Thays de Ataide e Silva

Manipulação de carboidrato endógeno com exercício prévio e jejum sobre a resposta ao bochecho de carboidrato e *performance*

Recife

2015

Thays de Ataide e Silva

Manipulação de carboidrato endógeno com exercício prévio e jejum sobre a resposta ao bochecho de carboidrato e *performance*

Tese apresentada ao Programa de Pós-Graduação em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, para obtenção do título de Doutor em Nutrição.

Orientador: Profº Drº Adriano Eduardo Lima da Silva

Co-Orientadora: Profª Drª Carol Virgínia Góis Leandro

Recife
2015

Ficha catalográfica elaborada pela
Bibliotecária: Mônica Uchôa, CRB4-1010

S586m Silva, Thays de Ataide e.
Manipulação de carboidrato endógeno com exercício prévio e jejum sobre a resposta ao bochecho de carboidrato e performance / Thays de Ataide e Silva.– Recife: O autor, 2015.
94 f.: il.; tab.; 30 cm.

Orientador: Adriano Eduardo Lima da Silva.
Tese (doutorado) – Universidade Federal de Pernambuco, CCS. Programa de Pós-Graduação em Nutrição, 2015.
Inclui referências, apêndices e anexos.

1. Maltodextrina. 2. Estado pós-prandial. 3. Glicogênio. 4. Eletromiografia. 5. Ciclismo. I. Silva, Adriano Eduardo Lima da (Orientador). II. Título.

612.3 CDD (23.ed.) UFPE (CCS2016-011)

Thays de Ataide e Silva

Manipulação de carboidrato endógeno com exercício prévio e jejum sobre a resposta ao bochecho de carboidrato e *performance*

Tese aprovada em 09 de Dezembro de 2015.

_____
Adriano Eduardo Lima da Silva, Doutor em Biodinâmica do Movimento Humano, UFPE

_____
João Henrique da Costa Silva, Doutor em Fisiologia, UFPE

_____
Raquel da Silva Aragão, Doutora em Nutrição, UFPE

_____
Elizabeth do Nascimento, Doutora em Nutrição, UFPE

_____
André dos Santos Costa, Doutor em Biodinâmica do Movimento Humano, UFPE

Recife
2015

À Maria José, Cristiane e Rodrigo,

meus amores.

AGRADECIMENTOS

A Deus, meu pai e alicerce de minha vida;

A minha mãe, Maria José do Nascimento Silva, pelo apoio incondicional, orações, amor e dedicação;

A minha irmã, Cristiane de Ataide Green, que mesmo longe fisicamente está sempre ao meu lado, torcendo por mim e me incentivando de todas as formas;

Ao meu esposo, Rodrigo Alexandre Leal da Silva, que esteve sempre comigo nessa longa jornada, por seu companheirismo, paciência, generosidade e motivação;

A minha família pela compreensão e apoio durante esse período, em particular as minhas tias Renilde e Ruth Ataide, pelo acolhimento fraterno e palavras de carinho.

Ao meu orientador, Adriano Eduardo Lima da Silva, por ter acreditado em mim, antes mesmo que eu mesma. Por ter me apresentado caminhos e me possibilitado escolhas. Por ter estado presente, em todas as etapas desse processo. Por ter me orientado e me permitido crescer.

A minha orientadora, Carol Leandro, pelo exemplo de professora e pesquisadora e por ter confiado no meu trabalho e estar sempre disposta a ajudar.

Ao meu orientador estrangeiro, Christos Stathis, pela orientação e acolhimento no período de doutorado sanduíche na Victoria University, Melbourne, Australia.

A minha companheira de laboratório, Thaysa Ghiarone, pelos momentos que compartilhamos, trabalhos e conquistas... agradeço pela dedicação no andamento deste estudo, que também é seu;

Ao professor, Rômulo Bertuzzi, pela colaboração no desenvolvimento deste trabalho.

Ao grupo de pesquisa, Nutrição, atividade física e plasticidade fenotípica, e ao Grupo de Pesquisa em Ciências do Esporte, pelas experiências compartilhadas;

Ao Núcleo de Educação Física e Ciência do Esporte do Centro Acadêmico de Vitória (CAV), UFPE, pela viabilidade da estrutura e recursos do laboratório de Fisiologia do Esforço.

Aos técnicos de laboratório do CAV, Geivianni Andrade, por sua assistência técnica excelente durante a coleta de dados sanguínea, e, Gabriel Locatelli, pelo suporte com as técnicas de análises laboratoriais.

Aos meus colegas de doutoramento, em especial, aos meus amigos Maria Claudia Alheiros Lira e José Antônio dos Santos, pela força e palavras de incentivo.

Ao Programa de Pós Graduação em Nutrição da UFPE, por essa oportunidade, e as secretárias, Cecília Arruda e Neci Nascimento, pela dedicação e suporte aos pós-graduandos.

Ao CNPq, pela bolsa concedida.

A todos que contribuíram direta ou indiretamente para o desenvolvimento deste trabalho.

E, em especial, aos voluntários, pela disponibilidade e interesse em participar desse estudo.

Os meus sinceros agradecimentos!

RESUMO

O bochecho de carboidrato tem demonstrado efeito positivo sobre a *performance* durante ciclismo contrarrelógio com duração de 30-60 min. No primeiro artigo dessa tese, intitulado "*Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review*" foi identificado significante efeito global do bochecho de carboidrato sobre a *performance* entre os estudos analisados. Ademais os principais mecanismos que envolvem o potencial efeito benéfico do bochecho de carboidrato sobre a *performance* também foram explorados. A ativação dos receptores sensoriais na cavidade oral que ativam algumas áreas do cérebro associadas com prazer e controle motor, como a ínsula, opérculo frontal, córtex órbitofrontal e estriado, tem sido proposta. Apoiando esta suposição, tem-se demonstrado que o bochecho de carboidrato aumenta potenciais motores e melhora a saída corticomotora aos músculos exercitados. No entanto, este efeito positivo parece ser acentuado quando a disponibilidade de carboidrato endógena está reduzida. Somando-se a isso, tem sido discutido que há uma relação direta entre a disponibilidade de carboidrato endógena e a magnitude da melhora da *performance* com bochecho de carboidrato. No segundo artigo dessa tese, intitulado "*CHO mouth rinse ameliorates neuromuscular response with lower endogenous CHO stores*" foi investigado o efeito do bochecho de carboidrato sobre a atividade neuromuscular (EMG), respostas metabólicas (glicose e lactato plasmático e taxas de oxidação de carboidrato e gordura), e *performance* durante ciclismo iniciado com diferentes níveis de disponibilidade de carboidrato endógeno pela manipulação do jejum e exercício anterior. Com um desenho duplo-cego, randomizado, controlado por placebo, oito indivíduos do sexo masculino fisicamente ativos completaram seis ensaios experimentais bochechando periodicamente carboidrato (6,4% maltodextrina) ou placebo no estado alimentado (FED), no estado de 12-h de jejum (FAST) ou depois de um protocolo de depleção de glicogênio muscular e 12-h de jejum (DEP). O ensaio experimental consistiu de 30 min de ciclismo a 90% do primeiro limiar ventilatório, seguido por uma prova contrarrelógio de 20-km. Lactato e glucose plasmática, consumo de oxigênio, taxa de oxidação de carboidrato e gordura, e atividade EMG do músculo vasto-lateral foram mensurados. Durante o exercício de carga constante, o bochecho de carboidrato manteve os níveis de glicose plasmática altos ao longo do exercício ($p = 0,023$). O estado DEP apresentou redução ($p = 0,05$) na atividade EMG na condição de placebo, que foi completamente restaurada com o bochecho de carboidrato ($p = 0,010$). O tempo de *performance* durante o exercício contrarrelógio foi mais rápido com o bochecho de carboidrato comparado ao placebo apenas no estado DEP ($p = 0,019$). A inferência qualitativa do efeito do bochecho de carboidrato sobre a *performance* do exercício foi "benefício muito provável" para DEP, "possivelmente benéfico" para FAST e "negligenciável ou trivial" para FED. A potência e atividade EMG ao longo do contrarrelógio foi reduzida na condição DEP com placebo, mas foi parcialmente restaurada com o bochecho de carboidrato. Em conclusão o bochecho de carboidrato influencia a *performance* do exercício quando a disponibilidade de carboidrato é baixa e sugere-se que o principal mecanismo que governa esta resposta é o aumento na atividade neuromuscular.

Palavras-chave: Maltodextrina. Estado pós-prandial. Glicogênio. Eletromiografia. Ciclismo.

ABSTRACT

The carbohydrate mouth rinse during the exercise has demonstrated a positive effect on performance during cycling time trial lasting 30-60 min. In the first paper of this thesis, entitled "*Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review*" has identified a significant overall effect of carbohydrate mouth rinse on performance among the analyzed studies, as well as the main mechanisms that involve the potential beneficial effect of carbohydrate mouth rinse on performance were explored. It has been purposed that carbohydrate mouth rinse stimulates a group of sensory receptors in the oral cavity, which actives some brain areas associated with reward and motor control as insula/frontal operculum, orbitofrontal cortex and striatum. Supporting this assumption, it has been reported that carbohydrate mouth rinse increases motor evoked potentials and improves corticomotor output to the exercised muscles. Adding to this, it has been argued there is a direct relationship between pre-exercise endogenous carbohydrate availability and the magnitude of the improvement in the exercise performance with carbohydrate mouth rinse. In the second paper of this thesis, entitled "*CHO mouth rinse ameliorates neuromuscular response with lower endogenous CHO stores*" was investigated the effect of carbohydrate mouth rinse on neuromuscular activity (EMG), metabolic responses (plasma glucose and lactate and carbohydrate and fat oxidation rates), and performance during cycling exercise starting with different levels of endogenous carbohydrate availability by manipulation of fast and prior exercise. In a double-blind, randomized, placebo-controlled design, eight physically active males completed six experimental trials mouth rinsing periodically carbohydrate (6.4% maltodextrin) or placebo in fed state (FED), 12-h fasted state (FAST) or after an exercise-depleting muscle glycogen plus 12-h fast (DEP). Experimental trial consisted of 30 min cycling at 90% of the gas exchange threshold, followed by a 20-km cycling time-trial. Plasma lactate and glucose, oxygen uptake, carbohydrate and fat oxidation rates, and EMG activity were measured. During the constant load exercise, rinsing the mouth with carbohydrate maintained higher plasma glucose levels as the exercise progressed ($p = 0.023$). The DEP carried to a reduction ($p = 0.05$) of the EMG activity in the placebo condition, which was fully restored with the carbohydrate mouth rinse ($p = 0.010$). The performance time during the time-trial was faster with carbohydrate compared to placebo only in DEP condition ($p = 0.019$). The qualitative inference of carbohydrate mouth rinse effect on exercise performance was 'benefit very likely' for DEP, 'possibly benefit' for FAST and 'negligible or trivial' for FED. The power output and EMG activity throughout the time trial were reduced in DEP condition with placebo, but both were partially rescued with carbohydrate mouth rinse. In conclusion, the carbohydrate mouth rinse influences exercise performance when endogenous carbohydrate availability is low, and the main mechanism governs it might be an enhanced central motor drive.

Key words: Maltodextrin. Postprandial state. Glycogen. Electromyography. Cycling.

LISTA DE ILUSTRAÇÕES

voluntary contraction, MVC) during the 20-km cycling time trial. FEDPLA and FEDCHO: fed with placebo and carbohydrate mouth rinse, respectively; FASTPLA and FASTCHO: fasted with placebo and carbohydrate mouth rinse, respectively; DEPPLA and DEPCHO: endogenous CHO depleted state through pre exercise with placebo and carbohydrate mouth rinse, respectively. Values are means ± SEM, $p < 0.05$. * Significant main effect of pre exercise CHO availability ($P < 0.05$), FED was higher than FAST and DEP for power output (a) and FAST higher than FED and DEP for EMG activity (b); ** Significant main effect of solution ($P < 0.05$), CHO was higher than PLA; *** Significant main effect of distance ($P < 0.05$), decreased from 5 km to 15 km and then increased until the end of the exercise; † Significant interaction between pre exercise CHO availability, solution and distance ($P < 0.05$), CHO attenuated progressively the reduction of the power output (a) and EMG activity (b) in DEP.

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

CAAE: certificado de apresentação para apreciação ética
CEP: comitê de ética em pesquisa
CHO: carbohydrate / carboidrato
CHO-E: electrolyte solution at carbohydrate; solução eletrolítica de carboidrato
CHOFS: carbohydrate rinse in fed state; bochecho de carboidrato em estado alimentado
CL: constant load / carga constante
CVM: contração voluntária máxima
DEP: after an exercise protocol to deplete muscle glycogen plus 12-h fasting / depois de um protocol de exercício de depleção de glicogênio muscular e 12 h de jejum
DEPCHO: glycogen depleted with carbohydrate mouth rinse / glicogênio depletado e bochecho de carboidrato
DEPPLA: glycogen depleted with placebo mouth rinse glicogênio depletado e bochecho de placebo
DP: desvio padrão
EDTA: ethylenediamine tetraacetic acid / etilenodiaminotetracético
EMG: sinal eletromiográfico
ET: endurance trained; treinamento de *endurance*
FAST: 12-h fasted state / estado de 12 h de jejum
FASTCHO: fasted with carbohydrate mouth rinse / jejum e bochecho de carboidrato
FASTPLA: fasted with placebo mouth rinse / jejum e bochecho de placebo
FC: frequência cardíaca
FCHO: carbohydrate rinse in fasted state / estado de bochecho de carboidrato em jejum
FED: fed state / estado alimentado
FEDCHO: fed with carbohydrate mouth rinse / alimentado e bochecho de carboidrato
FEDPLA: fed with placebo mouth rinse / alimentado e bochecho de placebo
FMALT: maltodextrine rinse in fast state / bochecho de maltodextrina em estado de jejum
FPLA: placebo rinse in fasted state/ bochecho de placebo em estado de jejum
GDEP: glycogen-depleting exercise protocol / protocolo de exercício de depleção de glicogênio
GET: the gases exchange threshold / limiar de trocas gasosas
GLU: glucose / glicose
h: hour / hora
Lan: limiar anaeróbio;
M: men / homem
MALT: maltodextrin / maltodextrina
MALTFS: maltodextrine rinse in fed state / bochecho de maltodextrina em estado alimentado
MR: mouth rinse / bochecho
MVC: maximum voluntary contraction / contração voluntária máxima
NA: nonathletic / não atletas
PAR-q: questionário de riscos cardiovasculares
PLA: placebo
PLAFS: placebo rinse in fed state / bochecho de placebo em estado alimentado

PO: power output / potência
PPO: peak power output / potência de pico
RA: recreationally active / ativo recreacional
RER: respiratory exchange ratio / razão de trocas respiratórias
RMS: the root mean square / media do valor quadratic médio
rNTS: rostral nucleus of the solitary tract / região rostral do núcleo do trato solitário
RPE: rating of perceived exertion / percepção subjetiva ao esforço
SD: standard deviation / desvio padrão
SEM: standard error of the mean / erro padrão da media
TCLE: termo de consentimento livre e esclarecido
TT: time Trial /contrarrelógio
UFPE: universidade federal de Pernambuco
UV: ultraviolet / ultravioleta
$VCO_{2:}$ carbon dioxide consumption / consumo de dióxido de carbônico
$VCO_{2:}$ oxygen consumption / consumo de oxigÊnio
VL: vasto lateral
VO_{2max} maximum oxygen consumption / máximo consume de oxigênio
VO_{2peak}: peak oxygen uptake / consumo de oxigênio de pico
VPMpc: ventral posterior medial nucleus of the thalamus / núcleo ventral posterior medial do tálamo
W: women / mulheres

LISTA DE SÍMBOLOS

ARTIGO ORIGINAL

* Significant main effect of pre exercise CHO availability ($P < 0.05$) *or* Significant faster than PLA in DEP condition ($p < 0.05$); *conforme indicação*.

** Significant interaction between pre exercise CHO availability and solution ($P < 0.05$) *or* Significant main effect of solution ($P < 0.05$); *conforme indicação*.

*** Significant main effect of distance ($P < 0.05$);

¦ Significant interaction between pre exercise CHO availability, solution and distance ($P < 0.05$).

