 2010). In

contradiction with the results of the present study, Balshaw et al (2013) investigated the effect of acute ingestion of 1 g of taurine on maximal 3-km time trial (3KTT) performance in trained middle-distance runners, and they found that the ingestion of taurine improved 3KTT performance in 1.7 %.

Factors such as taurine dose and timing, athletes ability and exercise protocol may have contributed to whether or not taurine enhanced endurance performance (BALSHAW et al, 2013). In regards time of administration, maximum plasma taurine concentration can be found two hours post intake (GHANDFOROUSH-SATTARI et al, 2010). Higher availability of plasmatic taurine would be interesting to athletes performance seeing that taurine can increase calcium transport to myofibrillar contractile proteins and enhance skeletal muscle function (Schaffer et al 2010), however the aim of our study was to investigate the action of taurine as a post exercise recovery aid, considering that taurine can modulate carbohydrate metabolism and consequently increase carbohydrate up take into muscle cells (CARVALHEIRA et al, 2002; ROPELLE et al, 2005). The higher nutrients availability will promote glycogen synthase enzyme activation and consequently increase glycogen synthesis and restore hepatic and muscle stores. And when considering oxidative stress, the intake of taurine post exercise would be interesting to promote taurine concentration peak only more than one our post exercise, in order to not down regulate physiological free radical production post exercise and interrupt aerobic adaptations (POWERS and JACKSON, 2008). Overall, there was observed only anti-oxidant protection with the TAUchoc treatment proposed.

Some authors discuss that only chocolate milk can benefit performance (FERGUSSON-STEGAL et al, 2011; PRITCHETT et al, 2009). Fergusson-Stegal et al (2011) compared the effects of chocolate milk and carbohydrate isocaloric beverages intake post exercise on training adaptations occurring over 4.5 weeks of aerobic exercise training. The authors concluded that the supplementation with chocolate milk post exercise improved aerobic power more effectively than carbohydrate alone. By this way, perhaps only chocolate milk may have benefited both groups.

Although no significant treatment effect weas observed in the aerobic parameters, our results indicates that there may be potential performance benefits attributed to taurine supplementation. From a practical perspective, there was observed, in average, an increase of 1% in the speed developed in the LT of the subjects in the TAUchoc group comparing the test speed developed before treatment. Therefore, the association of taurine and chocolate milk as a post exercise recovery beverage may offer some practical performance benefits for an athlete during a competition.

CONCLUSION

The results of the present study indicated that taurine supplementation did not improved aerobic parameters, but was effective in decreasing lipid peroxidation, which suggests that taurine can prevent oxidative imbalance in triathletes. Additional studies are warranted to examine the potential advantage of taurine supplementation on indices of exercise recovery and muscle damage, since that some evidence of performance improvement was observed in the present study.

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Capítulo III

Artigo II

Supplementation of taurine associated with low fat chocolate milk post exercise did not improved body composition and carbohydrate metabolismo, but prevented protein catabolism in triathletes

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ABSTRACT

Chocolate milk has been suggested to be an effective post exercise recovery beverage due to the ideal carbohydrate to protein ratio, while taurine supplementation may increase lipid catabolism and favor carbohydrate metabolism. Therefore, research examining the combination of taurine with chocolate milk after exercise on insulin action in order to favor carbohydrates metabolism regulation, assist with muscle recovery, and prevent protein catabolism is warranted. The purpose of this study was to evaluate the effects of taurine and chocolate milk supplementation on body composition, on markers of blood carbohydrate and urinary protein metabolism in triathletes. A double-blind, crossover study was conducted with 9 male triathletes, age 25 to 35 years. Taurine (3 grams) (TAUchoc) or placebo 3 grams (CHOC) associated to 400ml of chocolate milk was ingested daily post exercise for a 8-week period. A 2-week washout was implemented in between trials. Body composition, blood glucose and insulin levels, HOMA and 24 hour urinary nitrogen, creatinine and urea excretion were measured before and after 8 weeks of training and supplementation with TAUchoc or CHOC. TAUchoc treatment during the 8 weeks resulted in a significant ($p= 0.03$) reduction in urinary nitrogen excretion (-33%) and promoted a positive nitrogen balance ($p=0.01$), while CHOC provided significant reductions in body weight ($p=0.03$) and body fat percentage ($p=0.04$). In conclusion, the intake of taurine associated with chocolate milk post exercise preserved muscle mass, while chocolate milk alone resulted in a more favorable body composition.

INTRODUCTION

Athletes performance can be influenced by an adequate nutritional supply and is related to the amount and also type of nutrients. There are nutritional recommendations for athletes that includes macronutrients but also the utilization of specific nutrients in order to support high energy requirements and ensure energy requirements during practice or competitions, but also to prevent muscle damage, especially at recovery post exercise (BENTLEY et al, 2008, HERNANDEZ and NAHAS, 2009).

Nowadays there are many different kinds of nutritional supplements available in the markets that can be used as a post exercise nutrient replenishment, however some researchers have discussed the possibility of using foods as recovery aids and one food that is on evidence actually is chocolate milk (CM) due to its carbohydrate and protein content, which are similar to some fluid replacement drinks, and thus become a source of nutrients to replenish depleted glycogen in the muscles and favors recovery post intense exercise. Also, CM presents good taste (KARP et al, 2006; PRITCHETT et al, 2009). Moreover, CM is a good source of leucine, which is an amino acid that participates in muscle mass synthesis and may benefit body composition.

In regards to specific nutrients, taurine had gained attention for sports nutrition researchers and athletes. Taurine presents antioxidant and anti-inflammatory actions thus may prevent inflammatory and oxidative damage in consequence of intense exercise (TAPPAZ, 2004; SUN et al, 2012; ZHANG et al, 2004). And also, taurine can regulate carbohydrate (VETTORAZZI et al, 2014; CARNEIRO et al, 2009), and lipid metabolism (MURAKAMI, 2015; TSUBOYAMA-KASAOKA et al, 2006). According to Carneiro et al (2009) taurine can stimulate insulin cascade, favoring glucose transport into the cells and increase glucose availability. And taurine can regulate lipid metabolism by increasing PGC-1 α expression and consequently increase lipid utilization (TSUBOYAMA-KASAOKA et al, 2006).

Thus taurine supplementation may benefit athlete's performance by increasing de availability of energy substrates provided from carbohydrates, by this way it could favors glycogen replenishment in the muscles and prevent protein catabolism, and also taurine can increase lipids oxidation, which may benefit specially sports of low intensity and long duration as triathlon. These facts may benefit both athlete's body composition and performance.

Further research is warranted to examine the effect of the association of taurine with chocolate milk after exercise on insulin action in order to favor carbohydrates metabolism regulation, assist with muscle recovery, and prevent protein catabolism. Therefore, this study has three purposes, to examine the effects of taurine associated to chocolate milk on body composition; and to examine the

effectiveness of taurine associated to chocolate milk on markers of carbohydrate and protein metabolism.

METHODS

Subjects:

Nine well-trained male triathletes with ages between 25 to 35 years who competed in semiprofessional triathlon were recruited toparticipate in the study. The participants were invited to go to the lab and all the experimental procedure, the associated risks and benefits were explained; those who agree to participate were asked to give written consent. Approval for this study was granted by the Human Ethics Committee of State University of Sao Paulo (CAAE 06191512.9.0000.5426).

Subjects were asked to record their diet 1 day before performing the data collection and record three days during the each treatment. Participants were required to arrive at the laboratory in the morning after 12 hours fast, in a rested state, having avoided strenuous physical activity and anti-inflammatory drugs for at least 48 h and having not taken any nutritional supplements in the previous 2 months.

Individuals reporting cardiovascular diseases, or if they had experienced a muscle injury in the past six months and/or were currently taking chronic or daily doses of anti-inflammatory medication or nutritional supplements were excluded from the study (McBRIER et al., 2010).

Study design and treatment

A randomized, double blind, crossover study design with volunteers serving as their own control was conducted. The study was consisted of taurine or placebo supplementation associated with chocolate milk post exercise during eight weeks, followed by 2-week washout period and then the protocol was repeated with the other treatment.

Subjects were invited to attend the laboratory for 8 days. On the first visit (PRE), anthropometric measures including height and weight, blood samples were taken and body fat percentage was determinate by deuterium method. The participants were asked to collect 24 hours urine in the day before the data collection. After these all procedures the first period of treatment was started. Following the 8 weeks of treatment, the subjects were invited to the lab and retake all the evaluations (POST). A 2-week wash out was done and then the protocol was repeated with the other treatment (PRE and POST second trial) (fig. 1).

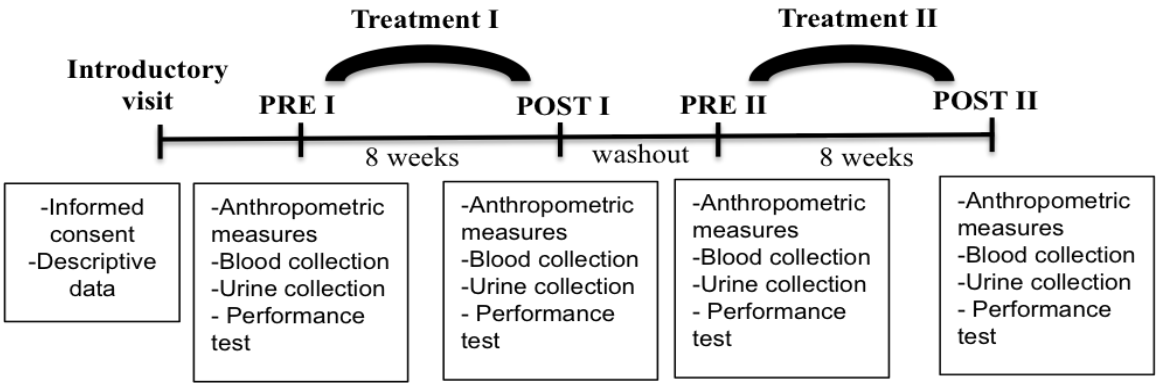

Fig. 1 Schematic representation of the experimental trial. Subjects were reported for an Introductory visit, and then at PRE I, after data collection and performance test were done, the first treatment was started. After 8-weeks of treatment, all the measurements were repeated (POST I). A 2-week wash out was done and then the protocol was repeated with the other treatment (PRE II and POST II).