[a] Main effect of pre exercise CHO availability ($P < 0.05$);

[b] Main effect of time *or* distance ($P < 0.05$); *conforme indicação*.

[c] Interaction between pre exercise CHO availability and time *or* distance ($P < 0.05$); *conforme indicação*.

[d] Interaction between solution and time ($P < 0.05$);

SUMÁRIO

APRESENTAÇÃO

1 APRESENTAÇÃO

O bochecho de carboidrato (CHO) durante o exercício tem demonstrado um efeito positivo (~3%) sobre o desempenho durante ciclismo contrarrelógio (TT) de 30-60 min (De Ataide E Silva *et al.*, 2014). O possível mecanismo de ação do bochecho de CHO sobre o desempenho físico envolve um grupo de receptores, ainda não identificados (Carter *et al.*, 2004), conectados a regiões encefálicas associadas com prazer e controle motor, como a ínsula/frontal, opérculo, córtex órbito-frontal e estriado (Chambers *et al.*, 2009). Apoiando esta suposição, foi demonstrado que o bochecho de carboidrato aumenta potenciais motores e melhora a saída corticomotora aos músculos exercitados (Gant *et al.*, 2010).

É interessante notar que, mesmo com uma diferença média global entre os estudos que demonstram significante efeito positivo do bochecho de CHO sobre o desempenho no exercício (De Ataide E Silva *et al.*, 2014), alguns estudos não relataram tal efeito (Whitham e Mckinney, 2007; Beelen *et al.*, 2009). Vale ressaltar que nesses estudos, os participantes realizaram o protocolo de exercício no estado alimentado (Whitham e Mckinney, 2007; Beelen *et al.*, 2009). Assim, argumentou-se que existe uma relação direta entre a disponibilidade de CHO endógeno pré-exercício e a magnitude da melhora no desempenho do exercício com o bochecho de CHO (Chambers *et al.*, 2009). No entanto, estudos que investigaram essa relação produziram resultados conflitantes e utilizaram apenas o jejum noturno para manipular os estoques de CHO endógeno. Lane *et al.* (2013) constataram que o bochecho de CHO melhorou em maior proporção o desempenho durante uma prova de ciclismo contrarrelógio de 60 min em estado de jejum (10 h jejum noturno), em comparação ao estado alimentado (2 h pós-prandial). Por outro lado, Fares e Kayser (2011) constataram que o bochecho de CHO melhorou a capacidade de *endurance* na mesma magnitude para ambos, no estado alimentado (3 h pós-prandial) e em estado de jejum (noturno). Apesar dessas discrepâncias, tem sido proposto, como o principal mecanismo explicando um maior efeito do bochecho de CHO sobre o desempenho em jejum, que quanto maior for a sensibilidade dos receptores orais de CHO, maior a ativação da unidade motora central superior (Lane *et al.*, 2013), embora nenhuma evidência experimental tenha sido fornecida para apoiar essa hipótese.

Assim, o objetivo da presente tese foi: 1) sintetizar, através de uma revisão sistemática e ferramentas de meta-análise, as principais evidências suportando o efeito positivo do

bochecho de carboidrato sobre o desempenho; 2) Investigar, através de um artigo original, os mecanismos pelos quais o bochecho de carboidrato melhora o desempenho. Especificamente, neste último, foi investigado, através de um estudo duplo-cego, randomizado e controlado por placebo, o efeito do bochecho de carboidrato sobre a atividade neuromuscular (EMG), respostas metabólicas (glicose e lactato plasmático e taxas de oxidação de carboidrato e gordura), e *performance* durante ciclismo iniciado com diferentes níveis de disponibilidade de carboidrato endógeno pela manipulação do jejum e exercício anterior. As hipóteses desse último estudo foram: (1) a magnitude de melhoria no desempenho do exercício com o bochecho de carboidrato é inversamente relacionada com a disponibilidade de carboidrato pré-exercício, (2) o mecanismo que governa isso é neural ao invés de metabólico.

REVISÃO DA LITERATURA

2 REVISÃO DA LITERATURA

A revisão de literatura do presente trabalho de tese resultou em um artigo de revisão intitulado *Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review* que foi publicado na revista "*Nutrients*" (2072 - 6643), Fator de Impacto (JCR 2014): 3.27, Qualis 2014: A2 (APÊNDICE A).

MÉTODOS

3 MÉTODOS

3.1.1 Participantes

A amostra foi composta por oito indivíduos saudáveis, fisicamente ativos, do sexo masculino, com idade entre 19 a 39 anos. Antes de iniciar o estudo, todos os voluntários foram informados sobre os possíveis riscos e benefícios, responderam a um questionário para excluir indivíduos com riscos cardiovasculares (PAR-q) (Anexo A) e assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) (Apêndice A). Este projeto foi aprovado pelo Comitê de Ética em Pesquisa (CEP) da Universidade Federal de Pernambuco (UFPE), com o número do Certificado de Apresentação para Apreciação Ética (CAAE): 06610112.5.0000.5208 e número do parecer consubstanciado do CEP: 103.919 (Anexo B).

Tabela 1. Características descritivas dos participantes, 8 homens, saudáveis, e fisicamente ativos.

	Média	± DP
Idade (ano)	31.5	7.3
Altura (cm)	172.9	5.2
Peso (kg)	74.1	11.7
Gordura corporal (%)	12.8	4.5
VO_{2pico} ($L.min^{-1}$)	3.3	0.2
VO_{2pico} ($mL.kg^{-1}.min^{-1}$)	46.1	8.2
Potência (W)	268.8	34.7

3.1.2 Desenho experimental

Cada participante completou sessões e procedimentos preliminares, incluindo uma avaliação antropométrica (estimativa de gordura corporal por Jackson e Pollock (1978), um recordatório alimentar de 24 h, um teste incremental e um uma familiarização. Depois disso, cada participante completou seis ensaios experimentais, duplo cego, contrabalanceado e controlado por placebo. Dois ensaios experimentais foram realizados num estado alimentado (ou seja, duas horas pós-prandial; FED). Dois ensaios experimentais foram realizados em

estado de jejum (isto é, após 12 h de jejum noturno; FAST). Finalmente, dois ensaios experimentais foram realizados após um protocolo de depleção de glicogênio muscular na noite anterior ao teste experimental, além de um 12 h jejum (DEP). Os testes experimentais tiveram um intervalo mínimo de 72 horas entre eles. A temperatura e umidade relativa durante os ensaios foram mantidos constantes (22,4 ± 1,7 ° C e 45,4 ± 7,4% de umidade relativa).

3.1.3 Teste incremental

O teste incremental foi realizado em um ciclo simulador (RacerMate, ComputrainerTM, Seattle, EUA; Peveler, 2013) para determinar o consumo de oxigênio de pico (VO_{2pico}) e a potência máxima (PPO). O teste consistiu de 3 min de aquecimento a 50 W, seguido por incrementos de 25 W a cada 1 minuto até à exaustão, que foi definida como uma incapacidade para manter a cadência de pedalada entre 60 e 70 revoluções por minuto (rpm). O consumo de oxigênio (VO_2) e produção de dióxido de carbono (VCO_2) foram medidas respiração a respiração durante todo o teste usando um analisador de gases (Cortex Metamax 3B, CortexBiophysik, Leipzig, Alemanha). A fração de O_2 expirado foi analisada com um sensor de zircónio e de CO_2 por absorção de infravermelho. Ambos os sensores foram calibrados antes de começar o teste com um cilindro de gás contendo concentrações conhecidas de O_2 (12%) e CO_2 (5%). O volume de ar expirado foi medido por um sensor de fluxo bidirecional, calibrado com uma seringa de 3 litros.

O VO_{2pico} foi registrado como os valores médios medidos de VO_2 durante os últimos 30 segundos do teste, enquanto PPO como a maior potência (PO) durante o ensaio. O limiar de trocas gasosas (primeiro limiar ventilatório, GET) foi identificado por três investigadores de acordo com os seguintes critérios: 1) um aumento desproporcional da curva de VCO_2 versus a curva VO_2; 2) um aumento no equivalente ventilatório para VO_2 sem um aumento no equivalente ventilatório para VCO_2 e; 3) um primeiro aumento da pressão O_2 sem queda na pressão de CO_2 ao final da expiração (Whipp *et al.*, 1981).

3.1.4 Familiarização

Os sujeitos compareceram ao laboratório com o objetivo de se familiarizar com o procedimento experimental. Os participantes realizaram três séries de contração voluntária máxima de uma perna só (CMV) de 5 s de duração, intercaladas por 60 s de repouso passivo. Posteriormente, eles realizaram 30 min de exercício de carga constante (CL) em uma carga de trabalho fixada em 90% da GET, seguido por CMV de uma perna. Imediatamente antes e nos tempos 5, 15 e 25 min do CL, os sujeitos realizaram o bochecho com água apenas para se

familiarizar com o procedimento. Nos tempos 5, 10, 15, 20, 25 e 30 min do CL eles responderam a escala de percepção subjetiva ao esforço (Anexo B).

Imediatamente após, os participantes realizaram 20 km de ciclismo TT. Para os 20 km TT os sujeitos foram orientados a concluírem a prova no menor tempo possível e foram informados apenas sobre a quilometragem completada. A configuração do ciclo simulador foi ajustada verticalmente e horizontalmente para se adequar ao participante, e as posições de assento e guidão foram registradas e, em seguida, replicadas durante as sessões experimentais subsequentes. As sapatilhas de ciclismo foram usadas para fixar os pés nos pedais. A cada 5 km do teste (5 km, 10 km e 15 km) os sujeitos realizaram o bochecho com água e foram questionados quanto à escala de percepção subjetiva ao esforço (PSE).

Os modelos de exercício (CL e TT) foram construídos com duração total de ~ 60 min, já que este foi classificado como a duração ideal em que o bochecho de CHO afeta o desempenho do exercício (Carter *et al.*, 2004). O exercício CL foi realizado como o primeiro ensaio, porque ele fornece um ambiente experimental mais controlado para investigar as respostas metabólicas e fisiológicas sem efeitos de variações da potência (Garby e Astrup, 1987), enquanto TT foi escolhido para medir o desempenho do exercício porque é um teste mais motivacional e seu resultado de desempenho é menos variável (Laursen *et al.*, 2007).

3.1.5 Controle pré teste experimental

Os participantes foram orientados a abster-se de alimentos que possuem cafeína (Apêndice B), álcool, e exercício extenuantes 24 horas antes do teste experimental (uma lista com os principais alimentos que contem cafeína foi entregue aos participantes). Eles, também, foram orientados a seguir prescrições dietéticas e protocolos de exercício específicos anterior a cada dia de teste experimental.

Em todas as condições experimentais, as primeiras quatro refeições do dia anterior ao teste foi a mesma. Refeição 1 no dia anterior ao teste experimental foi o café da manhã consumido às 8:00 h (485 ± 277 kcal, 63.6 ± 13.1 % CHO, 11.7 ± 3.7 % proteína e 24.7 ± 9.6 % lipídio). Refeição 2 foi um lanche consumido as 10:00 h (336 ± 220 kcal, 67.9 ± 22.1 % CHO, 7.8 ± 6.8 % proteína e 24.3 ± 17.4 % lipídio). Refeição 3 foi o almoço consumido as 12:00 h (783 ± 101 kcal, 47.8 ± 10.4 % CHO, 28.3 ± 4.1 % proteína e 23.9 ± 8.3 % lipídio). Refeição 4 foi um lanche consumido às 16:00 h (253 ± 155 kcal, 76.4 ± 16.2 % CHO, 5.2 ± 3.6% proteína e 18.4 ± 15.5 % lipídio).

As diferenças nos protocolos de exercício e dietético para cada condição se deu no final do dia anterior ao teste experimental (refeição 5) e no café da manhã do dia do teste

experimental (café da manhã). Na condição FED, os participantes consumiram a refeição 5 as 20:00 h na noite anterior ao teste experimental e realizaram o café da manhã as 6:00 h no dia do teste experimental. Na condição FAST, os participantes realizaram a refeição 5 as 20:00 h na noite anterior ao teste experimental e posteriormente realizaram 12 h de jejum noturno. Na condição DEP, os participantes foram ao laboratório na noite anterior (as 18:00 h) e realizaram um protocolo de exercício para redução do conteúdo de glicogênio muscular. Este teste consistiu de 90 min de exercício de carga constante a 70% da PPO seguido por 5 min de repouso e posteriormente 6 x 1 min de exercício intermitente a 125 % da PPO, intercalado por 1 min de repouso passivo. Os participantes mantiveram a cadencia de pedalada entre 60 e 70 rpm durante ambos os exercícios. Este protocolo foi previamente validado para reduzir o conteúdo de glicogênio muscular entre ~ 50-70 % (Gollnick *et al.*, 1973; Gollnick *et al.*, 1974; Heigenhauser *et al.*, 1983). A refeição 5 foi oferecida depois do exercício (20:00 h), então, os participantes seguiram um jejum noturno de 12 h até o teste experimental no outro dia.

A refeição 5 foi um jantar isoenergético para as três condições (1082.2 ± 253.3 kcal), mas com o conteúdo normal de CHO para as condições FED e FAST (56.4 % CHO, 16.9% proteína e 26.7 % lipídio) e baixa em CHO para DEP (12.5 % CHO, 12.5 % proteína e 75.0 % lipídio). Este jantar baixo em carboidrato na condição DEP foi fornecido com essas características para prevenir a ressíntese de glicogênio depois do protocolo de depleção de glicogênio muscular.

3.1.6 Teste experimental

Ao chegar ao laboratório, os participantes descansaram por 15 min antes de um cateter intravenoso ser inserido na veia anticubital para coleta de sangue durante o repouso (1 ml) e ficar disponível para as coletas de sangue subsequentes. Um eletrodo de superfície bipolar Ag-AgCl (com distância de 20 mm entre os eletrodos) foi subsequentemente posicionado no músculo vasto lateral (VL) da perna dominante para o registro da EMG. O eletrodo de referência foi colocado na superfície anterior da tíbia. O preparo da pele e localização do eletrodo seguiu as recomendações de SENIAM (Hermens *et al.*, 2000). Imediatamente após a preparação da EMG, os participantes realizaram CMV de uma perna por 5 s (com ângulo de 90º entre a coxa e a perna) em uma cadeira extensora, separado por 60 s de intervalo. A EMG e a força foram simultaneamente registradas com uma taxa de amostragem de 2000 Hz (EMG System do Brasil, São José dos Campos, Brasil). Depois de 5 min de repouso, um exercício de CL de 30 min foi realizado a 90% do GET. Adicionalmente amostras de sangue (1 ml) foram

coletadas nos tempos de 10, 20 e 30 min do exercício de CL. VO_2 e VCO_2 foram continuamente registrados, mas a máscara foi tirada por curtos períodos durante o bochecho de CHO imediatamente antes e a 5, 15 e 25 min de realização do exercício. Foi fornecido aos participantes 25 ml de uma solução sem sabor a 6,4% de maltodextrin (Neonutri-Malto, CHO) ou água (PLA), que foi bochechada ao redor da cavidade oral por 10 s e posteriormente expectorada em um recipiente. Os participantes foram, então, imediatamente questionados sobre sua taxa de percepção subjetiva ao esforço (Escala de Borg de 15 pontos, RPE) antes da máscara ser novamente colocada. O sinal bruto da EMG foi registrado durante 10 s (começando 50 s depois do bochecho de carboidrato).

Após o CL, os participantes se deslocaram para a cadeira extensora, realizaram mais uma CMVe foi retirado o cateter venoso antes de realizarem os 20 km TT (aproximadamente 5 min separaram o exercício CL e TT). Eles foram instruídos a terminar os 20 km TT o mais rápido possível, simulando uma prova. Antes e nos pontos de 5, 10, 15 e 18 km do TT, os participantes bochecharam a solução oferecida (CHO ou PLA) e foram questionados sobre a RPE. O sinal bruto da EMG foi registrado nos mesmos pontos durante 10 s (começando 50 s depois do bochecho de carboidrato).

3.1.7 Análises sanguíneas

As amostras sanguíneas foram imediatamente transferidas para tubos a vácuo (Becton Dickinson, BD, Juiz de Fora, MG, Brasil) e centrifugadas a 3000 rpm por 15 min a 4°C para separação do plasma. O lactato e a glicose plasmática foram analisados em espectofotômetro UV (Quimis®, model Q798U2V5, São Paulo, Brasil) usando kits comerciais (kit Biotecnica, Varginha, Brasil).