After the baseline measurements, the subjects started the treatment with 3 grams of taurine (AminoethylsulfonicAcid, 99 % pure, Ajinomoto®) (Shao and Hathcock, 2008), or placebo (starch) in capsules orally associated to low fat chocolate milk (CM) (200 ml) immediately after practice and two hour later they had to drink more 200 ml of CM daily for 8 weeks. And after a 2-week washout period, the protocol was repeated with the other treatment (taurine or placebo capsules). During the supplementation period, subjects were asked not to change their dietary habits and keep their triathlon practice.

The low fat CM (Low Fat Pepsico Chocolate milk, Sao Paulo, SP) contained 92 kcal, 16 grams of carbohydrate, 4 grams of protein and 1,2 grams of lipids per serving (200 ml). The CM was selected considering the optimal ratio of carbohydrate to protein (4:1), in order to favor glycogen resynthesis (IVY et al., 2003; KERKSICK et al, 2008). Also, CM presents good taste and high acceptability by the athletes (KARP et al, 2006; PRITCHETT et al, 2009).

The participants were required to record daily training, including data about hours, kind of training, training zone, in order to calculate traing load during each treatment.

Dietary assessment

In order to control for any differences in dietary intake, participants were required to complete three-day food records in non-consecutive days during each trial and were asked to record dietary intake on the day before data collection. ESHA software (Esha Research Inc, Salem, OR) was used to calculate total calories and macro nutrient intake. Participants were instructed to maintain their habitual diet throughout the study.

MEASUREMENTS

Body composition

Body composition including body fat, water and lean mass percentage, were determinate by deuterium oxide dilution (2 H2O) technique (SCHOELLER, 1983). After an overnight (8-hour) fast, each volunteer received 1 g/kg deuterium oxide (99.9% deuterium oxide, Cambridge Isotope, USA) diluted to 7%, followed by 50 ml of natural water for complete ingestion of the deuterium. The subjects were instructed to collect urine (≈50 mL) into small sterile plastic cups. Urine samples were collected before and three, four and five hours of equilibrium time after ingestion of the deuterium and were stored at -10˚C until analysis. The deuterium enrichment of the urine samples was determined by isotope-ratio mass spectrometry (Europa Scientific Hydra System, Cheshire, United Kingdom) after equilibration with 100% hydrogen by the platinum-aluminum catalyst method (SCHOELLER, 1983).

Carbohydrate metabolism

Blood was collected (5 ml) at PRE and POST the two trials after a 12-hour fast. The samples were then stored in a freezer at –80º C until the time for analysis. Glucose concentrations were determined with kit *Glicose Liquiform Labtest diagnóstica*®. The glucose analyzes were done at least 8 hours after blood collection following the instructions in the kit.

Insulin quantifications were determined by immunochemiluminescence enzymatic method using Immulite® insulin. And insulin resistance (HOMA-IR) was calculated considering the formula proposed by Matthews et al (1985): [fast plasma glucose (mMol/L) x fast plasma insulin (μU/mL) /22.5].

Protein metabolism

Twenty-four hour urine samples were collected PRE and POST each trial in order to quantify urinary excretion of nitrogen, creatinine and urea.

Total nitrogen excretion was determinate using 20 µL of 24-hour diluted in 1000 µl of distillated water using a chemiluminescence nitrogen analyzer by the method proposed by (GRIMBLE, et al, 1987). The nitrogen balance was calculated considering total nitrogen excretion and protein intake [NB= (Protein intake (g) /6.25) –Total nitrogen excretion (g) + 4 (g)] (MOTA et al, 2003).

Creatinine and urea concentrations were determined by colorimetric reaction with spectrophotometer using the kit *Creatinine and Urea CE (Labtest diagnóstica*®).

STATISTICAL ANALYSES

A t-test for dependent samples was used to compare 3-day food records data, protein and carbohydrate markers, and body composition between PRE and POST trials.

Statistical Package for the Social Sciences for Windows software version 15.0 was used for all statistical analyses. All data was reported as means $\pm$ standard deviation. Statistical significance was set at $p < 0.05$ for all analyses.

RESULTS

The subjects characteristics reported as means ± ED for each participant are as follows: age (years): 30.9 ± 1.3, height (cm): 179.0 ± 0.01, weight (kg): 77.45 ± 2.4. According to the dietary analysis, there were no significant differences in macronutrient composition (kcalories, carbohydrate, protein and fat) between groups (Table 1) when the treatment beverages were include in the daily food intake.

Table 1. Average kcal and macronutrient intake for each group.

Nutrients intake	TAUchoc	CHOC	P value
Total Energy (Kcal/day)	2437.9±770.7	2226.9±704.2	0.31
Carbohydrate (g/Kg BW)	4.29 ± 1.35	4.19 ± 1.32	0.76
Protein (g/kg BW)	1.84 ± 0.58	1.54 ± 0.48	0.06
Fat (g/kg BW)	0.91 ± 0.28	0.92 ± 0.29	0.98

Note: Kcal and macronutrient intake from treatment beverages included in the average. TAUchoc: taurine and chocolate milk treatment, CHOC: placebo and chocolate milk treatment, g/Kg BW, grams

per kg of body weight. No significant differences were observed between trials. Data reported are means ± ED.

Body composition was measured by deuterium oxide dilution (2 H2O) technique. Table 2 shows body composition and percent of variation (Δ% POST-PRE) between PRE and POST treatment. There was observed a higher percentage of change in CHOC group, since that were found lower body weight (p= 0.03) and body fat percentage (p=0.04) post CHOC treatment.

Table 2. Body composition of triathletes PRE and POST treatments for each group.

Measurement	**TAUchoc**			**CHOC**		
	PRE	**POST**	**Δ % (POST-PRE)**	**PRE**	**POST**	**Δ % (POST-PRE)**
Weight (Kg)	76.1±2.3	76.8±2.4	0.89	77.4±2.4	76.5±2.3	-1.17*
BF %	15.5±1.5	15.0±1.5	-3.04	17.8±1.2	15.5±1.5	-14.04*
Water %	61.7±1.1	62.2±1.0	0.89	60.1±1.0	61.7±1.1	2.53
LM %	84.4±1.5	85.0±1.4	0.62	82.2±1.2	84.5±1.5	2.75*

Note: PRE: before treatment; POST: post treatment; BF, body fat mass; LM, lean mass. Data reported are means ± ED.Δ % (POST-PRE), percentage of change between POST and PRE.*significant difference in relation to TAUchoc group (pared T Test, $p \leq 0.05$).

The results for carbohydrate and protein metabolism markers are displayed in table 3. There were no significant changes in relation to blood glucose, insulin, HOMA-IR, creatinine and urea levels after TAUchoc or CHOC treatment.

Table 3. Carbohydrate and protein metabolism markers of triathletes PRE and POST treatments for each group.

Measurements	TAUchoc			CHOC		
	PRE	POST	Δ % (POST-PRE)	PRE	POST	Δ % (POST-PRE)
Glicose (mg/dl)	102.6±1.50	101.6±1.1	-1	94.8±2.2	95.6±2.0	0,8
Insulin (μIU/mL)	4.23±0.68	4.73±0.85	11,1	4.65±0.71	4.03±0.74	-13,3
HOMA-IR	1.08±0.17	1.20±0.22	11,8	1.11±0.19	0.94±0.16	-15,9
N ur (g/day)	26.91±30	16.70±1.9**	-37,9	24.2±2.7	20.20±1.90	- 16,5
Urea (g/24 h)	37.53±3.80	29.34±2.5	- 21,9	32.04±4.85	43.91±6.48	37,1
Creatinine (mg/Kg/24 h)	28,14±2.68	22.06±2,97	- 21,06	23.05±2.263	26.73±1.98	15,9
NB	-4.84±-1.4	6.20±1.79**	228,1	1.24±0.36	4.91±1.42	295,9

Note: PRE: before treatment; POST: post treatment; HOMA-IR, insulin resistence; N ur, 24 hours nitrogen excretion; NB, nitrogen balance. Data reported are means ± ED. **significant difference in relation to CHOC group (pared T Test, p≤0.05).

A significant reduction in 24 hours nitrogen excretion was observed after TAUchoc treatment (p= 0.03) and the percent of variation was higher post TAUchoc treatment (-37.9%) when compared to CHOC treatment (– 16.5%) (table 3) (p= 0.03). The nitrogen balance was calculated considering protein intake and total urinary nitrogen excretion, although no significant differences were found in protein intake, there was observed a significant change in nitrogen balance after TAUchoc treatment (p= 0.01) and the balance changed from negative (-4.84±-1.4) to positive (6.20±1.79).

Training intensity was controlled by a daily training report and analyzing the sum of the training load during the eight weeks TAUchoc group presented a training load of 2750 au and CHOC 2550 au, no differences were found between the trials (p=0,957), which means that the training intensity was similar in both treatments.