3.1.8 Taxas de oxidação de gordura e carboidrato

A razão de troca respiratória (RER), e as taxas de oxidação de gorduras e CHO durante o CL (em repouso e nos tempos de 5, 10, 15, 20, 15 e 30 min) e TT (em repouso e nas distâncias de 5, 10, 15 e 20 km) foram calculadas utilizando os valores médios de VO_2 e VCO_2 durante o intervalo de coleta de 1 min. As taxas de oxidação de gordura e CHO foram calculadas com base no quociente respiratório não-proteico (Frayn, 1983):

$$\text{Taxa de oxidação de gordura} = 1.67 \cdot VO_2 - 1.67 \cdot VCO_2 \quad (1)$$

$$\text{Taxa de oxidação de carboidrato} = 4.55 \cdot VCO_2 - 3.21 \cdot VO_2 \quad (2)$$

com VO_2 e VCO_2 mensurados em $l.min^{-1}$ e taxa de oxidação em $g.min^{-1}$.

3.1.9 Atividade Eletromiográfica

Os sinais brutos da EMG foram retificados e filtrados com filtro de segunda ordem *Butterworth band-pass* com frequências de corte estabelecidos em 10 e 400 Hz para remover artefatos de ruído e interferência de movimento externo. Foi calculada a média do valor quadrático médio (RMS) de 5 contrações consecutivas durante cada período do ciclismo e normalizada pelo valor máximo do RMS do CMV pré exercício (Hirai *et al.*, 2010).

3.2 Tratamento Estatístico

Os dados foram apresentados como média ± DP, salvo indicação do contrário. ANOVA de três caminhos para medidas repetidas foi utilizada para verificar o efeito da disponibilidade de CHO pré-exercício (FED, o FAST e DEP), solução (CHO e PLA) e tempo ou distância nas variáveis dependentes (glicose, lactato, VO_2, RER, taxas de oxidação de CHO e gordura, frequência cardíaca, RPE, PO, CMV e RMS). Quando um efeito significativo foi encontrado, o principal efeito foi identificado usando o teste de LSD. ANOVA de dois caminhos para medidas repetidas, seguida pelo teste de LSD foi usada para identificar o efeito do bochecho de CHO e a disponibilidade de CHO pré-exercício no desempenho do exercício. Como por Batterham e Hopkins (2006) (Batterham e Hopkins, 2006), os valores de *p* obtidos a partir do *teste t* foram usados para fazer inferências sobre os valores verdadeiros (população) do efeito do bochecho de CHO no desempenho do exercício. A incerteza no efeito foi expressa como a probabilidade de que o verdadeiro valor do efeito representa a mudança substancial (dano ou benefício). A menor alteração no padrão foi assumida como sendo 0,20. Para todas as análises, a significância foi aceita quando $p \leq 0,05$. Todas as análises foram realizadas com o software SPSS (versão 17.0; Chicago, IL), exceto a inferência qualitativa do efeito, que foi calculada usando uma planilha de excel (Batterham e Hopkins, 2006).

RESULTADOS

4 RESULTADOS

Os resultados, do presente trabalho de tese, foram submetidos, na forma de artigo científico original, à revista “Medicine and Science in Sports and Exercise” (0195 - 9131), Fator de Impacto 2014 (2015): 3.983, Qualis 2014: A1 (APÊNDICE D e ANEXO D).

CHO mouth rinse ameliorates neuromuscular response with lower endogenous CHO stores

INTRODUCTION

Carbohydrate (CHO) mouth rinse has demonstrated a positive effect (~3%) on performance during cycling time trial (TT) lasting 30-60 min (6, 11). The mechanism governing this improvement is not fully understood, but it is unlikely to be of metabolic origin (2). Instead, it has been proposed that CHO mouth rinse stimulates a group of sensory receptors in the oral cavity (6), which actives some brain areas associated with reward and motor control, including the insula / frontal operculum, orbitofrontal cortex and striatum regions (8). In support of this assumption, CHO mouth rinse increases motor evoked potentials and improves corticomotor output to the exercised muscles, although this has only been demonstrated in a small muscle group (i.e. first dorsal interosseous) (15). Surprisingly, no study has measured the effect of CHO mouth rinse on central motor drive using active muscle electromyography (EMG) during whole-body exercise.

A majority of CHO mouth rinse studies have found a positive effect on performance (11). However, some studies do not demonstrate this improved performance and it is worth noting in these studies that the participants often performed the exercise trial in a fed state (5, 38). Consequently, it could be argued there is a potential relationship between pre-exercise endogenous CHO availability and the exercise performance with CHO mouth rinse (8). However, studies investigating this relationship report conflicting results with the use of an overnight fast to manipulate endogenous CHO stores. Lane et al (26) found that the CHO mouth rinse improved performance during a 60-min, cycling time-trial to a greater extent in the fasted state (10 h overnight fast) compared with the fed state (2 h postprandial). On the other hand, Fares et al (12) reported similar improvements in endurance capacity with CHO

mouth rinse in both fed (3 h postprandial) and fasted (overnight) states. It is postulated that fasting sensitizes CHO receptors in the mouth, consequently stimulating a higher central motor drive and increasing the effect of CHO mouth rinse response on exercise performance (26). However, no experimental evidence has been provided to support this mechanism.

Liver, but not muscle glycogen, is reduced following an overnight fast (7). Although it remains unknown, it is plausible to suspect that a reduction in the overall level of pre exercise endogenous levels of CHO (muscle and liver) may have an additional effect on the CHO mouth rinse-induced improvement in the exercise performance, when compared to partial endogenous CHO depletion (liver). While it is yet to be investigated with CHO mouth rinse, evidence from studies of CHO ingestion improving exercise performance leads to presume that pre-exercise endogenous CHO status would influence the ergogenic benefit of CHO on exercise performance (39). Carbohydrate ingestion enabled participants to maintain their optimal pace longer with low initial endogenous CHO availability (i.e., previous exercise at 48 and 24h before the main trial and a low CHO diet over the 48h + 6h fasting) compared to placebo. However, when pre-exercise endogenous CHO was adequate (i.e., previous exercise at 48h, not 24h before the main trial, and a high-CHO diet 48h prior with only 6h fasting), CHO feedings during the exercise did not lead to an additional effect on exercise performance (39). An understanding of the influence of endogenous CHO availability on CHO mouth rinse response is of practical relevance, as CHO mouth rinse has been listed as a candidate, although it has never been tested, to ameliorate the reduced training intensity resulting from low endogenous CHO availability, which is an alternative training approach to maximize mitochondrial biogenesis (2, 3).

Therefore, the main aim of this study was to investigate the effect of CHO mouth rinse on neuromuscular activity (EMG), metabolic responses (plasma glucose and lactate, and fat and CHO oxidation rates), and exercise performance with different starting levels of

endogenous CHO availability by prior fasting and exercise manipulation. Our hypotheses were: (1) the magnitude of improvement on exercise performance with CHO mouth rinse would be inversely related to the extent of pre-exercise CHO availability, (2) the mechanism governing the CHO mouth rinse response would be neural rather that metabolic.

METHODS

Participants

Eight healthy, physically active males volunteered to participate in this study. The participants' age, height, weight, body fat, peak oxygen uptake (VO_2peak) and peak power output (PPO) were 31.5 ± 7.3 years, 172.9 ± 5.2 cm, 74.1 ± 11.7 kg, 12.8 ± 4.5 %, 46.1 ± 8.2 $mL.kg^{-1}.min^{-1}$, and 268.8 ± 34.7 W (mean ± SD), respectively. They were all injury free and completed a physical activity readiness questionnaire (Par-Q) elaborated by American College of Sports Medicine before performing the tests. The human research ethics committee of the Federal University of Pernambuco approved this study, and participants signed an informed consent form before starting the study.

Experimental Design

Each participant completed preliminary sessions to ascertain anthropometric assessment (body fat estimating by Jackson and Pollock, 1978), a 24 h diet recall, incremental and a familiarization trial (Figure 1). Following this, each participant completed six experimental trials in a double blind, randomized, placebo-controlled crossover design. Two experimental trials were performed in a fed state (FED; i.e. two hours post-prandial), two in a fasted state (FAST; i.e. after a 12-h overnight fasting) and two after an exercise-depleting muscle glycogen protocol performed in the evening before the trial, plus a 12-h overnight fast

(DEP). The temperature and relative humidity during the trials were maintained constant (22.4 ± 1.7°C, 45.4 ± 7.4% relative humidity).

INSERT FIGURE 1 HERE

Maximal incremental exercise

The incremental test to ascertain VO_2peak and PPO was performed on a cycle simulator (RacerMate, Computrainer^{TM}, Seattle, USA; Peveler, 2013). The test consisted of a 3 min, warm-up at 50 W, followed by increments of 25 W every 1 min until exhaustion, which was defined as an inability to maintain the pedal cadence between 60 and 70 revolutions per minute (rpm). Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured breath-by-breath throughout the test using a gas analyzer (Cortex Metamax 3B, CortexBiophysik, Leipzig, Germany). The fraction of expired O_2 was analyzed with a zirconium sensor and end tidal CO_2 by infrared absorption. Both sensors were calibrated before starting the test with gas containing known concentrations of O_2 (12%) and CO_2 (5%). The volume of expired air was measured by a bidirectional flow sensor, calibrated with a 3 liter syringe.

The VO_2peak was recorded as the mean VO_2 values measured during the last 30 s of the test, while PPO as the highest power output (PO) attained during the test. The gas exchange threshold (GET) was established according the following: 1) a disproportionate increase in VCO_2 versus VO_2 curve; 2) an increase in the ventilatory equivalent for VO_2 without an increase in the ventilatory equivalent for VCO_2 and; 3) a first increase in tidal O_2 pressure with no drop in end-tidal CO_2 pressure (37).

Familiarization session

Participants were familiarized with the experimental procedures of the entire experimental protocol. In chronological order, they performed 1) three sets of a 5 s, one-legged maximum voluntary contraction (MVC), interspaced by 60 s of passive rest; 2) a 30-min constant load (CL) exercise at a workload set at 90% of GET; 3) a one-legged MVC; and 4) a 20-km cycling TT. Exercise models (CL and TT) were designed to last a total of ~60 min, as this has been reported as the optimal duration in which CHO mouth rinse influences exercise performance (6). The CL exercise trial was designed first in the protocol to investigate metabolic and physiological responses in a more controlled environment without influence of power output variations (16), while TT was chosen to measure exercise performance because it is a more repeatable performance test and a good reliable test of motivation (27). The configuration of the cycle ergometer was adjusted vertically and horizontally to suit the participant, and the seat and handle bar positions were recorded and then replicated during the subsequent experimental sessions. Cycling shoes were used to secure the feet to the pedals.

Pre experimental trial control

Participants were asked to abstain from all dietary sources of caffeine, alcohol, and strenuous exercise in the 24 h preceding the experimental trials. They were also asked to follow a prescribed set of dietary and/or exercise protocols specific for each trial day.

In all pre-experimental conditions, the first four meals in the day before the trial were identical. Meal 1 on the day before experimental trial was a breakfast consumed at 8 am (485 ± 277 kcal, 63.6 ± 13.1 % CHO, 11.7 ± 3.7 % protein, and 24.7 ± 9.6 % lipid). Meal 2 was a snack consumed at 10 am (336 ± 220 kcal, 67.9 ± 22.1 % CHO, 7.8 ± 6.8 % protein, and 24.3 ± 17.4 % lipid). Meal 3 was a lunch consumed at 12 pm (783 ± 101 kcal, 47.8 ± 10.4 % CHO, 28.3 ± 4.1 % protein, and 23.9 ± 8.3 % lipid). Meal 4 was a snack consumed at 4 pm (253 ± 155 kcal, 76.4 ± 16.2 % CHO, 5.2 ± 3.6% protein, and 18.4 ± 15.5 % lipid).

The differences in the exercise and dietary requirements for the various conditions started at the end of the day before the trial (meal 5) and on the morning of the trial day (breakfast). In FED condition, participants had meal 5 at 8 pm in the evening before and a breakfast at 6 am in the trial day. In FAST condition, participants had meal 5 at 8 pm in the evening before and performed the trial in the next morning after a 12-h overnight fasting. In DEP condition, the participants attended to the laboratory in the evening before (at 6 pm) and performed an exercise protocol intended to reduce muscle glycogen content. This exercise consisted of a 90-min CL exercise at 70 % of PPO followed by a 5-min rest and then 6 x 1-min intermittent set at 125 % of PPO, interspaced by 1-min of passive rest. Participants maintained a pedaling cadence between 60 and 70 rpm during both exercise bouts. This protocol was previously validated to reduce muscle glycogen content by ~ 50-70 % (17-19). Meal 5 was offered after the exercise finished (8 pm) and then participants followed a 12-h overnight fasting until the experimental trial in the next morning.

Meal 5 was an isoenergetic dinner across the three conditions (1082.2 ± 253.3 kcal), but with normal CHO content for FED and FAST conditions (56.4 % CHO, 16.9% protein, and 26.7 % lipid) and low CHO content for DEP (12.5 % CHO, 12.5 % protein, and 75.0 % lipid). This low-CHO dinner in DEP condition was offered to prevent glycogen resynthesis after the exercise-depleting muscle glycogen protocol (10).

Experimental trials

Upon arrival at the laboratory, participants rested quietly for 15 min before an intravenous catheter was inserted into the antecubital vein to collect a resting blood sample (1 ml) and enable subsequent serial blood sampling. Bipolar Ag-AgCl surface electrodes (with inter-electrode distance of 20 mm) were subsequently positioned on vastus lateralis (VL) of the dominant leg for EMG record and the reference electrode was placed over the anterior

surface of the tibia. The skin preparation, placement and location of the electrodes were performed in accordance with the recommendations of SENIAM (20). Immediately after EMG preparation, participants performed three 5 s, one-legged MVC of the knee extensors (trunk-thigh at 90º angle and thigh-leg at 0º angle), separated by a 60 s interval. EMG and force were simultaneously recorded with a sample rate of 2000 Hz (EMG System of Brazil, São José dos Campos, Brazil). After a 5 min resting period, a 30 min CL exercise was performed at 90% of GET. Additional blood samples (1 ml) were taken at 10, 20 and 30 min of the CL exercise. VO_2 and VCO_2 were continually recorded, but the mask was taken off for short periods of mouth rinse immediately before and at 5, 15 and 25 min exercise. Participants were given a 25 ml bolus of either a tasteless 6.4% maltodextrin (Neonutri-Malto, CHO) or water (PLA), which they rinsed around their mouths for 10 s and then expectorated. Participants were then immediately asked about their rating of perceived exertion (15-point Borg's scale, RPE) before the mask was reestablished. Raw EMG signal was recorded during 10 s, starting 50 s after the mouth rinse procedure. Following the CL, participants moved to the knee-extensor chair, performed one more MVC and withdrew the catheter before performing a 20-km cycling TT. This was performed in the fasted possible time (approximately 5 min separating CL and TT). The mouth rinse procedure (CHO or PLA) was performed before and at the 5, 10, 15 and 18 km mark of TT. RPE and a 10-s EMG (starting 50 s post mouth rinse) were also recorded at the same marks.

Blood samples

Blood samples were immediately transferred to vacutainer tubes (Becton Dickinson, BD, Juiz de Fora, MG, Brazil) and centrifuged at 3000 rpm for 15 min at 4°C for plasma separation. Plasma lactate and glucose were analyzed in a UV spectrophotometer (Quimis®,

model Q798U2V5, São Paulo, Brazil) using commercial kits (kit Biotecnica, Varginha, Brazil).

Fat and carbohydrate oxidation rates

The respiratory exchange ratio (RER), and fat and CHO oxidation rates during the CL (at rest, and 5, 10, 15, 20, 15 and 30 min) and TT (at rest, and 0, 5, 10, 15 and 20 km) were calculated using the mean VO_2 and VCO_2 values collected in 1-min intervals. Fat and CHO oxidation rates were calculated using the non-protein respiratory quotient elaborated by Frayn, 1983:

$$\text{Fat oxidation rate} = 1.67 \cdot VO_2 - 1.67 \cdot VCO_2 \quad (1)$$

$$\text{Carbohydrate oxidation rate} = 4.55 \cdot VCO_2 - 3.21 \cdot VO_2 \quad (2)$$

with VO_2 and VCO_2 measured in $l.min^{-1}$ and oxidation rate in $g.min^{-1}$.

EMG analyses

The raw EMG signals were full-wave rectified and filtered with second order Butterworth band-pass filters with cut-off frequencies set at 10 and 400 Hz to remove external interference noise and movement artifacts. The Root Mean Square (RMS) for 5 consecutive contractions during each period of the cycling was calculated, averaged and normalized by the RMS value at the maximal pre exercise MVC (21).

Statistical Analysis

The data are reported as mean ± SD, unless otherwise noted. Three-way ANOVA with repeated measures was used to verify the effect of pre-exercise CHO availability (FED, FAST

and DEP), solution (CHO and PLA) and time or distance on dependent variables (glucose, lactate, VO_2, RER, CHO and fat oxidation rates, heart rate, RPE, PO, MVC and RMS). When a significant effect was found, the main effect was identified using the least significant difference test. Two-way ANOVA with repeated-measures followed by least significant difference test were used to identify the effect of the mouth rinse solution and pre-exercise CHO availability on exercise performance. As per Batterham and Hopkins (4), the p values obtained from *t* test were used to make inferences about true (population) values of the effect of CHO mouth rinse on exercise performance. The uncertainty in the effect was expressed as the likelihood that the true value of the effect represents substantial change (harm or benefit). The smallest standardized change was assumed to be 0.20. For all analyses, significance was accepted at $P \leq 0.05$. All analyses were performed using SPSS software (version 17.0; Chicago, IL), except for uncertainty in the effects, which were calculated using a spreadsheet as described previously (4).

RESULTS

Constant load exercise

Plasma glucose and lactate

There was a main effect of pre exercise CHO availability on plasma glucose ($p = 0.001$, table 1). Plasma glucose was always lower in DEP compared with both FED ($p = 0.014$) and FAST ($p = 0.001$) for both CHO and PLA solutions, but there was no difference ($p = 0.068$) between FAST and FED states. There was also a main effect for time ($p = 0.003$), with plasma glucose being lower in minute 10 compared with minute 20 ($p = 0.016$) and 30 minute ($p = 0.004$). There was no main effect of solution ($p = 0.210$). However, an interaction between solution and time for plasma glucose was found ($p = 0.023$). Plasma glucose was maintained slightly higher with CHO mouth rinse than with PLA during the exercise. There

was no interaction effect between pre exercise CHO availability and solution (p = 0.310). However, there was an interaction between pre exercise CHO availability and time for plasma glucose (p = 0.011). Plasma glucose increased from rest to the end of exercise during FAST condition, whilst maintained lower and relatively constant during the exercise for DEP condition. In FED condition, plasma glucose reduced at minute 10, but returned to rest values by minute 20 and minute 30.

There were no main effect of pre exercise CHO availability (p = 0.060), solution (p = 0.098) and time (p = 0.084) for lactate, but there was an interaction between the pre exercise CHO availability and time (p = 0.033). Lactate increased from rest to 10 min in all conditions, but this was attenuated in DEP condition (table 1).

EMG response

There was a main effect of pre exercise carbohydrate availability for EMG (p = 0.005), with values in DEP being lower than both FED and FAST (p = 0.007 and p = 0.012, respectively), but there was no difference between FED and FAST (p = 0.491). There was no effect of solution (p = 0.106) or time (p = 0.064), but there was an interaction between pre exercise carbohydrate availability and solution (p = 0.031), with EMG activity being severely reduced in DEP with PLA, but not in DEP with CHO (Figure 2).

Metabolic response, heart rate and rating of perceived exertion

There was a main effect of pre exercise CHO availability for VO_2 (p = 0.021), RER (p = 0.001), and CHO and fat oxidation rates (p = 0.001) (table 1). VO_2 and fat oxidation rates were higher and RER and CHO oxidation rates were lower in DEP compared with both FED and FAST. There was no difference between FED and FAST for VO_2, but fat oxidation was higher and RER and CHO oxidation lower in FAST than in FED. There was also a main

effect of time for all of these variables. VO_2, CHO oxidation rate end RER increased, while fat oxidation rate decreased, from rest to 5 minutes and then remained relatively constant throughout the exercise. An interaction between pre exercise CHO availability versus time for CHO and fat oxidation rates was also found. The CHO and fat oxidation rates were maintained relatively constant from minute 5 to the end of exercise in FED, while CHO oxidation rate was reducing and fat oxidation rate increasing from 5 to the end of the exercise in FAST and DEP states. There was no significant effect of solution, or significant interaction of solution vs pre exercise CHO availability or time.

There was a main effect of pre exercise CHO availability for heart rate (p = 0.005). The mean heart rate was higher in DEP than in both FED and FAST (p = 0.017 and p = 0.001, respectively), but there was no difference between FED and FAST (p = 0.927). There was a main effect of time (p = 0.001), with heart rate increasing quickly in the beginning and thereafter slowly until the end. There were no solution effect (p = 0.883) or significant interactions for heart rate.

There was a main effect of time for RPE (p = 0.03), with values increasing similarly with time in all three conditions, but there was no pre exercise CHO availability (p = 0.677), solution (p = 0.999) or significant interactions.

INSERT TABLE 1 HERE

INSERT FIGURE 2 HERE

Force and EMG during MVC

There was no effect of pre exercise carbohydrate availability, solution and time (p = 0.197, p = 0.590 and p = 0.295, respectively) or any significant interaction for MVC before

and after constant load exercise. There was no effect of pre exercise carbohydrate availability and solution (p = 0.317 and p = 0.256, respectively) or any significant interaction for maximal RMS. However, there was a main effect of time for maximal RMS (p = 0.005), with values post-test being lower than pre-test.

20-km time trial

Performance

There was a main effect for pre exercise CHO availability (p = 0.003), with slower performance time for DEP trial (46.34 ± 1.74 min) compared to both FAST and FED trials (42.43 ± 1.43 min and 40.77 ± 1.50 min, p = 0.044 and 0.007, respectively), but there was no significant difference between the FAST and FED (p = 0.058) states. There was also a main effect for mouth rinse solution (p = 0.009), with performance time with CHO being faster compared to PLA (42.47 ± 1.39 versus 43.90 ± 1.51 min, respectively). However, there was no interaction between pre exercise CHO availability and solution (p = 0.09).

When simple effect was assessed, the performance time was faster with CHO compared to PLA only in DEP condition (Figure 3, p = 0.019). The corresponding qualitative inference of CHO mouth rinse was 'benefit very likely' for DEP, 'possibly benefit' for FAST and "negligible or trivial" for FED.

INSERT FIGURE 3 HERE

There was also a main effect for pre exercise CHO availability (p = 0.015), solution (p = 0.027) and distance covered (p = 0.001) on power output during the 20-km TT (Figure 4a). Power output was higher in FED than in both FAST and DEP (p = 0.006 and 0.026, respectively), but there was no difference between FAST and DEP (p = 0.211). There was

also a main effect of solution (p = 0.027), with power output being higher with CHO compared to PLA. The power output decreased from beginning until km 15, but increased from km 15 to km 20 in all conditions. An interaction between pre exercise CHO availability, solution and distance was also observed (p = 0.001). CHO mouth rinse was progressively attenuating the DEP-induced reduction in power output as distance progressed (Figure 4a).

INSERT FIGURE 4 HERE

EMG response

There was a main effect of pre exercise carbohydrate availability for EMG (p = 0.006), with FAST values being higher than in both FED and DEP (p = 0.026 and p = 0.015, respectively), but there was no difference between FED and DEP (p = 0.101) (Figure 4b). There was no distance effect (p = 0.154). A main effect of solution with higher EMG activity in the CHO than in the PLA conditions (p = 0.05) was found, which was dependent of the pre exercise carbohydrate availability (interaction between solution and pre exercise carbohydrate availability, p = 0.010). CHO mouth rinse had more effect on EMG signal in DEP (+ 162 %), followed by FAST (+ 20 %) condition (Figure 4b).

Metabolic, heart rate and rating of perceived exertion response

There was a main effect of pre exercise CHO availability for VO_2 (p = 0.001), RER (p = 0.001), CHO oxidation (p = 0.001) and fat oxidation (p = 0.001). Fat oxidation was higher, while VO_2, RER and CHO oxidation were lower in DEP compared with both FED and FAST. Fat oxidation was also higher and VO_2, RER and CHO oxidation lower in FAST than FED. There was a main effect for distance covered (p = 0.001). VO_2, CHO oxidation rate end RER increased, while fat oxidation rate decreased, from rest to 5 km and then remained relatively

constant throughout the exercise. However, there was no effect of solution or interactions for any of these variables (all $p > 0.05$, table 2).

There was a main effect of pre exercise CHO availability and distance for heart rate ($p = 0.028$ and $p = 0.001$, respectively), but there was no effect of solution ($p = 0.953$). The heart rate was higher in FED than in both FAST and DEP ($p = 0.048$ and 0.037, respectively), but there was no differences between FAST and DEP ($p = 0.369$). The heart rate increased from rest to 5 km and then was maintained relatively constant until the end. There was also an interaction between pre exercise CHO availability and distance for heart rate ($p = 0.001$), with a larger increase from km 15 to km 20 in DEP condition (table 2).

There was a main effect of solution and distance for RPE ($p = 0.038$ and $p = 0.001$, respectively), but there was no effect of pre exercise CHO availability ($p = 0.851$) or any interaction ($p > 0.05$). The RPE was slightly lower in PLA than in CHO during the trial, with values increasing progressively with the distance in all conditions.

INSERT TABLE 2 HERE

DISCUSSION

In the present study, a reduction of pre exercise endogenous CHO availability induced by prior exercise and fasting (DEP condition) resulted in a greater physiological stress (i.e. higher VO_2 and HR, and lower plasma glucose) during a set exercise task (i.e., a CL exercise), when compared to post prandial (FED) or partially depleted (FAST) conditions. This elevated physiological stress observed during the CL in DEP condition was concomitant with a lower EMG activity, indicating reduced muscle recruitment in the PLA condition. However, there was a restoration of the EMG activity with CHO mouth rinse but it had no influence on metabolic responses (plasma glucose and lactate, and fuel oxidation rates). The 20-km time

trial power output was progressively greater with CHO mouth rinse compared with PLA as the trial progressed in DEP state. This was not different in other trials and it was accompanied with increases in EMG activity (greater muscle recruitment) with CHO rinse protocol. When qualitative inference was considered, the CHO mouth rinse effect on the time to complete the 20-km time trial was categorized as 'benefit very likely' for DEP, 'possibly benefit' for FAST and "negligible or trivial" for FED. Together, these results indicate a centrally mediated response of CHO rinse with improved exercise performance rather than metabolic mechanisms.

Constant load exercise

An elevated plasma glucose was measured in the first 10 minutes of CL exercise with CHO mouth rinse (Table 2) and the mechanism by which such an increase occurs early in exercise is unknown. A few studies have evaluated the effect of the CHO mouth rinse on plasma glucose response during time trial performances and found no effect of CHO mouth rinse on plasma glucose (9, 14, 24, 26, 31, 32, 36, 38). However, it is difficult to draw a physiological inference from the time trial performance results as a higher power output with CHO mouth rinse reported in those studies (9, 26, 31) potentially increases muscle glucose uptake. No other study has investigated CHO mouth rinse with CL exercise and, as the exercise power output is constant in this model, the effect of CHO mouth rinse on plasma glucose can be compared. It is unlikely that any considerable amount of glucose could be absorbed directly from the mouth (23). However, the CHO mouth rinse potentially actives brain areas as the insula (8), a region that increases sympathetic activity when stimulated (29, 34, 40). Functional studies have demonstrated such activation of the hepatic and pancreatic sympathetic nerve fibers increases liver glucose output as well as stimulates the release of glucagon and inhibitions of insulin release from the pancreas (25). Nonetheless, the

physiological relevance of the ~3% increase in average of the plasma glucose with CHO mouth rinse is unlikely to explain subsequent improvement on exercise performance as no change in CHO oxidation rate was observed.

A reduction in EMG activity during DEP condition with PLA mouth rinse was ameliorated with CHO mouth rinse. While the interpretation of EMG activity during dynamic exercise is difficult, it is the only practical way to measure muscle activation during whole body exercise. Changes in EMG signal reflect changes in muscle activation during controlled-experimental conditions, such as a CL exercise model (13). A reduced EMG activity in DEP condition with placebo mouth rinse might indicate that a portion of vastus lateralis fibers was silenced due to a reduced glycogen content. Alternatively, the CHO mouth rinse may have activated some brain areas, which might favor a higher motor output. Gant et al (15) using transcranial magnetic stimulation of primary motor cortex detected an increase in the corticomotor excitability during voluntary muscle activation and maximal voluntary force when non-sweet CHO solutions were present in the mouth. They suggested that afferent signals from oral receptors are integrated with descending motor outputs and might affect muscle force production. Interesting to note this effect appeared greater with fatigue, suggesting that the CHO mouth rinse effect may be potentiated when cell energy status is low. Supporting this assumption, Turner et al (35) showed that oral CHO combined with a motor task increased activation within the primary sensorimotor cortex and the anterior cingulate gyrus. As the anterior cingulate gyrus is a region responsible to drive emotional and behavioural responses to rewarding food stimuli, these authors suggested that exposing CHO in the mouth would be considered high reward value during exercise, given that during intense muscular work fuels become gradually sparse and muscle glycogen is rapidly depleted (35). Our findings expand on these studies by showing that for a given whole body exercise task, CHO mouth rinse rescues central motor drive and muscle recruitment when exercise

starts with reduced endogenous CHO availability, but not when exercise starts with CHO stores intact or only partially depleted.

Time-trial

A reduced pace was observed in the DEP condition for the TT, in which the volunteers were free to regulate their power output aiming to finish the trial as soon as possible. The difference in performance between the trials was not considered to be influenced by the previously performed CL exercise, as the MVC, a general indicator of muscle fatigue (28), was not different before and after the CL exercise in all experimental conditions. Furthermore, 30 minutes of cycling at a moderate exercise intensity (90% of GET) is likely to have no homeostatic disturbance and thus have a minimal effect on subsequent performance (30). Prior exercise and fasting (DEP condition) resulted in an elevated physiological strain (higher VO_2 and HR) during CL. However, where participants are free to adjust their pace during TT, there was a reduction in these parameters (i.e., lower VO_2 and HR). This finding indicates that participants chose to reduce their pace during DEP condition rather than exacerbate physiological stress triggered by a reduced CHO availability.

A lower EMG activity accompanied the reduced power output in DEP condition. However, the capacity to apply power output during the trial in the DEP condition was partially recovered with CHO mouth rinse, and this was associated with a concomitant increase in the EMG activity. A reduction of the power output and EMG activity during a cycling time trial is associated with a reduction in the ability of the motor cortex to drive the knee extensor muscles, probably by an inhibitory somatosensory feedback from working muscles on the central motor drive (1, 33). A reduction in motor output, and consequently in external power output, might act as a protective mechanism that results from the integration of multiple feedback signals informing a reduced CHO availability in the peripheral tissues

(liver and muscle). Nevertheless, data in this study suggests that this reduced motor output can be ameliorated by the central stimulatory effect of the CHO mouth rinse. A large increase in the EMG activity and a concomitant increase in the power output with CHO mouth rinse in DEP condition supports previous suggestion that the greater the reduction in CHO stores the greater the effectiveness of the CHO mouth rise (26). These results also support the suggestion that CHO mouth rinse could be a nutritional intervention to reduce the drop-off in training intensity when training in a low muscle glycogen state (22). Training with reduced muscle glycogen has been adopted to maximize the mitochondrial adaptation to endurance exercise (2, 3). Our results indicate that the benefit of the CHO mouth rinse during a muscle glycogen depleted state is due to a neural modulation with minimal metabolic alteration. Therefore, this intervention would not affect induced-low muscle glycogen training adaptations, as it would not be expected to alter the metabolic state of skeletal muscle or affect signaling effects induced by training with reduced muscle glycogen.