DISCUSSION

The main objective of the present study was to examine the effects of TAUchoc vs. CHOC in body composition and indices of blood carbohydrate and protein metabolism markers. Exercise performance was controlled by a daily training report in order to minimize differences in training between trials. Our results suggest that the supplementation of taurine associated to low fat chocolate milk for a 8-week period provide no additional benefits on body composition and carbohydrate metabolism markers, but only chocolate milk benefited body composition. However, there was found a reduction of urinary nitrogen excretion and positive nitrogen balance, indicating that taurine supplementation may have preserved muscle mass. Our study was the first to investigate the effect of taurine associated with chocolate milk specifically on carbohydrate and protein metabolism markers

in triathletes.

The triathletes presented an adequate body fat percentage (15.5±1.5 %), according to Jackson and Pollock (1985) classification. Similar results were found by Rüst et al (2012) that evaluated 82 "ironman" triathletes and found an average of 15.7 ± 4.6 body fat percent. A reduction in body fat percentage after TAUchoc treatment was expected since that taurine can increase energy expenditure by increasing mRNA expression of proteins that increase lipid oxidation (PGC-1α and PPAR) on adipose tissue (TSUBOYAMA-KASAOKA et al, 2006) also can stimulate mRNA expression of lipolitic enzymes as lipoprotein lipase, acyl-Coa oxidase, acyl-Coa synthetase (MURAKAMI, 2015). Martiniano et al (2015) evaluated the effect of 11-week taurine (2%) supplementation associated with exercise training in obese rats fed with high fat diet and found a reduction on visceral fat and epididymal fat weight after taurine supplementation, by this way, taurine promoted benefits totheir body composition. However, in the present study no significant changes in body composition were found after TAUchoc treatment and only the CHOC treatment promoted a significant reduction in body weight and body fat percentage.

Corroborating with our results but using only CM, Ferguson-Stegall et al (2011) compared the effects of a chocolate milk and an isocaloric carbohydrate beverage associated to aerobic exercise training during 4 weeks and found that chocolate milk post exercise improved performance and body composition more effectively than carbohydrate alone. These results were associated with chocolate milk nutritional composition, since that it contains carbohydrates and proteins, which favors recovery post exercise and may have affected body fat percentage.

In regards to carbohydrate metabolism, no significant changes were found after TAUchoc and CHOC treatment and the results indicates that the triathletes presented adequate levels of blood glucose and insulin. Since that taurine can modulate insulin action and promote carbohydrate metabolism (CARNEIRO et al, 2009), the purpose of supplementing taurine associated with CM post exercise and CM intake again two hours later was to provide nutrients in the moment that muscle cells presents higher sensitivity to nutrients uptake (JENTJENS and JEUKENDRUP, 2003) also, according to Ghandforoush-Sattari et al (2003), maximum plasma taurine concentration can be reached by 1.5±0.6 hours post administration. By this way, the TAUchoc treatment would increase the availability and utilization of nutrients post exercise and consequently stimulate glycogen synthesis, prevent muscle catabolism and promote protein synthesis.

In the present study, no significant changes were found in regards to blood markers of carbohydrate metabolism, indicating that the intake of taurine associated to CM had no impact blood glucose regulation, and glucose and insulin remained in appropriate levels. However, the results related to urinary protein metabolism showed that TAUchoc treatment induced lower urinary nitrogen

excretion and positive nitrogen-balance. The nitrogen-balance shows how much nitrogen is coming into to the body as is being excreted, and a positive balance indicates that more nitrogen is being retained than excreted, which suggests that muscle is being gained (BENARDOT, 2006).

Although other authors have shown changes in urea levels post intense exercise training (BENARDOT, 2006; HARTMANN and MESTER, 2000), the present study did not found changes in urea after TAUcho and CHOC and 8 weeks of triathlon practice. Corroborating with our study, but with different sports modalities, no significant changes in urinary urea levels were found after a periodized training in a study with soccer players (SANCHES et al, 2006) and with cyclists (HALTSON et al, 2002).

According to Haltson et al (2006) creatinine and urea can be used as markers of muscle damage, changes in lean body mass and dehydration (RIEHL et al, 2004). Since that no changes were found in urea and creatinine levels, and that nitrogen balance was positive after TAUchoc treatment, the results of the present study suggests that taurine can contribute to preserve muscle mass and may favor muscle recovery by ensuring adequate nutrients supply to muscle cells post exercise. Although the urinary nitrogen excretion was lower and nitrogen balance was positive after taurine treatment which could promote an increase in lean mass, no benefits were found in body composition after TAUchoc treatment.

CONCLUSION

The current research findings suggest that the association of taurine and low fat chocolate milk post exercise decreased urinary nitrogen excretion and promoted a positive nitrogen balance, indicating that taurine supplementation can contribute to preserve muscle mass and may favor muscle recovery, while chocolate milk alone resulted in a more favorable body composition. By this way, taurine supplementation may be considered a possible ergogenic aid in order to promote the maintenance of lean body mass, prevent muscle breakdown and benefit triathletes performance.

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Capítulo IV

Introdução e Artigo III

O Capítulo IV é composto por uma breve introdução à respeito do uso da suplementação de flavonóides de cacau para atletas de alto rendimento e inclui um artigo científico que foi elaborado com os resultados do projeto de pesquisa intitulado "*Effects of cocoa flavonols supplementation on oxidative stress and muscle recovery markers in rugby players"* desenvolvido durante o Doutorado Sanduíche – PDSE CAPES, realizado no *Department of Nutrition, Exercise, and Health Sciences* na *Central Washington University*, Ellensburg- WA/EUA, sob orientação da Profa Dra Kelly Pritchett no período de Fevereiro à Julho de 2015.

INTRODUÇÃO

Exercícios de alta intensidade induzem ao aumento da taxa de consumo de oxigênio e, consequentemente, elevam a produção de radicais livres, assim como os níveis de estresse oxidativo, podendo comprometer o desempenho de atletas (BENTLEY et al, 2008; MCBRIER et al, 2010; SUN et al, 2011). Entre os esportes que podem ocasionar alterações no metabolismo oxidativo pode-se destacar o rugby, visto que se trata de um esporte em que os jogadores passam a maior parte do tempo em atividades aeróbicas, porém também há momentos em que se envolvem em atividades anaeróbicas (PERELLA et al, 2005).

A prática de rugby resulta em combinadas e repetitivas corridas de alta intensidade que resultam em diversas respostas fisiológicas em seus jogadores podendo ocasionar desequilíbrios oxidativos, além de apresentar freqüêntes contatos corporais resultando em alta incidência de colisões e lesões musculares (SCOTT et al, 2003). Desta forma, são necessários cuidados nutricionais que vão desde a adequação da ingestão calórica, para garantir a alta demanda energética destes atletas, mas também a utilização de nutrientes específicos visando a proteção antioxidante e anti-inflamatória a fim de minimizar as alterações oxidativas e inflamatórias consequentes da prática desta modalidade esportiva (SCOTT et al, 2003, FINAUD et al, 2006).

Atualmente o consumo de flavonóides proveniente da alimentação, principalmente os flavonoides de cacau, ganharam atenção de pesquisadores devido às suas propriedades antioxidantes e o seu potencial de minimizar o estresse oxidativo e, assim, auxiliar na recuperação muscular pós-exercício (MORILLAS-RUIZ et al, 2006). Os antioxidantes evitam que radicais livres se transformem em radicais prejudiciais às células, e também podem converter espécies reativas de oxigênio em versões menos ativas, promovendo equilíbrio entre a produção de substâncias pró-oxidantes e anti-oxidantes após o exercício (PEAKE et al, 2006; POWERS et al, 2004).

Particularmente as bebidas à base de cacau, como os leites achocolatados, têm se mostrado efetivos para a recuperação após o treino (KARP et al, 2006; GILSON et al, 2010; PRITCHETT et al, 2009; THOMAS et al, 2009). Além de seu conteúdo de flavonóides de ação anti-oxidante, estas bebidas contêm teor de carboidratos e proteínas semelhantes à algumas bebidas repositoras comumente utilizada por atletas, e desta forma, tornam-se um

meio eficaz para o reabastecimento de glicogênio depletado nos músculos, favorecendo a recuperação após o exercício (KARP et al, 2006; PRITCHETT et al, 2009).

O estudo realizado por Karp et al (2006) comparou o efeito ergogênico para a recuperação após o treino utilizando-se leite achocolatado, bebida para a reposição de líquidos ou bebida rica em eletrólitos, e os autores constataram que a bebida achocolatada resultou em maior tempo para atingir a exaustão em relação às demais bebidas. O consumo de leite achocolatado após exercícios de resistência pode favorecer ao aumento da taxa de síntese de proteína muscular, a sinalização de moléculas para *turnover* de proteína muscular esquelética, a cinética da leucina, e assim beneficiar o desempenho de atletas (LUNN et al, 2012). De acordo com Ferguson-Stegall et al (2011) a suplementação de achocolatado após o exercício pode melhorar a potência aeróbia e a composição corporal de forma mais eficaz do que bebidas que contém apenas carboidratos.

Além dos benefícios nutricionais provenientes do consumo de achocolatados relatados anteriormente, Miller et al (2008) sugerem que os flavonoides de cacau também oferecem benefícios para a saúde cardiovascular, tais como reduzir a pressão arterial e melhorar a circulação sanguínea. Desta forma, estes compostos podem favorecer a distribuição de oxigênio e de nutrientes para as células musculares, além de remover de forma mais eficiente os resíduos metabólicos (dióxido de carbono, ácido lático, entre outros) gerados pelos músculos durante o exercício (MCBRIER et al, 2010).

O uso de bebidas achocolatadas, principalmente devido ao seu conteúdo de flavonóides de cacau, pode beneficiar a circulação sanguínea, promover a absorção de nutrientes de ação antioxidante e, conseqüentemente, otimizar a recuperação de atletas após os treinos. No laboratório do *Department of Nutrition, Exercise, and Health Science* na *Central Washington University*, foi realizado um estudo que avaliou os efeitos da suplementação aguda de flavonóides de cacau sobre marcadores de lesão e dor muscular utilizando-se de leite achocolatado de baixo teor de gordura, no entanto, não foram constatados benefícios adicionais na recuperação pós-treino (PESCHEK et al, 2014). Portanto, é necessário avaliar o efeito da suplementação crônica de flavonóides de cacau a fim de investigar os benefícios relatados na literatura.