CONCLUSIONS

This study demonstrated that reductions of pre exercise endogenous CHO availability (with prior exercise and fasting) produced an elevated physiological strain (VO_2 and HR) and concomitant reductions in muscle recruitment (EMG activity) during a fixed intensity exercise and reduced power output and EMG activity during a subsequent 20-km TT. However, CHO mouth rinse restored the EMG activity during the DEP condition across both CL and 20-km TT and attenuated the reductions in performance observed. These findings indicate that the CHO mouth rinse effect may be more salient when endogenous CHO status is low and the main mechanism governs it is likely to be centrally mediated.

ACKNOWLEDGMENTS

Thays Ataide-Silva is grateful to National Council for Scientific and Technological Development (CNPq) for her PhD scholarship. The authors thank all cyclists who took part in this study, and Geivianni Andrade, Marcos Silva-Cavalcante, João Lopes-Silva and Carlos Rafaell Correia-Oliveira for their technical support.

CONFLICT OF INTEREST

No financial support was received.

The authors declare no conflicts of interest.

The results of this study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

1. Amann M, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol*. 2009;587(Pt 1):271-83.

2. Baar K. Nutrition and the adaptation to endurance training. *Sports Med*. 2014;44 Suppl 1:S5-12.

3. Bartlett JD, Louhelainen J, Iqbal Z, Cochran AJ, Gibala MJ, Gregson W, et al. Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. *Am J Physiol Regul Integr Comp Physiol*. 2013;304(6):R450-8.

4. Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. *Int J Sports Physiol Perform*. 2006;1(1):50-7.

5. Beelen M, Berghuis J, Bonaparte B, Ballak SB, Jeukendrup AE, van Loon LJ. Carbohydrate mouth rinsing in the fed state: lack of enhancement of time-trial performance. *Int J Sport Nutr Exerc Metab*. 2009;19(4):400-9.

6. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc*. 2004;36(12):2107-11.

7. Casey A, Mann R, Banister K, Fox J, Morris PG, Macdonald IA, et al. Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by (13)C MRS. *Am J Physiol Endocrinol Metab*. 2000;278(1):E65-75.

8. Chambers ES, Bridge MW, Jones DA. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *J Physiol*. 2009;587(Pt 8):1779-94.

9. Che Muhamed AM1 MN, Ismail N, Aziz AR, Singh R. Mouth rinsing improves cycling endurance performance during Ramadan fasting in a hot humid environment. *Appl Physiol Nutr Metab*. 2014;39(4):458-64.

10. Costill DL, Sherman WM, Fink WJ, Maresh C, Witten M, Miller JM. The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. *Am J Clin Nutr*. 1981;34(9):1831-6.

11. de Ataide e Silva T, Souza MEDCAD, Amorim JFD, Stathis CG, Leandro CG, Lima-Silva AE. Can carbohydrate mouth rinse improve performance during exercise? a systematic review. *Nutrients*. 2014;6:1-10.

12. Fares EJ, Kayser B. Carbohydrate mouth rinse effects on exercise capacity in pre- and postprandial States. *J Nutr Metab*. 2011;2011:385962.

13. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. *J Appl Physiol (1985)*. 2004;96(4):1486-95.

14. Gam S, Guelfi KJ, Fournier PA. Opposition of carbohydrate in a mouth-rinse solution to the detrimental effect of mouth rinsing during cycling time trials. *Int J Sport Nutr Exerc Metab*. 2013;23(1):48-56.

15. Gant N, Stinear CM, Byblow WD. Carbohydrate in the mouth immediately facilitates motor output. *Brain Res*. 2010;1350:151-8.

16. Garby L, Astrup A. The relationship between the respiratory quotient and the energy equivalent of oxygen during simultaneous glucose and lipid oxidation and lipogenesis. *Acta Physiol Scand*. 1987;129(3):443-4.

17. Gollnick PD, Armstrong RB, Sembrowich WL, Shepherd RE, Saltin B. Glycogen depletion pattern in human skeletal muscle fibers after heavy exercise. *J Appl Physiol*. 1973;34(5):615-8.

18. Gollnick PD, Karlsson J, Piehl K, Saltin B. Selective glycogen depletion in skeletal muscle fibres of man following sustained contractions. *J Physiol*. 1974;241(1):59-67.

19. Heigenhauser GJ, Sutton JR, Jones NL. Effect of glycogen depletion on the ventilatory response to exercise. *J Appl Physiol*. 1983;54(2):470-4.

20. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol*. 2000;10(5):361-74.

21. Hirai DM, Roseguini BT, Diefenthaeler F, Carpes FP, Vaz MA, Ferlin EL, et al. Effects of altering pedal frequency on the slow component of pulmonary VO2 kinetics and EMG activity. *Int J Sports Med*. 2010;31(8):529-36.

22. Hulston CJ, Venables MC, Mann CH, Martin C, Philp A, Baar K, et al. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med Sci Sports Exerc*. 2010;42(11):2046-55.

23. Hussain MM, Pan X. Circadian Regulation of Macronutrient Absorption. *J Biol Rhythms*. 2015.

24. Ispoglou T, O'Kelly D, Angelopoulou A, Bargh M, O'Hara JP, Duckworth LC. "Mouth-Rinsing with Carbohydrate Solutions at the Postprandial State Fail to Improve Performance During Simulated Cycling Time Trials". *J Strength Cond Res*. 2015.

25. Jarhult J, Falck B, Ingemansson S, Nobin A. The functional importance of sympathetic nerves to the liver and endocrine pancreas. *Ann Surg*. 1979;189(1):96-100.

26. Lane SC, Bird SR, Burke LM, Hawley JA. Effect of a carbohydrate mouth rinse on simulated cycling time-trial performance commenced in a fed or fasted state. *Appl Physiol Nutr Metab*. 2013;38(2):134-9.

27. Laursen PB, Francis GT, Abbiss CR, Newton MJ, Nosaka K. Reliability of time-to-exhaustion versus time-trial running tests in runners. *Med Sci Sports Exerc*. 2007;39(8):1374-9.

28. Merletti R, Knaflitz M, De Luca CJ. Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions. *J Appl Physiol (1985)*. 1990;69(5):1810-20.

29. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. *Neurology*. 1992;42(9):1727-32.

30. Poole DC, Jones AM. Oxygen uptake kinetics. *Compr Physiol*. 2012;2(2):933-96.

31. Pottier A, Bouckaert J, Gilis W, Roels T, Derave W. Mouth rinse but not ingestion of a carbohydrate solution improves 1-h cycle time trial performance. *Scand J Med Sci Sports*. 2010;20(1):105-11.

32. Rollo I, Cole M, Miller R, Williams C. Influence of mouth rinsing a carbohydrate solution on 1-h running performance. *Med Sci Sports Exerc*. 2010;42(4):798-804.

33. Sidhu SK, Bentley DJ, Carroll TJ. Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. *J Appl Physiol (1985)*. 2009;106(2):556-65.

34. Tanida M, Sakatani K, Takano R, Tagai K. Relation between asymmetry of prefrontal cortex activities and the autonomic nervous system during a mental arithmetic task: near infrared spectroscopy study. *Neurosci Lett*. 2004;369(1):69-74.

35. Turner CE, Byblow WD, Stinear CM, Gant N. Carbohydrate in the mouth enhances activation of brain circuitry involved in motor performance and sensory perception. *Appetite*. 2014;80:212-9.

36. Watson P, Nichols D, Cordery P. Mouth rinsing with a carbohydrate solution does not influence cycle time trial performance in the heat. *Appl Physiol Nutr Metab*. 2014;39(9):1064-9.

37. Whipp BJ, Davis JA, Torres F, Wasserman K. A test to determine parameters of aerobic function during exercise. *J Appl Physiol Respir Environ Exerc Physiol*. 1981;50(1):217-21.

38. Whitham M, McKinney J. Effect of a carbohydrate mouthwash on running time-trial performance. *J Sports Sci*. 2007;25(12):1385-92.

39. Widrick JJ, Costill DL, Fink WJ, Hickey MS, McConell GK, Tanaka H. Carbohydrate feedings and exercise performance: effect of initial muscle glycogen concentration. *J Appl Physiol (1985)*. 1993;74(6):2998-3005.

40. Williamson JW, McColl R, Mathews D, Ginsburg M, Mitchell JH. Activation of the insular cortex is affected by the intensity of exercise. *J Appl Physiol (1985)*. 1999;87(3):1213-9.

FIGURES CAPTIONS

FIGURE 1. (a) Overall experimental design and (b) Experimental trial day protocol. IT: incremental test; Fam: familiarization trial; FED: experimental trial in fed state; FAST: experimental trial after a 12-h fasting; DEP: experimental trial after an exercise-depleting muscle glycogen protocol (DEP) and 12-h fasting; CHO: carbohydrate; PLA: placebo; MVC: maximal voluntary contraction; b: blood sample; GET: gas exchange threshold; MR: mouth rinse; RPE: rating of perceived exertion; EMG: electromyography; TT: time trial.

FIGURE 2. Electromyography activity (Root Mean Square, RMS) of the vastus lateralis muscle (relative to maximal RMS at pre-exercise maximal voluntary contraction, MVC) during constant load test (90% of the gas exchange threshold). FEDPLA and FEDCHO: fed with placebo and carbohydrate mouth rinse, respectively; FASTPLA and FASTCHO: fasted with placebo and carbohydrate mouth rinse, respectively; DEPPLA and DEPCHO: endogenous CHO depleted state through pre exercise with placebo and carbohydrate mouth rinse, respectively. Values are means ± SEM. * Significant main effect of pre exercise CHO availability ($P < 0.05$) with DEP lower than FED and FAST; ** Significant interaction between pre exercise CHO availability and solution ($P < 0.05$), with EMG activity reduced in DEPPLA, but not in DEPCHO.

FIGURE 3. Performance time during a 20-km cycling time trial with CHO or placebo mouth rinse after different pre exercise carbohydrate availability conditions. FED: fed state; FAST: fast state; DEP: endogenous CHO depleted state through pre exercise; PLA: placebo mouth rinse; CHO: carbohydrate mouth rinse. *Significant faster than PLA in DEP condition ($p < 0.05$). Qualitative inference of CHO mouth rinse effect are shown.

FIGURE 4. (a) Power output profile and (b) electromyography activity (Root Mean Square, RMS) of the vastus lateralis muscle (relative to maximal RMS at pre-exercise maximal voluntary contraction, MVC) during the 20-km cycling time trial. FEDPLA and FEDCHO: fed with placebo and carbohydrate mouth rinse, respectively; FASTPLA and FASTCHO: fasted with placebo and carbohydrate mouth rinse, respectively; DEPPLA and DEPCHO: endogenous CHO depleted state through pre exercise with placebo and carbohydrate mouth rinse, respectively. Values are means ± SEM, $p < 0.05$. * Significant main effect of pre exercise CHO availability ($P < 0.05$), with FED higher than FAST and DEP for power output (a) and FAST higher than FED and DEP for EMG activity (b); ** Significant main effect of solution ($P < 0.05$), with CHO higher than PLA; *** Significant main effect of distance ($P < 0.05$), decreased from 5 km to 15 km and then increased; † Significant interaction between pre exercise CHO availability, solution and distance ($P < 0.05$), with CHO attenuating progressively the reduction of the power output (a) and EMG activity (b) in DEP.