Sabe-se que exercícios físicos naturalmente aumentam o estresse oxidativo, e já está estabelecido que a produção de quantidades baixas a moderadas de oxidantes desempenham

várias funções celulares como a regulação de vias de sinalização celular e modulação de células musculares esqueléticas, por outro lado, altos níveis de radicais livres podem danificar componentes celulares (POWERS e JACKSON, 2008), desta forma, o uso de compostos antioxidantes após exercício tem a finalidade de evitar a produção excessiva de radicais livres, sem comprometer a produção fisiológica de substâncias pró-oxidantes. Holt et al (2002) descreveu a cinética de flavonoides de cacau no plasma humano após o consumo de uma bebida rica em flavonoides de cacau (375 mg) e os flavonoides foram detectados no plasma inicialmente 30 minutos após o consumo e atingiram concentração máxima 2 horas após o consumo. Assim, se considerarmos que as concentrações plasmáticas máximas de flavonoides são atingidas duas horas após o consumo, imediatamente após o exercício seria um bom momento para a suplementação de flavonóis de cacau visto que estes nutrientes provavelmente não irão comprometer metabolismo oxidativo após o exercício, mas poderão evitar a produção exacerbada de radicais livres.

Mais pesquisas são necessárias a fim de verificar se a suplementação crônica de bebidas achocolatadas à base de flavonoides de cacau pode ser utilizada como recurso ergogênico eficaz para a recuperação pós-exercício. Mais especificamente, pesquisadores precisam avaliar quais os efeitos que o consumo de flavonoides de cacau pode proporcionar sobre os níveis de estresse oxidativo e sobre marcadores de recuperação muscular após treino em atletas de alto rendimento.

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Artigo III

Additional cocoa flavanols has limited benefit to a post exercise recovery beverage on markers of oxidative stress and recovery indices in elite college rugby players.

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Abstract

Athletic performance can be impaired following strenuous sport specific movements due to localized muscular inflammation and oxidative stress. Antioxidants such as those found in cocoa flavanols have been supplemented to aid in reduction of oxidative stress, however, the benefits of dietary antioxidants for athletic performance following muscle soreness (MS) is unclear. In this study, cocoa flavanols effects were examined after a MS protocol. In a randomized double blind design, 13 male collegiate rugby players consumed either chocolate milk (CHOC) or chocolate milk with additional cocoa flavanols (CocoaCHOC) during a 7 day loading phase. Muscle soreness was induced on Day 5 of the intervention by way of 100 strenuous eccentric drop jumps. Muscle function was assessed with vertical jump height, average peak torque and a rugby performance (Yo-Yo) test as well as subjective measures of soreness. Blood biomarkers of inflammation were assessed Pre, 24 and 48 hours post MS. Urinary samples were also analyzed for oxidative stress markers at baseline and 48 hours post MS. No significant differences were found between groups in performance measures of peak isometric force, vertical jump height or Yo-Yo running distance. Additionally, no significant interaction was found between the flavanols for either blood or urinary markers of oxidative stress. Although not significant, the CocoaCHOC group ran 9.85% further than the CHOC group in the Yo-Yo test. This study indicates that the addition of cocoa flavanols to a post-exercise recovery beverage has no oxidative stress or athletic performance benefits.

Key words: oxidative stress, cocoa flavanol, rugby, chocolate milk, muscle damage, recovery.

Introduction

Intense exercise increases the rate of oxygen consumption and consequently increases the production of free radicals and oxidative stress, which may compromise an athlete's performance (BENTLEY et al. 2008; MCBRIER et al. 2010; SUN et al. 2011). Recently, dietary flavanols have gained attention because their antioxidant properties and potential to reduce oxidative stress, and thus aid in post exercise muscle recovery (MORILLAS-RUIZ et al. 2006). Antioxidants can prevent free radicals from transforming to damaging radicals, therefore limiting the conversion of reactive oxygen species into less active versions (PEAKE et al. 2006; POWERS et al. 2004).

In particular, cocoa-based beverages, such as chocolate milk have been found to be an effective post exercise recovery aid (KARP et al. 2006; GILSON et al. 2010; PRITCHETT et al. 2009; THOMAS et al. 2009). Besides the antioxidants flavanols content, these foods contain carbohydrate and proteins similar to some fluid replacement drinks, and thus become effective for

replenishing depleted glycogen in the muscles, thus promoting recovery after high intensity exercise. Also, chocolate milk presents good taste and high acceptability by the athletes (KARP et al. 2006; PRITCHETT et al. 2009). Karp et al. (2006) compared the effects of using chocolate milk, a fluid replacement drink and a carbohydrate replacement beverage as a post exercise recovery aid, following endurance cycling. The authors' suggested that the chocolate beverage increased time to exhaustion when compared to other drinks. Furthermore, chocolate milk consumption after endurance exercise may benefits post exercise muscle protein fractional synthetic rate, molecules of skeletal muscle protein turnover signaling, leucine kinetics, and performance measures (LUNN et al. 2012).

Pritchett et al. (2009) compared chocolate milk and commercial carbohydrate replacement beverage and they concluded that chocolate milk promoted the same benefits that carbohydrate beverage in post exercise recovery for cyclists. And according to Ferguson-Stegall et al (2011) chocolate milk supplementation post exercise may improve aerobic power and body composition more effectively than carbohydrate alone.

In addition to nutritional benefits chocolate milk intake previously reported, Miller et al (2008) suggested that the cocoa flavanols also provide cardiovascular health benefits such as increasing vasodilation and coronary arterial output, decrease blood pressure and improve blood circulation. Thus, this improvement in blood circulation may favor a better delivery of oxygen and nutrients to working muscle cells as well as a more efficient removal of metabolic waste products (carbon dioxide, lactic acid, and others) generated by muscles during exercise (MCBRIER et al. 2010). Therefore, the use of cocoa flavanols may enhance blood pressure, promote anti-oxidant nutrient uptake and consequently favor athlete's recovery after workouts. However, according to Miller et al. (2008) the cocoa found in chocolate milk has gone through the alkalization process therefore reducing the antioxidant potential. Perhaps the addition of cocoa flavanols (308 mg immediately, and 2-h post exercise) could enhance the antioxidant capabilities of low fat chocolate milk and benefit exercise recovery. In a previous we found that an acute addition of cocoa flavanols to low-fat chocolate milk did not provide an additional recovery benefit (PESCHEK et al. 2014), therefore it can be assumed that a cocoa flavanols loading phase is needed to investigate any benefits for high levels of free radicals in athletes that have a chronically high training volume.

Exercise naturally increases oxidative stress and low-to-moderate levels of oxidants play multiple regulatory roles in cells such as the regulation of cell signaling pathways and skeletal muscle modulation (POWERS and JACKSON, 2008). Furthermore, high levels of free radicals can damage cellular components (POWERS and JACKSON, 2008), therefore, the use of antioxidant compounds post exercise could prevent high levels of free radicals without limiting oxidative stress production. Holt et al. (2002) described cocoa flavanol kinetics in human plasma after the

consumption of a flavanol-rich cocoa and detected cocoa flavanols as early as 0.5 hours post ingestion and reached maximal concentrations by 2 hours. Thus, considering two hours for the maximal concentration to develop, immediately post exercise ingestion would be ideal for cocoa flavanol supplementation to prevent free radicals overproduction and oxidative damage.

Further research is warranted to examine the efficacy of a one-week loading phase with a cocoa-based beverage with various flavanol contents as a post-exercise recovery aid. More specifically, research needs to focus on cocoa's effect on muscle recovery and oxidative stress levels. Therefore, this study has two purposes, 1) to examine the effectiveness of natural cocoa on markers of muscle recovery and 2) to examine the efficacy of cocoa on oxidative stress levels.

Methods:

Subjects:

Elite level male rugby players (n = 13) who were members of the Central Washington University rugby team, were recruited for this study. All subjects were between the ages of 18–26 years of age. Human Subjects Review Committee (HSRC) approval was obtained from Central Washington University. All participants read and signed a written consent form approved by HSRC. Participants were excluded if they had experienced a lower extremity injury in the past six months and/or were currently taking chronic or daily doses of anti-inflammatory medication or nutritional supplements. Participants who had history of a recent illness were excluded from the study (McBRIER et al., 2010).

Experimental Design

A randomized double blind study design was conducted. Participants were assigned to one of two independent groups: (i) Chocolate milk + cocoa flavanols (CocoaCHOC) and (ii) Chocolate milk (CHOC). The study consisted of 7-day supplementation period with the assigned recovery beverage based on the 1 g/ carbohydrate/kg of body weight given daily to the subjects immediately after and at 2-h post rugby practice.

Subjects reported to the lab four times in total. During the introductory lab visit, anthropometric measures were taken, including height, weight, and body fat percentage. Body composition was determined using a three-site skin fold measurement on the right side of the body, using standard procedures described by Jackson and Pollock in 1985 (ACSM's Guidelines, 2010). All measures were taken by the same trained technician using Lange skin fold calipers (Country Technology, Inc., Gays Mills, WI, USA). Measurements were repeated three times per skin fold site

(chest, abdomen, and thigh) using the average results to calculate body density (ACSM's Guidelines, 2010). Body fat percentage was calculated using the Siri formula (Siri, 1961).

Performance tests included the Yo-Yo test, vertical jump, Muscle function protocol and subjective measures of muscle soreness. All tests were performed during the introductory visit (Baseline).