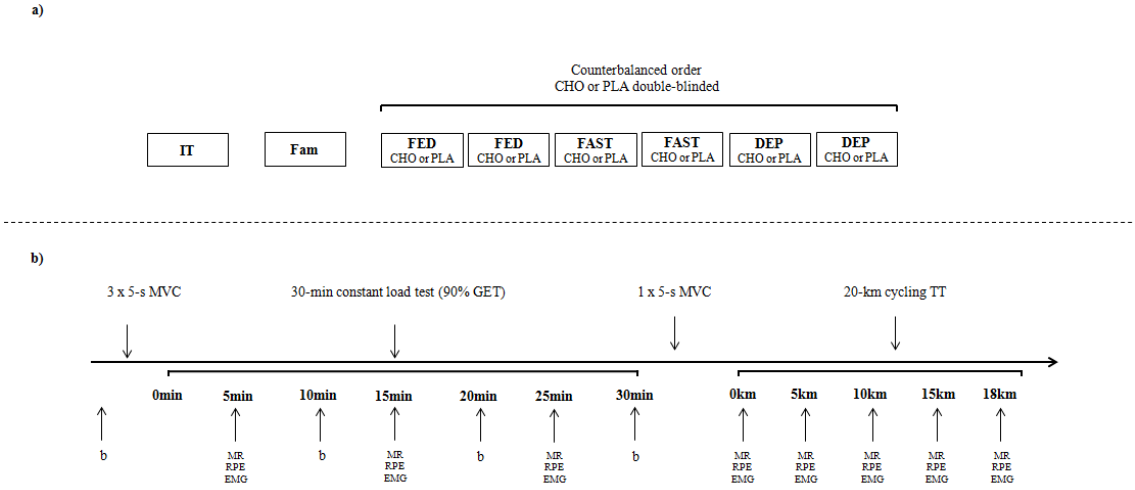


FIGURA 1

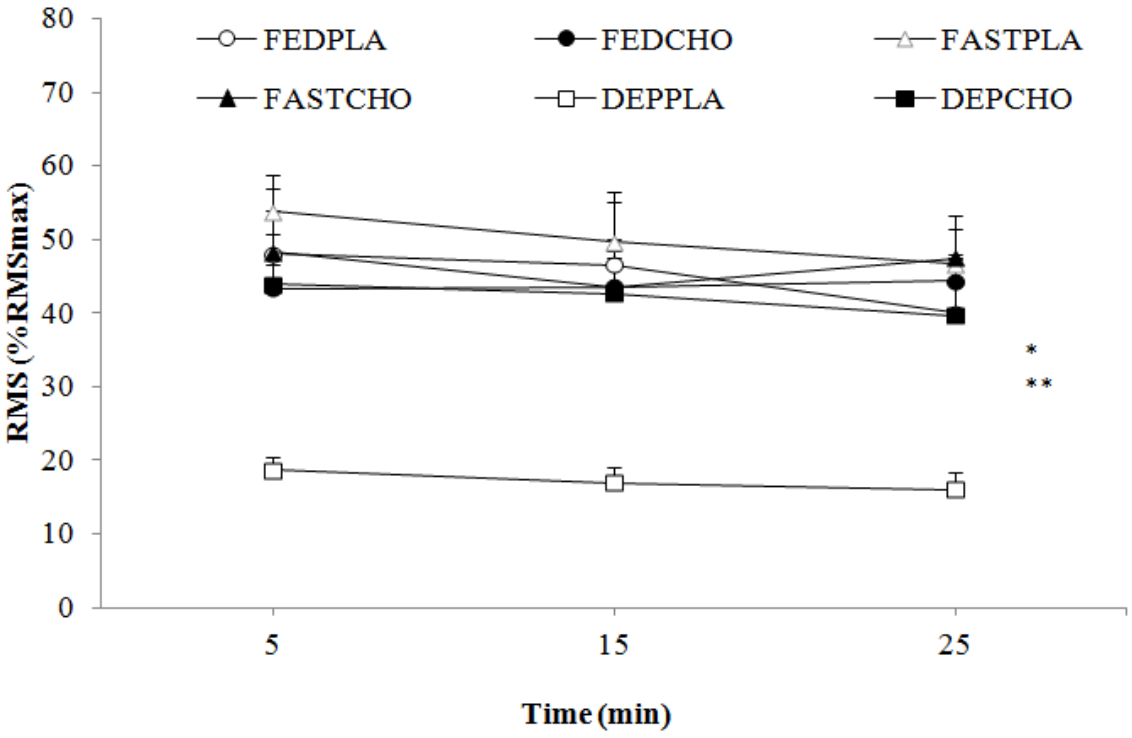


FIGURA 2

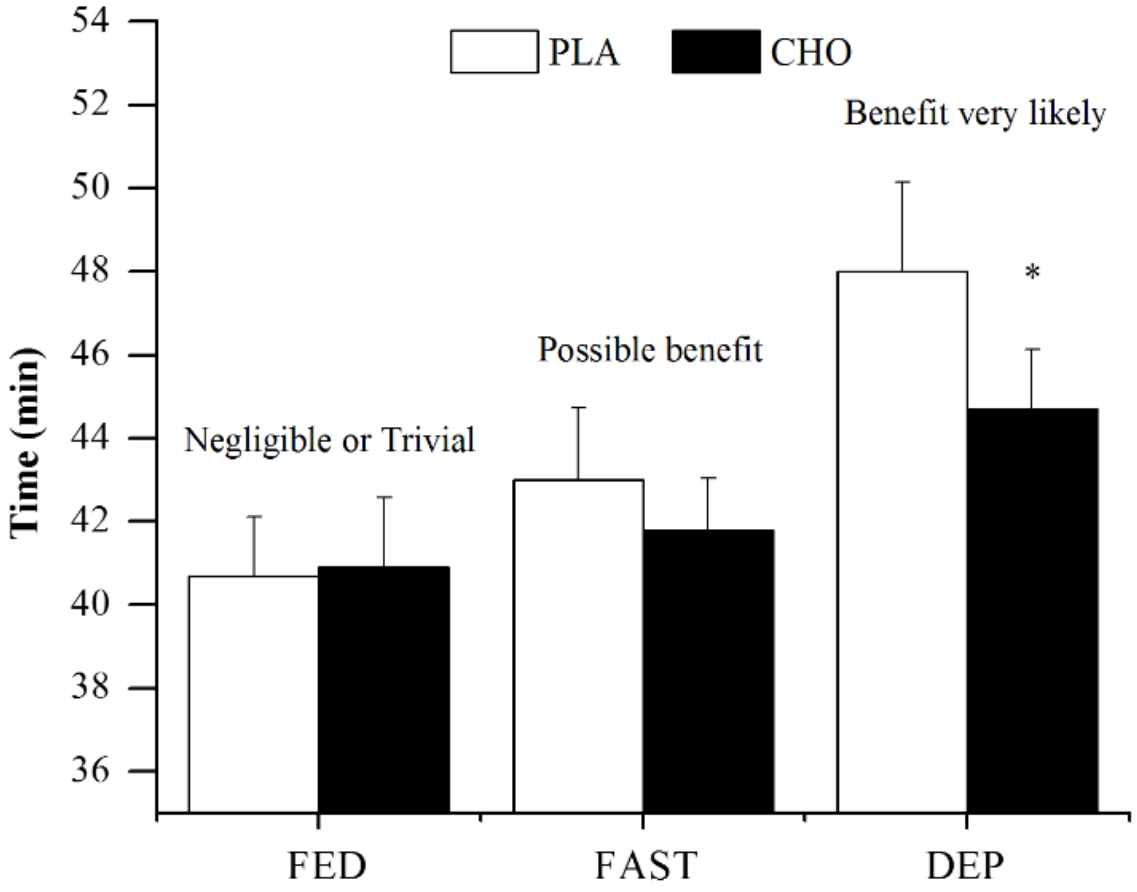

54
52
50
48
46
44
42
40
38
36
Time (min)
PLA
CHO
Benefit very likely
*
Possible benefit
Negligible or Trivial
FED
FAST
DEP

FIGURE 3

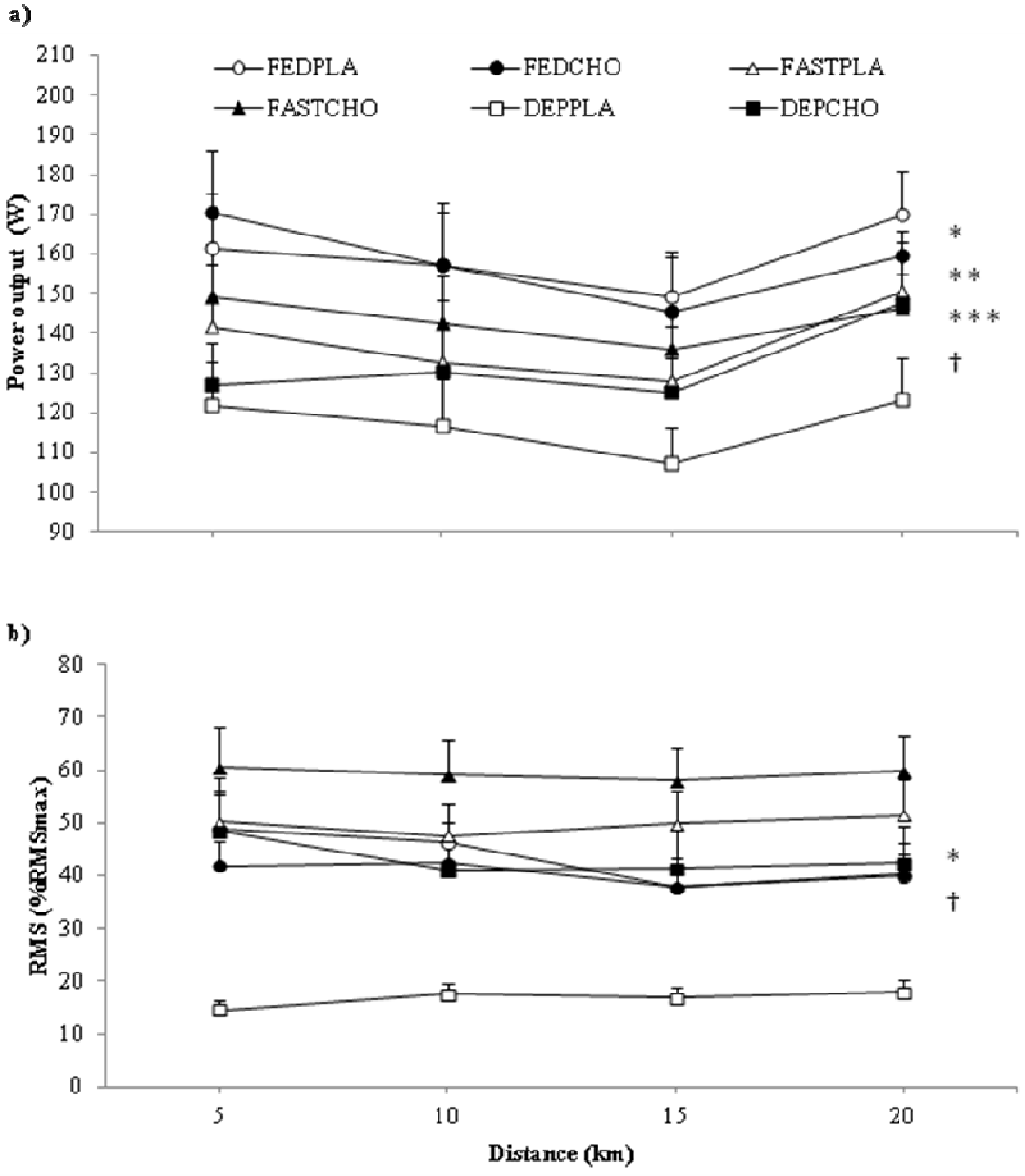

a)
FEDPLA
FEDCHO
FASTPLA
FASTCHO
DEPPLA
DEPCHO
Power output (W)
210
200
190
180
170
160
150
140
130
120
110
100
90
*
**
***
†
b)
RMS (%RMSmax)
80
70
60
50
40
30
20
10
0
5
10
15
20
Distance (km)
*
†

FIGURE 4

TABLE 1. Metabolic response during the constant load exercise (90% of the gas exchange threshold) with carbohydrate or placebo mouth rinse after different pre exercise endogenous CHO availability conditions.

	PLA							CHO						
	Time (min)							Time (min)						
	0	**5**	**10**	**15**	**20**	**25**	**30**	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Glucose (mmol.L^{-1})^{a,b,c,d}														
Fed	4.97±0.93		4.08±0.43		5.03±0.78		5.12±0.59	4.88±0.61		4.26±0.75		4.89±0.77		4.97±0.58
Fast	4.88±0.54		4.67±0.61		4.90±0.88		5.41±0.98	5.02±0.67		5.45±0.61		5.83±0.65		5.45±0.67
Dep	4.38±0.63		3.79±0.66		3.92±0.57		4.26±0.43	4.14±0.61		3.99±0.93		3.96±0.68		4.35±0.77
Plasma Lactate (mmol.L^{-1})^{c}														
Fed	1.51±0.32		2.59±1.71		2.50±2.04		2.55±2.07	1.71±0.50		2.92±2.36		2.57±2.33		2.68±2.37
Fast	1.24±0.63		2.31±1.90		2.43±1.98		2.57±2.15	1.32±0.34		2.45±1.59		2.62±1.93		2.58±1.90
Dep	1.27±0.50		2.06±1.97		1.98±1.88		1.90±1.65	1.31±0.35		2.05±1.52		1.89±1.42		1.97±1.34
VO_2(l.min)^{a,b}														
Fed	1.26±0.10	2.01±0.28	2.06±0.27	2.09±0.23	2.12±0.22	2.08±0.27	2.07±0.26	1.19±0.20	2.04±0.23	2.04±0.18	2.02±0.24	2.05±0.23	2.05±0.20	2.06±0.20
Fast	1.21±0.08	2.02±0.26	1.98±0.27	2.05±0.28	2.05±0.29	2.07±0.30	2.03±0.26	1.24±0.10	2.06±0.29	2.05±0.27	2.03±0.27	2.07±0.25	2.11±0.32	2.08±0.20
Dep	1.24±0.17	2.06±0.31	2.09±0.36	2.25±0.30	2.23±0.35	2.20±0.39	2.19±0.36	1.28±0.14	2.19±0.29	2.17±0.26	2.24±0.30	2.26±0.32	2.24±0.37	2.22±0.43
RER^{a,b}														
Fed	0.74±0.03	0.96±0.06	0.95±0.04	0.95±0.04	0.94±0.05	0.94±0.04	0.94±0.04	0.74±0.03	0.96±0.05	0.96±0.04	0.95±0.04	0.95±0.05	0.96±0.05	0.95±0.06
Fast	0.70±0.05	0.92±0.06	0.92±0.06	0.92±0.06	0.91±0.06	0.89±0.08	0.89±0.08	0.71±0.05	0.94±0.06	0.94±0.06	0.94±0.04	0.93±0.04	0.92±0.05	0.91±0.04
Dep	0.70±0.01	0.87±0.06	0.86±0.06	0.85±0.07	0.85±0.07	0.84±0.07	0.84±0.07	0.71±0.025	0.88±0.04	0.87±0.03	0.86±0.04	0.85±0.04	0.85±0.03	0.85±0.03
Carbohydrate Oxidation (g.min^{-1})^{a,b,c}														
Fed	0.22±0.18	2.34±0.61	2.30±0.58	2.33±0.62	2.31±0.66	2.27±0.66	2.20±0.68	0.19±0.18	2.41±0.18	2.35±0.48	2.23±0.47	2.27±0.51	2.34±0.56	2.27±0.65
Fast	0.04±0.26	1.98±0.84	2.00±0.85	2.09±0.92	1.93±0.83	1.83±1.04	1.78±0.99	0.05±0.28	2.26±0.80	2.21±0.84	2.23±0.69	2.15±0.63	2.13±0.75	2.00±0.70
Dep	0.33±0.22	1.57±0.73	1.57±0.76	1.56±0.83	1.52±0.91	1.36±0.89	1.37±0.87	0.28±0.22	1.81±0.60	1.70±0.51	1.63±0.57	1.51±0.55	1.52±0.57	1.46±0.52
Fat oxidation (g.min^{-1})^{a,b,c}														
Fed	0.54±0.07	0.13±0.18	0.17±0.11	0.17±0.14	0.19±0.15	0.19±0.13	0.21±0.13	0.51±0.07	0.12±0.19	0.14±0.14	0.18±0.14	0.17±0.17	0.15±0.17	0.18±0.20
Fast	0.61±0.07	0.26±0.21	0.24±0.20	0.24±0.21	0.30±0.19	0.35±0.25	0.35±0.26	0.59±0.10	0.19±0.19	0.20±0.20	0.18±0.14	0.23±0.14	0.26±0.15	0.29±0.12

Dep	0.73±0.09	0.44±0.17	0.45±0.15	0.53±0.23	0.54±0.22	0.58±0.25	0.57±0.24	0.73±0.10	0.41±0.10	0.45±0.07	0.51±0.09	0.56±0.08	0.54±0.05	0.56±0.09
Heart Rate (bpm)[a,b]														
Fed	76±6	133±12	126±14	137±12	136±13	141±13	138±12	73±8	131±9	130±12	137±12	128±21	138±10	136±12
Fast	71±7	131±13	129±15	134±16	132±17	140±17	135±16	72±5	132±14	131±13	137±14	134±13	141±14	139±13
Dep	74±7	138±12	137±11	143±12	142±13	147±13	145±12	74±4	137±10	136±11	143±11	141±10	148±11	146±11

Values are presented as means ± SD. [a] Main effect of pre exercise CHO availability; [b] Main effect of time; [c] Interaction between pre exercise CHO availability and time; [d] Interaction between solution and time (all $P < 0.05$). PLA: placebo mouth rinse; CHO: carbohydrate mouth rinse; FED: fed state; FAST: fast state; DEP: muscle glycogen deplete and fasted; RER: respiratory exchange ratio.

TABLE 2. Metabolic response during the 20-km cycling time trial with carbohydrate or placebo mouth rinse after different pre exercise endogenous CHO availability conditions.

	PLA					CHO				
	Distance (km)					Distance (km)				
	0	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
VO_2 (l.min)[a,b]										
Fed	1.77±0.30	2.42±0.37	2.40±0.48	2.28±0.43	2.33±0.31	1.85±0.39	2.33±0.48	2.22±0.50	2.13±0.45	2.36±0.38
Fast	1.77±0.34	2.08±0.58	1.99±0.46	2.02±0.51	2.07±0.41	1.69±0.32	2.21±0.53	2.18±0.33	2.07±0.37	2.15±0.32
Dep	1.55±0.17	1.91±0.46	1.92±0.61	1.87±0.49	2.12±0.47	1.61±0.15	1.81±0.54	1.96±0.52	1.84±0.50	2.00±0.38
RER										
Fed	0.78±0.06	0.94±0.04	0.96±0.06	0.92±0.04	0.92±0.06	0.79±0.09	0.96±0.05	0.94±0.10	0.91±0.07	0.92±0.04
Fast	0.73±0.06	0.89±0.07	0.89±0.07	0.87±0.05	0.89±0.05	0.74±0.08	0.92±0.07	0.89±0.04	0.89±0.06	0.89±0.05
Dep	0.71±0.06	0.82±0.07	0.83±0.10	0.80±0.06	0.82±0.06	0.73±0.05	0.82±0.07	0.81±0.05	0.79±0.05	0.84±0.08
Carbohydrate oxidation ($g.min^{-1}$)[a,b]										
Fed	0.65±0.56	2.64±0.76	2.87±1.22	2.28±0.75	2.35±0.90	0.80±0.82	2.82±0.93	2.42±1.21	2.07±0.96	2.36±0.69
Fast	0.19±0.48	1.94±1.29	1.81±1.13	1.61±0.87	1.79±0.91	0.30±0.54	2.30±1.40	1.86±0.70	1.78±0.90	1.83±0.76
Dep	0.29±0.39	1.07±0.95	1.29±1.46	0.88±0.86	1.22±0.93	0.26±0.35	1.04±1.04	1.04±0.83	0.76±0.64	1.33±0.98
Fat oxidation ($g.min^{-1}$)[a,b]										
Fed	0.63±0.18	0.22±0.14	0.13±0.23	0.28±0.13	0.28±0.20	0.62±0.23	0.11±0.14	0.12±0.32	0.29±0.20	0.29±0.16
Fast	0.80±0.22	0.31±0.21	0.31±0.22	0.41±0.12	0.36±0.15	0.72±0.23	0.25±0.26	0.39±0.11	0.36±0.17	0.38±0.14
Dep	0.87±0.11	0.55±0.16	0.47±0.32	0.59±0.12	0.59±0.13	0.89±0.13	0.51±0.20	0.58±0.12	0.63±0.10	0.49±0.21
Heart Rate (bpm)[a,b,c]										
Fed	95±6	154±16	158±120	155±18	160±17	92±10	154±16	152±22	153±23	154±20
Fast	96±10	143±24	140±24	142±24	147±23	93±12	154±20	150±18	148±20	148±21
Dep	95±11	141±22	141±23	140±24	150±23	94±4	141±22	139±23	141±27	144±22

Values are presented as means ± SD. [a] Main effect of pre exercise CHO availability; [b] Main effect of distance; [c] Interaction between pre exercise CHO availability and distance (all $P < 0.05$). PLA: placebo mouth rinse; CHO: carbohydrate mouth rinse; FED: fed state; FAST: fast state; DEP: muscle glycogen deplete and fasted; RER: respiratory exchange ratio.