Participants started the CocoaCHOC or CHOC treatment after baseline urine data collection on Day 1 (Figure 1). Urine was collected to quantify oxidative stress (isoprostanes) markers. At Day 5, participants reported to the laboratory for blood draw (PRE) and then performed a muscle soreness (MS) protocol. Another blood draw was done 24h-POST MS protocol for muscle damage (creatine kinase) markers quantification and subjective measures of muscle soreness was collected. The rugby players had practice daily during the treatment except for Day 6, which was the recovery day, to isolate effects of MS protocol.

On Day 7, blood and urine samples were taken (48H-POST MS) and participants completed the performance and subjective measure of muscle soreness tests (Figure 1).

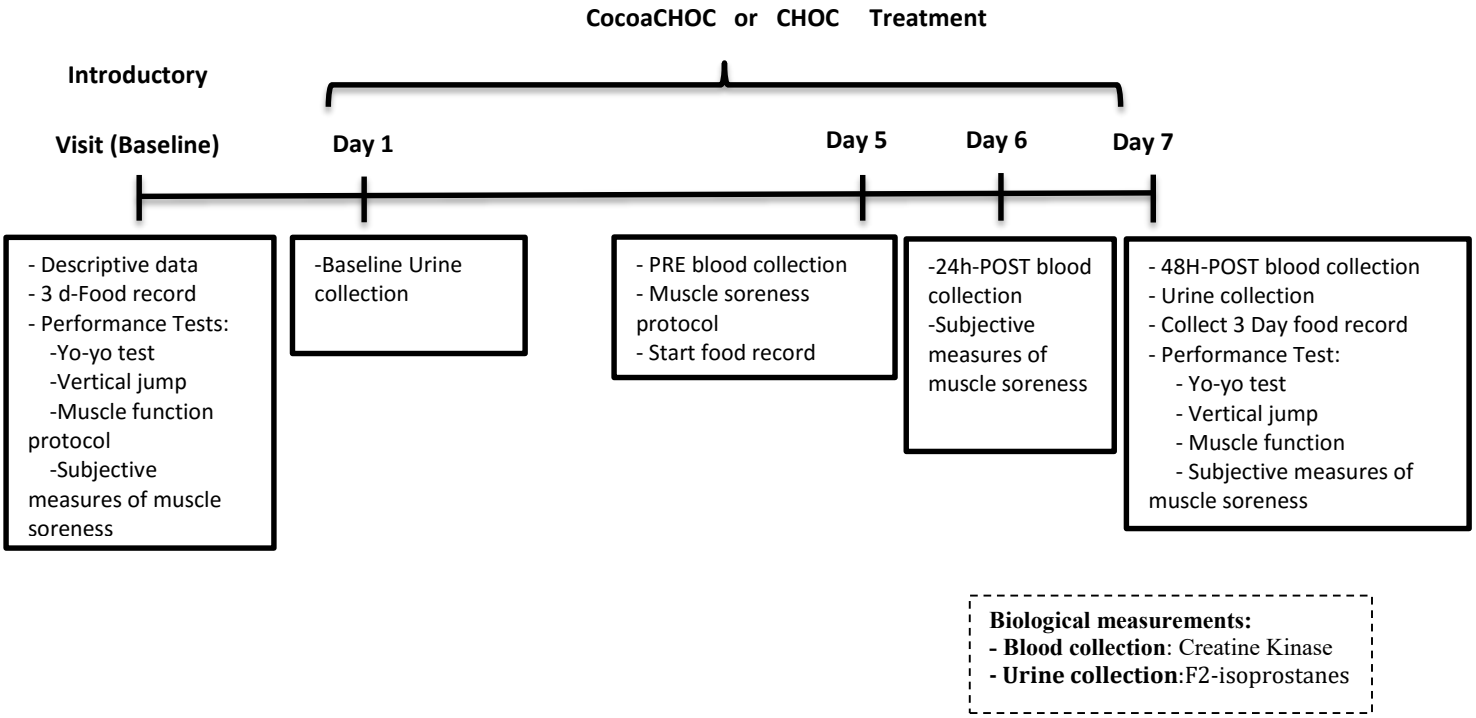

Fig. 1: Schematic representation of the study design.

Treatment

Two recovery beverages were examined: a cocoa- based (processed with alkali) carbohydrate protein beverage (0 mg of flavanols) vs. a carbohydrate protein beverage plus natural cocoa (308 mg of flavanols). Subjects consumed their assigned beverage immediately after exercise and again at 2-h post exercise, the volume of chocolate milk per serving was calculated based on 1g of CHO/kg of body weight daily for a 7 day period, and the total amount of cocoa flavanols provided was 616 mg per day (KARP et al. 2006; PRITCHETT et al. 2009; THOMAS et al. 2009, PRITCHETT et al. 2010). The CHOC used in this study (LowFat Darigold Chocolate milk, Seattle, WA) contained sucrose (glucose plus fructose), cocoa processed with alkali, salt, carrageenan, vanillin, vitamin A palmitate, and vitamin D3. CocoaCHOC (Unsweetened Cocoa, Hersheys Company, Hershey, PA) contained cocoa powder (processed with alkali), maltodextrin, natural flavor, carrageenan, cocoa powder (unprocessed), salt, and soy lecithin. Nutrient content (Kcal, CHO, Pro, Fat, per 240 mL) of the test drink is listed in Table 1. All drinks were packaged in unmarked bottles. During the recovery period, subjects were permitted to engage in simple activities of daily living such as walking, reading and studying. Furthermore, the participants were instructed to consume a similar diet during the testing period.

Table 1. Macronutrient content of the recovery beverages per 240 ml.

Total energy and macronutrient content	CHOC	CHOC Cocoa
Energy (Kcals)	246	246
Carbohydrate (g)	36	36
Protein (g)	11	10
Fat (g)	12	12
Volume (mL)	240	240

Note: Amount of beverage ingested immediately after exercise and 2-h post exercise was based on 1 g CHO/kg body mass.

Muscle Soreness (MS) protocol

Participants performed a total of 100 drop-jumps from a height of 0.6 m, which has previously been suggested to induce muscle damage in rugby players and other similar sports (GOODAIL and HOWATSON, 2008; NOSAKA et al. 2006). Upon landing, participants were encouraged to immediately jump vertically with maximal effort. Five sets of 20 drop-jumps were performed with a

10 s interval between each jump and a 2 min rest between sets. The MS protocol was performed on Day 5.

Dietary assessment

Subjects were instructed on the protocol for completing three-day food records and were asked to record dietary intake during the trial starting the morning of the MS protocol (Day 5) and ending on Day 7, to control for any differences in dietary intake.

Measurements

Muscle function protocol (isometric contractions)

Muscle function was assessed in the dominant-limb knee extensors (based on kicking preference) against the lever arm of the isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) at introductory visit (Baseline) and 48 h POST MS protocol (Day 7). Participants were secured to the dynamometer chair in a seated position using chest, waist, and thigh straps. The lever arm was attached to the dominant limb ankle, 2cm above the medial malleolus. The subjects performed three maximum voluntary contractions (MVC) lasting 3 seconds, with 60 seconds rest in between. Each MVC was performed with the knee at an angle of 90°. The average peak torque value was recorded as Newton meters (Nm). Every subject received strong verbal encouragement during each MVC (HOWATSON et al. 2012).

Vertical Jump

Using a 60x40c m force plate (AMTI, Watertown, USA), participants were required to place their hands on their hips to minimize the impact of arm swing and, in one flowing movement, to bend their knees to approximately 90° and then jump vertically for maximum height. Vertical jump performance was assessed at introductory visit (Baseline) and 48 h POST MS protocol. Flight time and ground reaction forces were recorded using Cortex analysis software (Motion Analysis, Denver, USA) with a frequency of 1000 hz. Each participant performed three attempts with a minute rest in between each jump. The participant's jump height was calculated from flight time, and the mean of three jumps was used for the analysis (LINTHORNE, 2001). The coefficient of variation for this protocol from reliability trials conducted in Northumbria University Laboratories is 1.9%. Vertical jump test is commonly used when assessing performance in team-based field sports (GABBETT, 2006).

Rugby Performance Test: Yo-Yo test

Subjects completed the Yo-Yo test that consisted of repeated 2 x 20-meter shuttle runs (i.e. shuttle pairs) back and forth between the starting, turning, and finishing line at a progressively increased speed controlled by audio beeps from a pre-recorded compact disc (BANGSBO et al. 2008). The test was completed when the player failed twice to reach the finishing line in time. The distance covered (in meters) was recorded and this represented the test result. The Yo-Yo test was performed indoors on running lanes and was done during the introductory visit (Baseline) and 48 h POST MS protocol (KRUSTRUP et al. 2015). This performance test is familiar to these rugby players as the coaches use it in season.

Subjective measurements of muscle soreness

Subjective measurements of muscle soreness were assessed at the introductory visit (Baseline), 24h POST and 48 h POST MS protocol, and again after the performance tests (Day 6 and 7). Self-perceived muscle soreness was assessed using a 10 cm visual analog scale (DANNECKER et al., 2003) with anchor points "no pain at all" at the left end and "unbearable pain" at the right end. Based on McBrier et al. (2010), muscle soreness was assessed using a lower extremity functional scale (LEFS) at introductory visit (Baseline), 24h POST and 48 h POST MS protocol, to determine the participant's level of difficulty with various activities of daily living (McBRIER et al. 2010). The LEFS is a validated survey consisting of 20 questions. It asks the participants to rate the perceived level of difficulty if they were to perform tasks on a 0–4 scale with 0 being unable to perform the activity, and 4 having no difficulty at all (BINKLEY et al. 1999). Upon completion of the questionnaire, percent of muscle function can be assessed. The higher the number, the higher percent of perceived muscle function the participant has.

Creatine kinase

Blood samples for creatine kinase (CK) were obtained before (PRE) the muscle soreness protocol (MS), 24 h post the MS (24 h-POST) and 48 h post the MS, for both treatments. The samples consisted of 0.025 mL of blood from the fingertip using a lancet. The blood sample was collected at the fingertip using a plasma separator tube before the beginning of the first exercise session (PRE) to determine baseline CK levels. Blood samples were analyzed for the average CK absorbance difference per minute at 340 nm using a spectrometer (Genesys 10 Series Thermo Spectronic, Rochester, NY, USA).CK levels were examined at 24 and 48-h after the MS protocol based off of the literature that describes that peak accumulation for CK levels occurs between 12 and 24 h after

exercise, with peaks of subjective measures of muscle soreness at 48 h post exercise (SAUDERS et al. 2004; PESCHEK et al. 2014).

Isoprostanes

Urine samples were collected into sterilized 100 mL cups at the introductory visit (Baseline) and 48 h POST MS protocol in order to determine Urinary F2-isoprostanes, which are oxidative stress markers. Urine samples were kept in a portable, insulated bag with ice packs (at ~0^{o} – 4^{o} C), processed in ≤6 h of collection, and immediately stored at -80^{o} C until laboratory analyses. Urinary F2-isoprostane concentration was determined by immunoassay using commercially available ELISA kits according to the manufacturer's instructions (Oxford Biomedical Research, Oxford, MI).

Statistical analysis:

3-day diet records were analyzed using ESHA software (Esha Research Inc, Salem, OR) to examine kcal, carbohydrate, protein and fat content. Data from the 2 trials was compared with a t-test for independent samples. A 1-Way Repeated Measures ANOVA (treatment x time) was used to compare CK, VAS, LEFS. A t-test was used to compare isoprostane levels and performance test between baseline and 48 h POST trials. Statistical Package for the Social Sciences for Windows software version 15.0 was used for all statistical analyses. All data was reported as means $^{\pm}$ standard deviation. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Descriptive characteristics reported as means ± SD for each participant are as follows: age (years): 20.69 ± 1.49, height (cm): 180.0 ± 0.05, weight (kg): 87.02 ± 8.03, and body fat percentage: 12.91 ± 4.20. According to the dietary analysis, there were no significant differences in macronutrient composition (kilocalories, CHO, PRO, and FAT) between groups (Table 2) when the treatment beverages were include in the daily food intake.

Table 2. Average kcal and macronutrient intake for each group.

Nutrients intake	CocoaCHOC	CHOC	P value
Total Energy (Kcal/day)	3178.9±312.4	3269.2±574.5	0.75
Carbohydrate (g/Kg BW)	4.41 ± 0.79	4.82 ± 0.28	0.22
Protein (g/kg BW)	1.87 ± 0.45	1.69 ± 0.29	0.64
Fat (g/kg BW)	1.40 ± 0.49	1.33 ± 0.45	0.90

Note: Kcal and macronutrient intake from treatment beverages included in the average.
No significant differences were observed between trials. Data reported are means ± SD.
g/Kg BW, grams per kg of body weight.

Performance was measured using accumulated shuttle distance (meters) by the subjects in the Yo Yo test. There was no significant difference ($p = 0.57$) between baseline and 48 h POST MS protocol (CocoaCHOC Baseline 1405.71 ± 373.52 m and 48 h POST MS 1468 ± 378.25 m, CHOC PRE 1453.33 ± 355.28 m, 48 h POST MS 1293.33 ± 397.77 m (fig. 2). However, total distance increased by 9.85% (62.86 ± 539.48 m) in the CocoaCHOC trial and decreased by 5.8% (160.0 ± 583.48 m) in the CHOC trial from Baseline to 48 h POST MS in the Yo Yo test performance, respectively.

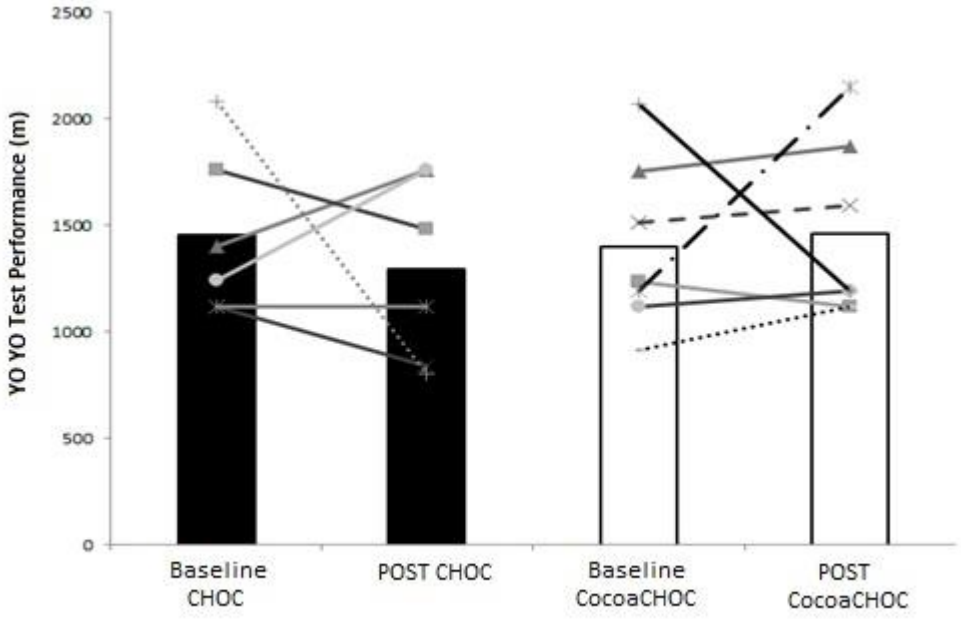

Fig. 2 Yo-Yo test performance of trained rugby players (n = 13) on two treatments (CocoaCHOC and CHOC). Individual data and mean data are presented. No significant differences were observed between trials.

There was no significant difference (p = 0.63) found in regards to isometric torque overtime between trials (CocoaCHOC Baseline 311.71 ± 48.91Nm,and 48 h POST MS 321.53 ± 56.45 Nm; CHOCBaseline299.34 ± 43.97Nm, 48 h POST MS 332.29 ± 60.25Nm, respectively). No significant changes (p = 0.39) were found for vertical jump performance between trials (CocoaCHOC Baseline 0.47 ± 0.11 m, and 48 h POST MS 0.41 ± 0.89 m; CHOC Baseline 0.52 ± 0.55 m, and 48 h POST MS 0.45 ± 0.05 m).

Perceived muscle soreness using a LEFS, as well as a VAS, was examined at Baseline, 24 h-POST and 48 h POST. No interaction (treatment × time) (p = 0.23) for muscle soreness using the VAS was observed (fig. 3). No interaction-time (treatment × time) (p= 0.82) for muscle soreness using the LEFS was observed. The results for LEFS are shown in figure 4.

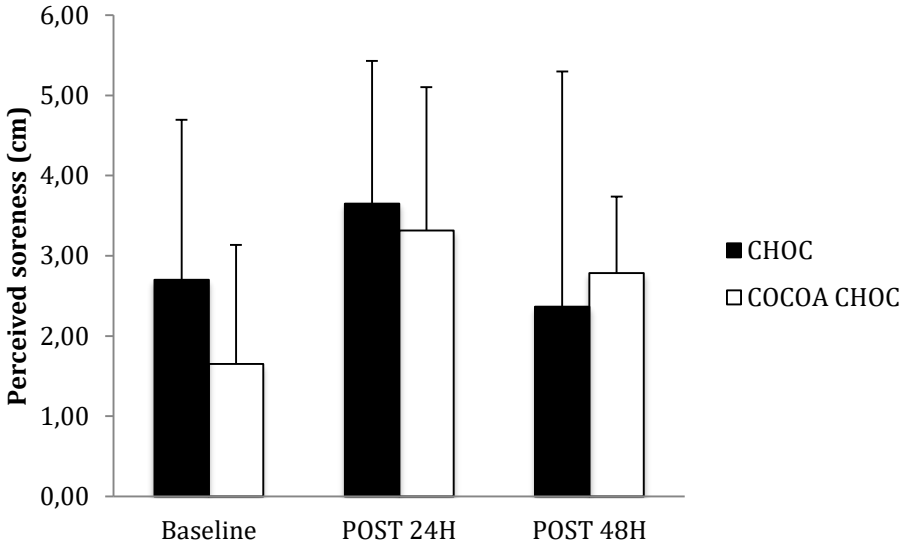

Fig. 3 Mean ± SD perceived muscle soreness using the visual analog scale (VAS) at Day 1, Baseline, 24 h-POST and 48 h-POST MS protocol for each beverage (CHOC, CocoaCHOC). No significant differences were observed between trials.