CONSIDERAÇÕES FINAIS

5 CONSIDERAÇÕES FINAIS

O presente trabalho de tese por meio da realização de uma revisão sistemática com ferramentas de meta-análise, concluiu que o bochecho de CHO durante exercícios de moderada a alta intensidade (~60% a 75% VO_{2pico}) demonstra um efeito positivo (~3%) sobre o desempenho durante ciclismo contrarrelógio (TT) de 30-60 min. É provável que o mecanismo que envolva esta melhora não seja metabólico mas sim neural, por via de possíveis receptores de CHO (glicose e maltodextrina) que ativam regiões cerebrais relacionadas a sensação de prazer e centro motor. Estes receptores parecem ser especialmente responsivos em condição metabólica de reduzidos estoques de CHO endógeno (glicogênio muscular e hepático).

Desse modo, através de um estudo duplo-cego, randomizado e controlado por placebo, a presente tese investigou o efeito do bochecho de carboidrato sobre a atividade neuromuscular (EMG), respostas metabólicas (glicose e lactato plasmático e taxas de oxidação de carboidrato e gordura), e *performance* durante ciclismo iniciado com diferentes níveis de disponibilidade de carboidrato endógeno pela manipulação do jejum e exercício anterior. Sendo demonstrado que a redução da disponibilidade de CHO endógeno pré teste (com o exercício anterior e jejum) produziu elevado esforço fisiológico (VO_2 e FC) e redução concomitante no recrutamento muscular (atividade EMG) durante o exercício de intensidade (CL) fixa, bem como, reduziu a potência e atividade EMG durante o subsequente 20 km TT. Além disso, foi demonstrado que o bochecho de CHO restaurou a atividade EMG durante a condição de DEP entre ambos CL e 20 km TT e atenuou as reduções no desempenho observadas. Estes resultados indicam que o efeito do bochecho de CHO pode ser mais saliente quando o *status* de CHO endógeno está baixo e o mecanismo principal que governa parece ser mediado por influências centrais sobre a atividade EMG.

Assim, o bochecho de CHO pode ser uma alternativa a ingestão, em especial, quando o *status* de CHO endógeno está baixo. Isso é importante na aplicação da nova abordagem de treinamento proposta recentemente, onde os indivíduos são submetidos a baixa disponibilidade de CHO durante os treinamentos com o objetivo de maximizar a biogênese mitocondrial. O bochecho, desse modo, poderia restaurar a intensidade do treino nessa condição. Além disso, pode evitar possível prejuízo no desempenho decorrente a problema gastrointestinal quando o CHO é ingerido durante exercício de alta intensidade ou com

duração de aproximadamente 1 h. Assim, os atletas com histórico de problemas gastrointestinais induzidos pela ingestão de CHO durante a prova podem se beneficiar do bochecho de CHO. Entretanto, a precisa identificação dos receptores orofaríngeos, o mecanismo de ativação das regiões cerebrais, bem como a padronização e o controle do protocolo de oferta do mesmo são necessários para elucidar os mecanismos e magnitude pelo qual o bochecho de CHO promove melhora no desempenho.

É prudente, também, salientar que bochechar bebida de CHO exclusivamente pode comprometer o abastecimento de substrato energético, hidratação e manutenção dos níveis séricos de glicose e comprometer o desempenho durante eventos com duração superior a 1 h. Portanto, é necessária uma investigação mais aprofundada para desempenho em provas com maior duração.

REFERÊNCIAS

REFERÊNCIAS

BATTERHAM, A. M.; HOPKINS, W. G. Making meaningful inferences about magnitudes. **Int J Sports Physiol Perform,** v. 1, n. 1, p. 50-7, Mar 2006.

BEELEN, M. et al. Carbohydrate mouth rinsing in the fed state: lack of enhancement of time-trial performance. **Int J Sport Nutr Exerc Metab,** v. 19, n. 4, p. 400-9, Aug 2009.

CARTER, J. M.; JEUKENDRUP, A. E.; JONES, D. A. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. **Med Sci Sports Exerc,** v. 36, n. 12, p. 2107-11, Dec 2004.

CHAMBERS, E. S.; BRIDGE, M. W.; JONES, D. A. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. **J Physiol,** v. 587, n. Pt 8, p. 1779-94, Apr 15 2009.

DE ATAIDE E SILVA, T. et al. Can carbohydrate mouth rinse improve performance during exercise? a systematic review. **Nutrients,** v. 6, p. 1-10, 2014.

FARES, E. J.; KAYSER, B. Carbohydrate mouth rinse effects on exercise capacity in pre- and postprandial States. **J Nutr Metab,** v. 2011, p. 385962, 2011.

FRAYN, K. N. Calculation of substrate oxidation rates in vivo from gaseous exchange. **J Appl Physiol Respir Environ Exerc Physiol,** v. 55, n. 2, p. 628-34, Aug 1983.

GANT, N.; STINEAR, C. M.; BYBLOW, W. D. Carbohydrate in the mouth immediately facilitates motor output. **Brain Res,** v. 1350, p. 151-8, Sep 2 2010.

GARBY, L.; ASTRUP, A. The relationship between the respiratory quotient and the energy equivalent of oxygen during simultaneous glucose and lipid oxidation and lipogenesis. **Acta Physiol Scand,** v. 129, n. 3, p. 443-4, Mar 1987.

GOLLNICK, P. D. et al. Glycogen depletion pattern in human skeletal muscle fibers after heavy exercise. **J Appl Physiol,** v. 34, n. 5, p. 615-8, May 1973.

GOLLNICK, P. D. et al. Selective glycogen depletion in skeletal muscle fibres of man following sustained contractions. **J Physiol,** v. 241, n. 1, p. 59-67, Aug 1974.

HEIGENHAUSER, G. J.; SUTTON, J. R.; JONES, N. L. Effect of glycogen depletion on the ventilatory response to exercise. **J Appl Physiol,** v. 54, n. 2, p. 470-4, Feb 1983.

HERMENS, H. J. et al. Development of recommendations for SEMG sensors and sensor placement procedures. **J Electromyogr Kinesiol,** v. 10, n. 5, p. 361-74, Oct 2000.

HIRAI, D. M. et al. Effects of altering pedal frequency on the slow component of pulmonary VO2 kinetics and EMG activity. **Int J Sports Med,** v. 31, n. 8, p. 529-36, Aug 2010.

JACKSON, A. S.; POLLOCK, M. L. Generalized equations for predicting body density of men. **Br J Nutr,** v. 40, n. 3, p. 497-504, Nov 1978.

LANE, S. C. et al. Effect of a carbohydrate mouth rinse on simulated cycling time-trial performance commenced in a fed or fasted state. **Appl Physiol Nutr Metab,** v. 38, n. 2, p. 134-9, Feb 2013.

LAURSEN, P. B. et al. Reliability of time-to-exhaustion versus time-trial running tests in runners. **Med Sci Sports Exerc,** v. 39, n. 8, p. 1374-9, Aug 2007.

WHIPP, B. J. et al. A test to determine parameters of aerobic function during exercise. **J Appl Physiol Respir Environ Exerc Physiol,** v. 50, n. 1, p. 217-21, Jan 1981.

WHITHAM, M.; MCKINNEY, J. Effect of a carbohydrate mouthwash on running time-trial performance. **J Sports Sci,** v. 25, n. 12, p. 1385-92, Oct 2007.

APÊNDICE A – Artigo de Revisão - *Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review.*

Nutrients 2014, **6**, 1-10; doi:10.3390/nu6010001

OPEN ACCESS

nutrients

ISSN 2072-6643

www.mdpi.com/journal/nutrients

Review

Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review

Thays de Ataide e Silva [1,2], Maria Eduarda Di Cavalcanti Alves de Souza [1], Jamile Ferro de Amorim [1], Christos G. Stathis [3], Carol Góis Leandro [2] and Adriano Eduardo Lima-Silva [1,*]

[1] Sport Science Research Group, Department of Physical Education and Sports Science (CAV), Federal University of Pernambuco, Alto do Reservatório street, Bela Vista, Vitória de Santo Antão, Pernambuco zip code: 55608-680, Brazil; E-Mails: thays_de_ataide@hotmail.com (T.A.S.); dicavalcanti@hotmail.com.br (M.E.D.C.A.S.); jamiferro@hotmail.com (J.F.A.)

[2] Laboratory of Physiology and Pharmacology, Department of Physical Education and Sports Science (CAV), Federal University of Pernambuco, Alto do Reservatório street, Bela Vista, Vitória de Santo Antão, Pernambuco 55608-680, Brazil; E-Mail: carolleandro22@gmail.com

[3] College of Health and Biomedicine and Institute of Sport Exercise and Active Living (iSEAL), Victoria University, Melbourne zip code: 8001, Australia; E-Mail: christos.stathis@vu.edu.au

* Author to whom correspondence should be addressed; E-Mail: adrianosilva@usp.br; Tel./Fax: +55-81-35233351.

Received: 11 October 2013; in revised form: 5 December 2013 / Accepted: 10 December 2013 / Published: 19 December 2013

Abstract: The purpose of this review was to identify studies that have investigated the effect of carbohydrate (CHO) mouth rinse on exercise performance, and to quantify the overall mean difference of this type of manipulation across the studies. The main mechanisms involving the potential benefit of CHO mouth rinse on performance was also explored. A systematic review was conducted in the following electronic databases: PubMed, SciELO, Science Direct, MEDLINE, and the Cochrane Library (Cochrane Central Register of Controlled Trials), without limit of searches. Eleven studies were classified as appropriate and their results were summarized and compared. In nine of them, CHO mouth rinse increased the performance (range from 1.50% to 11.59%) during

moderate- to high-intensity exercise (~75% Wmax or 65% VO_2max, ~1 h duration). A statistical analysis to quantify the individual and overall mean differences was performed in seven of the 11 eligible studies that reported power output (watts, W) as the main performance outcome. The overall mean difference was calculated using a random-effect model that accounts for true variation in effects occurring in each study, as well as random error within a single study. The overall effect of CHO mouth rinse on performance was significant (mean difference = 5.05 W, 95% CI 0.90 to 9.2 W, $z = 2.39$, $p = 0.02$) but there was a large heterogeneity between the studies ($I^2 = 52\%$). An activation of the oral receptors and consequently brain areas involved with reward (insula/operculum frontal, orbitofrontal cortex, and striatum) is suggested as a possible physiological mechanism responsible for the improved performance with CHO mouth rinse. However, this positive effect seems to be accentuated when muscle and liver glycogen stores are reduced, possibly due to a greater sensitivity of the oral receptors, and require further investigation. Differences in duration of fasting before the trial, duration of mouth rinse, type of activity, exercise protocols, and sample size may account for the large variability between the studies.

Keywords: maltodextrin; glucose; mouthwash; performance

1. Introduction

Carbohydrate (CHO) mouth rinse is defined as a CHO fluid distribution around the mouth for 5 to 10 s with subsequent expulsion by spitting. The utilization of either a low-concentrated 6.0% to 6.4% glucose [1] or partially hydrolyzed maltodextrin are the most common CHO used, with the latter being colorless and tasteless when dissolved in water [2]. CHO mouth rinse has been investigated as a potential ergogenic resource for improved performance during moderate- to high-intensity exercises (~75% VO_2peak, ~1 h duration). Carter *et al.* [3] first studied the effect of CHO mouth rinse on performance after results demonstrated CHO ingestion improved performance during high-intensity exercise and was not accompanied by an increased CHO oxidation [4]. Furthermore, they showed the positive mouthwash effect was eliminated when glucose was infused instead ingested [3]. Together these results indicated that an oral CHO rinse may exert its effects during high-intensity exercise through a central action mediated by receptors in the mouth or GI tract, improving motor drive or motivation. Furthermore, CHO ingestion during high intensity exercise increases the potential incidence of gastrointestinal problems [5,6] and the CHO mouth rinse might be an alternative strategy to reduce any potentially debilitating incidence of gastrointestinal problems in endurance events lasting approximately one hour.

Several studies have reported that CHO mouth rinse improves both cycling [1,2] and running performance [7], and included different protocols to assess performance. For example, Carter *et al.* [2] reported a faster cycling time trial performance covering a set amount of work (914 ± 40 kJ, ~1-h TT), while Rollo *et al.* [7] found that CHO mouth rinse increased the distance covered during a time-based, running time trial, compared to placebo (PLA). Other studies that adopted similar exercise protocols also showed improvements in performance with CHO mouth rinse [1,8]. The mechanism by which

CHO mouth rinse increases performance is not fully understood, but it may involve a group of receptors in the oral cavity with connections to the reward areas in the brain [9].

The activation of reward areas in the brain, such as the insula/frontal operculum, orbitofrontal cortex and striatum was suggested to lower perception of exertion during the exercise [1,10], and possibly reduce the feeling of displeasure [11]. However, some evidence suggests that the magnitude of performance improvements with CHO mouth rinse may be dependent on several factors, including duration of fasting [12] and time of mouth rinse [13]. While there is a growing number of publications about the effect of CHO mouth rinse on performance [1,2,8,13,14,15], no systematic review and quantitative measurement of the magnitude of CHO mouth rinse effect on performance has been performed. In order to investigate whether CHO mouth rinse significantly improves performance during high-intensity exercises lasting ~1 h, we conducted a systematic review of the literature coupled with a quantification of the overall mean difference across the studies. The main mechanisms involving the CHO mouth rinse were explored and main bias among the studies was also identified.

2. Methods

A search of all articles up to May 2013, which have investigated the effect of CHO mouth rinse on performance, were examined with no publication date or language limits. The search encompassed the following electronic databases: Pubmed (National Library of Medicine U.S.), SciELO (Scientific Electronic Library Online), Science Direct, LILACS, MEDLINE (International Literature on Health Sciences), and the Cochrane Library (Cochrane Central Register of Controlled Trials). The following search terms were used: carbohydrate combined with mouth and rinse. We used the logical operator "and" to combine the descriptors. Original articles conducted in humans were considered, and any articles that reported CHO intake with no specific mouthwash protocol were excluded.

The systematic review procedures consisted of four steps: (1) to read the titles of the studies; (2) to verify duplication; (3) to read the papers fully; and (4) to check for exclusion criteria carried out by three independent researchers and complete a double check on reference lists. Each study was further categorized referring to authors, year of publication (reference), type of activity/exercise protocol, sample size (n), level of performance, duration of fasting, experimental design, mouth rinse protocol, solutions offered, results, and main conclusions.

In addition, the individual and overall mean differences between PLA and CHO mouth rinse were calculated in seven of the eleven eligible studies that reported power output (W) as the main performance variable. As power output was the most reported outcome, an overall mean difference analysis using mean power output was chosen instead of variables such as time to completion for a given distance or work, time to exhaustion, or distance covered in a given time.

3. Results

Following an initial search of the database, one hundred and thirty-two publications were identified as potentially eligible for inclusion. Twenty-eight articles remained following a titles analysis (step 1). Eliminating duplicity (step 2), and subsequent application of the exclusion criteria (step 4), eight studies were deemed appropriate. However, three more articles were identified from the reference lists

of these studies and considered eligible for inclusion. In the total, eleven articles were eligible for this review. The characteristics and main results from reviewed studies are displayed in Table 1.

Table 1. Summary of the studies investigating the effect of carbohydrate mouth rinse on performance during exercise.