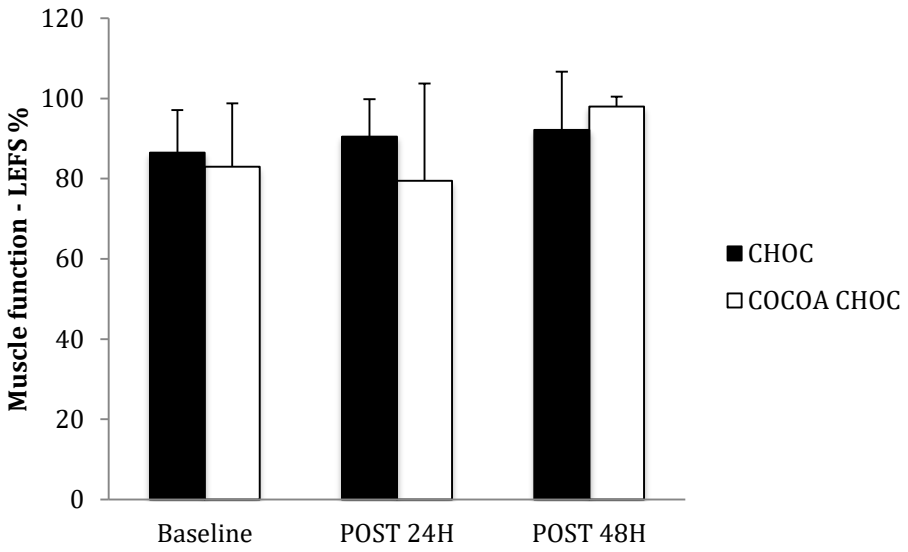

Fig. 4 Mean ± SD percent muscle function (LEFS) at Baseline, 24 h-POST, 48 h-POST MS protocol between treatment beverages. Note CHOC, chocolate milk; CocoaCHOC, cocoa beverage; LEFS, lower extremity functional scale.

The results for CK levels are displayed in figure 5. A one-way ANOVA revealed no significant changes between groups for CK PRE ($p = 0.36$) (CocoaCHOC 112.94 ± 64.82 U/L, CHOC 77.95 ± 56.09 U/L), for CK 24 h-POST ($p = 0.74$) (CocoaCHOC 92.06 ± 57.89 U/L, CHOC 97.06 ± 58.81 U/L), and for CK h POST ($p = 0.24$) (CocoaCHOC 41.97 ± 19.25 U/L, CHOC 56.41 ± 28.27 U/L). Furthermore, there was no main effect (treatment × time) ($p = 0.95$) for CK observed within groups.

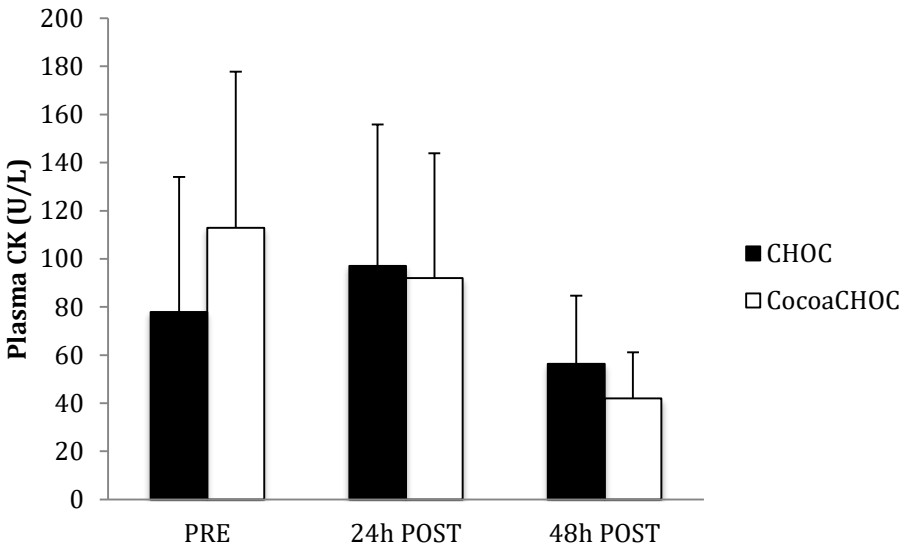

Fig.5 Creatine kinase (CK) levels for each trial (CK PRE, CK 24 h-POST, and CK 48 h-POST MS protocol). CK, creatine kinase; U/L, units/liter Note: CHOC, chocolate milk; CocoaCHOC, cocoa beverage; CK, creatine kinase; U/L, units/liter. No significant differences were observed between trials.

The results for isoprostanes are displayed in figure 6. No significant difference was found between trials (CocoaCHOC Baseline0.88 ± 0.38 ng/mL and 48 h POST 0.81 ± 0.53 ng/mL, p = 0.82; and CHOCBaseline 0.98 ± 0.96 ng/mL and 48 h POST0.96 ± 0.38 ng/mL, p = 0.59). And no main effect (treatment × time) (p = 0.58) was observed for Isoprostanes within groups.

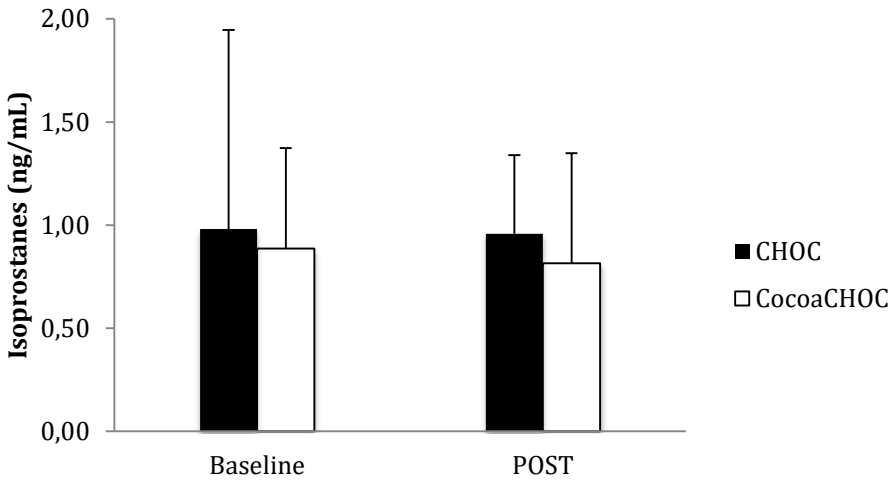

Fig. 6 Mean ± SD isoprostanes levels at Baseline and 48 h-POST MS protocol between treatment beverages. Note: CHOC, chocolate milk; CocoaCHOC, cocoa beverage.

Discussion

The main objective of this study was to examine the effects of CocoaCHOC vs. CHOC on indices of oxidative stress and muscle damage. Exercise performance was assessed using a Yo-Yo test, isometric torque and vertical jump height to compare the efficacy of the treatment beverages. Our results suggest that the addition of cocoa flavanols or placebo (cocoa- based processed with alkali, 0 mg of flavanols) to low fat chocolate milk for a week long period provide no additional benefits on markers of muscle recovery and perceived soreness. Furthermore, there were no differences in subsequent exercise performance following exercise induced muscle damage between the two treatments.

Although not significant, an increase in perceived muscle soreness from baseline at either 24 h or 48 h after the drop-jumps protocol indicated that the muscle soreness protocol was effective in inducing delayed onset muscle soreness (DOMS). In addition, CK levels were not different between PRE, 24 h POST and 48 h POST MS protocol in either of the groups of rugby players and was actually lower following the muscle damage protocol, but not significantly. Other researchers have investigated the effects of chocolate milk as a recovery beverage after exercise and found similar results (MCBRIER et al. 2010; PESCHEK et al. 2014). McBrier et al. (2010) supplemented a cocoa-based carbohydrate/protein beverage following exhaustive exercise and observed no changes from CK PRE to CK 48 h POST in both trials after exhaustive aerobic exercise. Peschek et al. (2014) added

350 mg flavanols to a low fat chocolate milk recover beverage and supplemented athletes immediately after the downhill run and again 2 h later, and no significant difference was found for creatine kinase levels, or muscle soreness after downhill treadmill running. Although the present study implemented a treatment loading phase, the results were similar to previous findings from McBrier et al. (2010) and Pescheck et al. (2014) using acute doses of cocoa flavanols.

In regards to oxidative stress, it is well known that intense training and competitions require eccentric muscle contractions and can increase oxidative metabolism and exacerbate free radicals production, thus negatively impacting performance (MCLEAY et al. 2012; PINGITORE et al. 2015). Therefore, the utilization of antioxidant nutrients following a muscle soreness protocol aimed to prevent inflammation, oxidative stress and muscle damage (KIM and LEE, 2014). Using quadriceps strenuous eccentric contractions MS protocol, McLeay et al. (2012) examined the effect of a blueberry smoothie or placebo with a similar antioxidant capacity prior to and then immediately, 12 and 36 hours after MS protocol on oxidative stress markers. In contradiction with the results of the present study, an increase was found in oxidative stress biomarkers following the damage protocol and a faster rate of decrease in oxidative stress was observed 36 hours after the MS protocol in the blueberry group. According to McLeay et al. (2012), this protection occurred due to the effect of antioxidant compounds (flavanols, anthocyanins and phenolic acids, 10.2 mg, 26 mg and 96.6 mg per 100 ml beverage, respectively) found in blueberries.

However, in the present study a drop-jumps MS protocol was performed five days after the CocoaCHOC or CHOC treatment was started and no significant differences were found in relation to oxidative stress between the treatments. It is necessary to note that increased isoprostane levels were not observed in either trial. Goodail and Howatson (2008) and Nosaka et al (2006) used the same MS protocol and suggested that muscle damage was induced. However, our study was the first to investigate the effect of the drop-jumps MS protocol specifically on oxidative stress markers. Perhaps, the MS protocol performed in the present study was not enough to induce reactive oxygen species overproduction and consequently lipids peroxidation, since isoprostanes are markers of lipid peroxidation.

The addition of cocoa flavanols to a post exercise recovery beverage offered no additional benefits to oxidative stress and muscle damage. The CocoaCHOC beverage utilized in the current study provided a total of 616 mg of flavanols per day during a seven-day loading phase. However, other studies have utilized different foods and doses of flavanols with athletes. Contrary to our findings, Nishizawa et al. (2011) utilized a flavanol-rich lychee fruit extract containing 50 mg of flavanols per day for two months and found a decrease in serum interleukin-6 levels, which suggests that the supplementation suppressed inflammation caused by high-intensity exercise training.

Connolly et al. (2006) supplemented tart cherry juice with 600 mg of flavanols two times per day for eight days and observed that strength loss and pain were significantly less in the cherry juice trial. By this way, the utilization of flavanols decreased the oxidative stress and consequently reduced muscle damage (NISHIZAWA et al. 2011; CONNOLLY et al. 2006).

Although no significant treatment effects were observed for the Yo-Yo test, our data indicates that there may be potential performance benefits attributed to cocoa flavanols. From a practical perspective, even perfoming the muscle damage protocol, 71% of the subjects in the CocoaCHOC group had a better Yo-Yo performance comparing the test performed in the introductory visit and the test performed 48 h POST MS protocol, while only 28% of the subjects performed better in the CHOC group (fig. 2). When looking at the percentage of change over time (Baseline to 48 h POST MS protocol), an improvement of 9.85% was found in the accumulated shuttle distance by the CocoaCHOC group, which means about 97 meters more than CHOC group, considering that CHOC group presented a decrease of 5.8% in the Yo-Yo test performed at 48 h POST MS protocol. Therefore, the addition of cocoa flavanols to a post exercise recovery beverage may offer some practical performance benefits following a MS protocol.

The results of the current study suggested that the MS protocol was effective to promote muscle soreness and may have induced some negative effect on subjects performance since 67% of the players of the CHOC trial presented lower distances in the Yo-Yo test performance.

Conclusion

The current research findings suggest that the addition of cocoa-flavanols to a post exercise recovery beverage resulted in no additional benefits in performance and oxidative stress when compared to consuming chocolate milk as a recovery aid. Additional studies are warranted to examine the potential advantage of cocoa flavanols on indices of exercise recovery and oxidative stress, since that some possible benefits were observed in performance, when considering individual athletic perspective. More specifically, future studies should investigate the consumption of cocoa flavanols for longer loading phases rather than seven days and must analyze the individual responses to treatment since from a practical perspective some evidence of performance improvement was found in the present study.

Acknowlegments

The authors would like to thank Darigold (Seattle, WA) for providing the chocolate milk and The Hershey Company (Hershey, PA) for providing the cocoa. CAPES PDSE for providing student financial support.

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Anexos

Anexo A. Carta da aprovação do trabalho no Comitê de Ética em Pesquisa da FCFAR- UNESP

FACULDADES DE CIÊNCIAS FARMACÊUTICAS DO CÂMPUS DE ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITOS DA SUPLEMENTAÇÃO DE TAURINA E ACHOCOLATADO SOBRE A COMPOSIÇÃO CORPORAL, PERFORMANCE E ESTRESSE OXIDATIVO EM TRIATLETAS DE ELITE DE RIBEIRÃO PRETO.

Pesquisador: Flávia Giolo De Carvalho

Área Temática: Área 9. A critério do CEP.

Versão: 2

CAAE: 06191512.9.0000.5426

Instituição Proponente: Faculdades de Ciências Farmacêuticas do Câmpus de Araraquara da UNESP

DADOS DO PARECER

Número do Parecer: 185.595

Data da Relatoria: 21/12/2012

Apresentação do Projeto:

A redação do projeto foi melhorada, tornando-o mais claro.

Objetivo da Pesquisa:

Avaliar o efeito da suplentação com Taurina em associação com leite achocolatado industr1alizado sobre a composição corporal, performance e estresse oxidativo em triatletas de elite. A taurina será administrada em cápsulas, em dose única de 3g/dia e o achocolatado em duas tomadas consecutivas de 200 mL cada, nos primeiros 30 minutos pós-treino. Está previsto o uso de placebo para controle do efeito da taurina e um período de washout entre dois períodos experimentais.

Avaliação dos Riscos e Benefícios:

Todas as informações fornecidas no projeto registram apenas benefícios da ingestão do aminoácido.

Comentários e Considerações sobre a Pesquisa:

As correções indicadas foram acatadas, recomendo aprovação do protocolo

Considerações sobre os Termos de apresentação obrigatória:

todos os termos atendidos

Recomendações:

Endereço: Rodovia Araraquara Jaú, Km 1
Bairro: Campus Universitário **CEP:** 14.801-902
UF: SP **Município:** ARARAQUARA
Telefone: 1633-0168 **Fax:** 1633-2200 **E-mail:** sta@fcfar.unesp.br; diretor@fcfar.unesp.br

Conclusões ou Pendências e Lista de Inadequações:

Permanece dúvida do relator, que a submete ao Colegiado, quanto ao adequado atendimento da Resolução 196/96, item VII e da carta circular 006/2011, diante da informação do autor de que "as coletas de sangue e a avaliação da composição corporal serão realizadas no Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto- Universidade de São Paulo (HCFMRP-USP, e de que o teste de desempenho físico em esteira ergométrica será realizado na Escola de Educação Física e Esportes de Ribeirão Preto.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O Comitê de Ética em Pesquisa, reunido em 12 de Dezembro de 2012, após análise do projeto supracitado, considerou que o mesmo encontra-se adequado em conformidade com as orientações constantes da Resolução 196/96 do Conselho Nacional de Saúde/MS.

Por essa razão, o Comitê de Ética em Pesquisa desta Faculdade, considerou o referido projeto estruturado dentro de padrões éticos manifestando-se FAVORAVELMENTE à sua execução.

Um relatório parcial deverá ser entregue em JANEIRO de 2014 e o relatório final juntamente aos Termos de Consentimento Livre Esclarecido dos sujeitos da pesquisa (originais e assinados em todas as folhas) deverão ser entregues em FEVEREIRO de 2015.

ARARAQUARA, 15 de Janeiro de 2013

Assinador por:
AMAURI ANTIQUERA LEITE
(Coordenador)

Endereço: Rodovia Araraquara Jaú, Km 1
Bairro: Campus Universitário **CEP:** 14.801-902
UF: SP **Município:** ARARAQUARA
Telefone: 1633-0168 **Fax:** 1633-2200 **E-mail:** sta@fcfar.unesp.br; diretor@fcfar.unesp.br

Anexo B. *Certificate of Approval – Central Washington University*

CWU Central Washington University
LEARN. DO. LIVE.

April 21, 2015

Flávia Giolo De Carvalho
Department of Nutrition, Exercise and Health Sciences
Mail Stop 7572

Dear Ms. Carvalho:

Thank you for submitting an HSRC application and revisions for your study, *Effect of Cocoa Flavanols Supplementation on Oxidative Stress and Muscle Recovery Markers in Rugby Players*. Your application was reviewed in a convened meeting of the Council on April 8, 2015 and your study was approved pending requested modifications which have now been satisfied. This approval is valid for one year [from April 8, 2015 through April 7, 2016] so long as the approved procedures are followed.

Please note that the enclosed approved consent form has been stamped and dated for the time period in which it may be used. This approved form should be used for all needed copies.

Your responsibilities with respect to keeping this office apprised of your progress include the following:

1. File a Project Modification Request form for HSRC approval before modifying your study in any way except formatting of documents (e.g. any change in recruitment, subjects, co-investigators, consent forms, any procedures). If there is a major change in purpose or protocol, you may be asked to submit a new application. Please call if you have questions.
2. File a Termination Report form with this office upon completion of your study.
3. Immediately contact the HSRC for further guidance should you encounter unanticipated problems with your research. Follow up with an Unanticipated Problems report may be required.
4. Provide a current contact address and phone number if either should change prior to termination of the study.

All forms are available at the HSRC website. Please refer to your HSRC study number (H15057) in all future correspondence with the committee. If you have any questions or concerns, feel free to contact us.

Thank you for your willingness to work closely with the HSRC in the approval process for your project. Best wishes for a productive research experience.

Sincerely,

Sandra M. Martinez

Sandra M. Martinez, MA
Human Protections Administrator

c: HSRC file
Dr. Leo D'Acquisto, HSRC Chair
Dr. Kelly Pritchett, faculty sponsor

Human Subjects Review Council Office
400 East University Way . Ellensburg WA 98926-7401 . Office: 509-963-3115 . Black Hall 225-17 . Fax: 509-963-1064 . Web: www.cwu.edu/hsrc
EEO/AA/TITLE IX INSTITUTION • TDD 509-963-2143

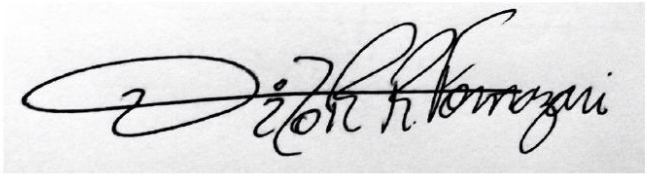
Anexo C. Cópia do certificado de apresentação de trabalho e classificação entre os dois melhores trabalhos da categoria "humanos" no "XIV Encontro AAARL de Medicina Esportiva".

XIV ENCONTRO A.A.A.R.L. DE MEDICINA ESPORTIVA

USP Ribeirão Preto - SP

Ribeirão Preto, 8 de novembro de 2014.

O **XIV Encontro A.A.A.R.L. de Medicina Esportiva**, da Associação Atlética Acadêmica "Rocha Lima" da Faculdade de Medicina de Ribeirão Preto certifica que **Flávia Giolo de Carvalho** foi selecionada para apresentação oral e finalista do "Prêmio Galeno 2014" na categoria **"TRABALHOS COM HUMANOS"** pela apresentação do trabalho: **EFEITOS DA SUPLEMENTAÇÃO DE TAURINA SOBRE MARCADORES DE ESTRESSE OXIDATIVO EM TRIATLETAS –** Carvalho FGD; Galán BSM, Dato CC, Santos PC, Marchini JS, Ovídio PP, Jordão Junior AA, Freitas EC.



Vitor Rodrigues Fornazari
Presidente da Comissão Organizadora do XIV Encontro AAARL de Medicina Esportiva