Reference	Type of activity/ exercise protocol	Sample (***n***)	**Fast (h)**	Design	Duration of mouth rinse/beverage concentration (%)	**Number of mouth rinses**	**Distinguish between the solutions** ***	Main results (mean ± SD)	**(% Enhanced Performance)**
Chambers *et al.*[1]	Cycling Time-trial ~1 h (914 ± 29 kJ) ~75% W_{max}	8 M (ET)	6	Double-Blinded	10 s/Glucose (6.4%) *vs.* PLA (saccharin + aspartame in water: 150 mL/1000 mL)	8	0	Time (min) 60.4 ± 3.7 *vs.* 61.6 ± 3.8	Yes, 1.99%
Chambers *et al.*[1]	Cycling Time-trial ~1 h (914 ± 29 kJ) ~75% W_{max}	6 M and 2 W (ET)	6	Double-Blinded	10 s/MALT (6.4%) + saccharin and aspartame *vs.* PLA (saccharin and aspartame in water: 150 mL/1000 mL)	8	0	Time (min) 62.6 ± 4.7 vs. 64.6 ± 4.9	Yes, 3.19%
Carter *et al.*[2]	Cycling Time-trial ~1 h (~914 ± 40 kJ) ~75% Wmax	7 M and 2 W (ET)	4	Blinded	5 s/MALT (6.4%) *vs.* Water	8	4 (4)	Time (min) 59.6 ± 0.5 vs. 61.4 ± 0.5	Yes, 3.02%
Rollo *et al.*[7]	Running Time-trial 30 min ~60% VO2max	10 * (ET)	Overnight fast	Double-Blinded	5 s/CHO (6%) *vs.* PLA	9	2 (**)	Distance (m) 6584 ± 520 *vs.* 6469 ± 515	Yes, 1.78%
Pottier *et al.*[8]	Cycling Time-trial ~1 h (975 ± 85 kJ) ~75% W_{max}	12 * (ET)	3	Double-Blinded	5 s/CHO-E (6%) *vs.* PLA	8	**	Time (min) 61.7 ± 5.1 *vs.* 64.1 ± 6.5	Yes, 3.89%
Pottier *et al.*[8]	Cycling Time-trial ~1 h (975 ± 85 kJ) ~75% W_{max}	12 * (ET)	3	Double-Blinded	Ingestion CHO-E (6%) *vs.* PLA	8	**	Time (min) 63.2 ± 6.9 *vs.* 62.5 ± 6.9	No, −1.11%
Beelen *et al.*[12]	Cycling Time-trial ~1 h (1.053 ± 48 kJ) ~75% W_{max}	14 M (ET)	2	Double-Blinded	5 s/MALT (6.4%) *vs.* Water	8	5 (4)	Time (min) 68.1 ± 0.3 *vs.* 67.5 ± 0.3	No, −0.91%
Sinclair *et al.*[13]	Cycling time trial 30-min	11 M	4	Blinded	5 s/MALT (6.4%) *vs.* Water	5	11 (5)	Power Output (W) 153 ± 17 *vs.* 146 ± 13	Yes, 4.34%
Sinclair *et al.*[13]	Cycling time trial 30-min	11 M	4	Blinded	10 s/MALT (6.4%) *vs.* Water	5	11 (6)	Power Output (W) 156 ± 17 *vs.* 146 ± 13	Yes, 6.36%
Fares and Kayser[14]	Cycling ~60% W_{max} until exhaustion	13 M(NA)	3	Blinded	5–10 s/CHOFS (6.4%) *vs.* PLAFS (water)	12	8 (4)	Time (min) 56.6 ± 12.2 *vs.* 54.7 ± 11.3	Yes, 3.47%

Table 1. *Cont.*

Reference	Type of activity/ exercise protocol	Sample (***n***)	**Fast (h)**	Design	Duration of mouth rinse/beverage concentration (%)	**Number of mouth rinses**	**Distinguish between the solutions** ***	Main results (mean ± SD)	**(% Enhanced Performance)**
Fares and Kayser[14]	Cycling ~60% W_{max} until exhaustion	13 M (NA)	Overnight fast	Blinded	5-10 s/FCHO (6.4%) *vs*. FPLA (water)	12	7 (4)	Time (min) 53.9 ± 12.8 *vs*. 48.3 ± 15.3	Yes, 11.59%
Rollo *et al*.[15]	Running Time-trial ~1 h ~60% VO_{2max}	10 M (ET)	~14	Double-Blinded	5 s/CHO-E (6.4%, mouth rinse without intake) *vs*. PLA (mouth rinse + intake)	4	**	Distance (m) 14283 ± 758 *vs*. 14190 ± 800	No, 0.65%
Rollo *et al*.[15]	Running Time-trial ~1 h ~60% VO_{2max}	10 M (ET)	~14	Double-Blinded	5 s/CHO-E (6.4%, mouth rinse + intake) *vs*. PLA (mouth rinse + intake)	4	**	Distance (m) 14515 ± 756 *vs*. 14190 ± 800	Yes, 2.29%
Whitham and Mckinney[16]	Running Time-trial 45 min (1.053 ± 48 kJ) ~65% VO_{2max}	7 M (RA)	4	Double-Blinded	5 s/ MALT (6% maltodextrin-97% polysaccharide, 2% disaccharide, 1% glucose + 3% lemon juice) *vs*. PLA (3% lemon juice)	10	1 (1)	Distance (m) 9333 ± 988 *vs*. 9309 ± 993	No, 0.26%
Rollo *et al*.[17]	Running Time-trial ~1 h ~60% VO_{2max}	20 M (ET)	~14	Double-Blinded	5 s/CHO-E (6.4%) *vs*. PLA	4	0	Distance (m) 14298 ± 685 *vs*. 14086 ± 732	Yes, 1.50%
Lane *et al*.[18]	Cycling Time-trial ~1 h	12 M	Overnight fast	Double-Blinded	10 s/MALTFS (10%) vs. PLAFS (water)	9	** (3)	Power output (W) 286 ± 6 *vs*. 285 ± 1	Yes, 1.8%
Lane *et al*.[18]	Cycling Time-trial ~1 h	12 M	Overnight fast	Double-Blinded	10 s/FMALT (10%) *vs*. FPLA (water)	9	** (3)	Power output (W) 282 ± 6 *vs*. 273 ± 6	Yes, 3.4%

* No gender specification; ** Not reported; *** Number of distinguishing (number of correct distinguishing is given in parentheses). M—men; W—women; ET—endurance trained; RA—recreationally active; NA—nonathletic; CHO-E—electrolyte solution at carbohydrate; GLU—glucose; MALT—maltodextrin; PLA—placebo; FCHO—carbohydrate rinse in fasted state; FPLA—placebo in fasted state; CHOFS—carbohydrate rinse in fed state; PLAFS—placebo in fed state; MALTFS—maltodextrine rinse in fed state; FMALT—maltodextrine rinse in fast state.

3.1. Type of Activity/Exercise Protocol

The most common exercise protocols used in the studies were either cycle time trial with fixed total work (~1 h duration, intensity ~75% VO_2max) or running time trial (~1 h duration, intensity between 60% and 65% VO_2max). One study performed time to exhaustion test to measure performance. Eight articles were double-blinded, while three studies were only single blinded.

3.2. Sample

The sample size (n) in any one study ranged from seven to sixteen individuals, totaling one hundred and thirty-four volunteers. Seven studies involved only males, two both genders, and two did not specify participant gender. A majority of the studies involved endurance trained volunteers, except in two studies in which participants were either moderately trained or untrained. The duration of fasting prior to the testing ranged between two and 15 h.

3.3. Mouth Rinse Protocols

There was a large variation in mouth rinse protocols between the studies, including: (1) duration of mouth rinse (5 or 10 seconds); (2) mouth rinse repetitions during the performance trial (4 to 12 times); and (3) solution (maltodextrin, lemon juice, glucose, artificial sweeteners, and saccharin). In addition, CHO solution was either mouth rinsed and expectorated (n = 10) or subsequently ingested (n = 1). In two studies, the volunteers were not able to distinguish the CHO mouth rinse solution. In addition, the volunteers noticed differences between CHO and PLA solutions in seven studies but only in two cases were able to distinguish correctly. Two studies did not report solution differentiation assessment.

3.4. Performance

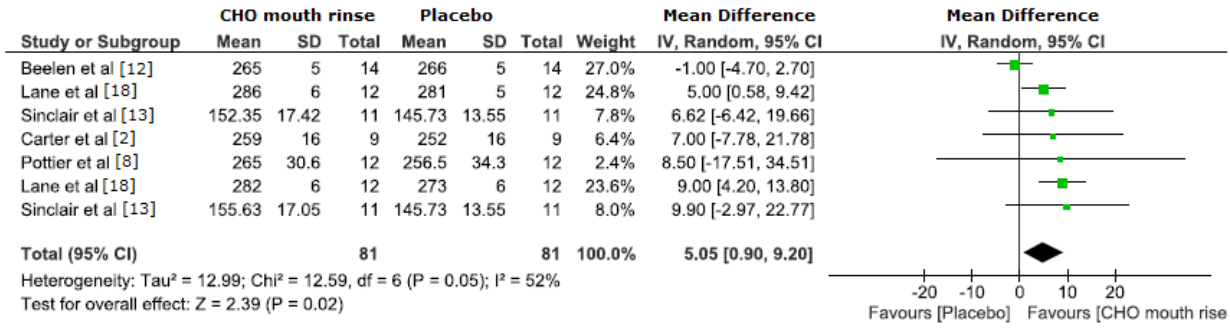
Eight of the eleven eligible studies found an improvement in exercise performance (decreased time to complete the time-trial, increased running distance, or increased time to exhaustion) with CHO mouth rinse (glucose or maltodextrin) (Table 1). However, one study reported that CHO mouth rinse influenced performance only when followed by ingestion [15], while another one found that mouth rinse, but not ingestion of CHO solution, had an effect on performance [8]. Three studies found no effect of CHO mouth rinse on performance [12,15,16]. Two of these studies used running to access performance [15,16]. Power output was the most reported outcome (seven studies), so an overall mean difference analysis using mean power output was performed. The overall effect of CHO mouth rinse on performance was significant with a mean difference of 5.05 W (95% CI 0.90 to 9.2 W, z = 2.39, p = 0.02). However, there was a large intra and inter study variability observed (I^2 = 52%), as displayed in Figure 1.

3.5. Rating of Perceived Effort

The rating of perceived exertion (RPE) did not differ between CHO and PLA mouth rinse in eight studies, while two studies did not report the RPE, and one study reported reduction in RPE with CHO mouth rinse. The brain areas activated by the CHO mouth rinse (glucose and maltodextrin) were

investigated in only one study, and it was found that brain regions associated with reward, including insula/frontal operculum, orbitofrontal cortex, and striatum were significantly activated [1].

Figure 1. The overall effect of CHO mouth rinse on power output (W) as the main performance outcome.



4. Discussion

The present review identified eleven studies investigating the influence of CHO mouth rinse on endurance exercise performance. A majority of these studies reported improved performance with either glucose or maltodextrin mouth rinses [1,2,7,8,13,14,17,18]. The pioneering study investigating CHO mouth rinse was conducted by Carter *et al*. [2], who found an improvement in the exercise performance when CHO was subsequently expelled and not ingested. The ergogenic effect derived from CHO mouth rinse does not seem to be a result of its absorption, as it has been reported that CHO mouth rinse is not associated with changes in blood glucose concentration [7,15,17].

Two studies did not find a beneficial effect of CHO mouth rinse on performance [12,16], while in one study the effect of mouth rinse was apparent when followed by ingestion [15]. It is noteworthy that participants in the first two studies [12,16] performed the exercise in a postprandial state. It has been suggested that the prior fasting period may be required for a positive effect of mouth rinse, suggesting that potential benefit of CHO mouth rinse is, at least partially, dependent on endogenous CHO (liver and muscle glycogen) stores [12]. Lane and colleagues [18] concluded that a CHO mouth rinse improves performance to a greater extent in a fasted compared with a fed state. Another study [14] found that CHO mouth rinse improved time to exhaustion in both pre (overnight fast) and postprandial (3 h after meal) states. However, individuals performed the trial listening to music in that study which may have masked the influence of fasting, as it has been shown that listening to music, *per se*, can improve performance [19]. Otherwise, other studies having participants fasting 4 h or less [2,8,13,14,18] found a beneficial effect of CHO mouth rinse on performance, suggesting that other factors than duration of fasting may have influenced the absence of CHO mouth rinse effect in some studies [12,16].

It has also been demonstrated that the sweetness of CHO does not influence the level of activation of the oral receptors. Glucose is a simple CHO with a sweet taste while maltodextrin is a complex CHO and tasteless. However, Chambers [1] revealed that both glucose and maltodextrin increased similarly the performance and insula/frontal operculum, orbitofrontal cortex, and striatum activation [1]. This indicates there may be a class of unidentified oral receptors that responds to CHO content of the solution independently of sweetness. Interestingly, these brain areas are associated with reward, which

probably leads to an increase in the exercise intensity mediated by a reduction in the perceived exertion and an increased pleasure.

The RPE was not different between PLA and CHO mouth rinses in eight studies and indicates that participants were able to produce more power for a given RPE in the CHO mouth rinse condition. Similarly, in the only study investigating the CHO mouth rinse on a constant-load exercise [14], RPE was reduced in CHO mouth rinse compared to PLA condition. The pathway by which reward areas in the brain are activated remains to be clarified further, but it seems to be plausible that rinsing the mouth with a CHO solution activates the chemoreceptors on the tongue and oral cavity, exciting first-order neurons that carry information to the Rostral Nucleus of the Solitary Tract (rNTS) [9]. The rNTS probably acts on the ventral posterior medial nucleus of the thalamus (VPMpc) neurons projecting to the insular cortex. The insular cortex could stimulate the motor cortex excitability, reducing RPE and influence any motor neural feedback to increase the power output during the exercise [9]. Additionally, the CHO mouth rinse may induce an increased pleasure via the lamina I spinothalamocortical system, which seems to influence interception and modification of neural feedback involved with emotion and motivation [20].

We also found that six of the seven articles reporting power output as the main outcome had a positive main effect favoring CHO mouth rinse (Figure 1). However, the calculated 95% CI was large and overlapped zero in four of these six studies. Similarly, even with an overall mean difference significantly favoring CHO mouth rinse, there was a large intra and inter study variability of CHO mouth rinse effect on power output. The large variability in these studies suggests that methodological factors should be considered and better controlled/reported, including: (1) duration of fasting; (2) duration and number of mouth rinse; (3) solution concentration; (4) type of activity and exercise protocol; (5) sample size; (6) intervenient factors (e.g., listening to music and muscle and liver glycogen levels before the trial). In particular, there is evidence pointing that a 10-s mouth rinse may be better than a 5-s mouth rinse on performance, suggesting a dose response to the duration of mouth rinse [13]. In addition, we observed a large range in the number of mouth rinses during the performance trial between the studies (from four to 12 times), but no study investigated if a higher number and/or a shorter interval between CHO mouth rinses would result in an improved performance. Therefore more studies using standardized protocols and larger sample sizes are necessary to ascertain both the effect of CHO mouth rinse on performance and its mechanism of action. Further studies should also investigate different forms of "placebo", e.g., water *versus* no water *versus* artificially flavored fluids. Recent evidence indicates that repeated mouth rinsing with water results in decreased performance relative to not rinsing at all, suppressing partially the CHO mouth rinse effect [21].

5. Conclusions

CHO mouth rinse seems to improve performance during moderate- to high-intensity exercise (~60% to 75% VO_2max), of at least 1 h duration. It is probable that the mechanism involved in this improvement may not be metabolic but neural, via oral CHO receptors (glucose and maltodextrin) that activate brain regions related to the sensation of reward and pleasure. These receptors appear to be especially responsive in metabolic conditions of reduced endogenous CHO stores (muscle and liver glycogen), but further investigation is required. The CHO mouth rinse might be an alternative to the intake, avoiding any potentially performance debilitating incidence of gastrointestinal problems when

CHO is ingested during high-intensity exercise or during competitions lasting ~1 h. Thus, athletes with historically problematic CHO induced GI issues may be benefited for CHO mouth rinse. However, the precise identification of the oropharyngeal receptors, the mechanism of activation of the brain regions, as well as more standardized and controlled protocols are necessary to clarify the mechanism and magnitude in which the CHO mouth rinse promotes improvement in the performance.

It is prudent to also point out that spitting out CHO/fluid replacement drink may compromise energy substrate supply, hydration and blood glucose maintenance and jeopardize performance during events lasting longer than 1 h. Therefore, further investigation for performance over longer duration is required.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Chambers, E.S.; Bridge, M.W.; Jones, D.A. Carbohydrate sensing in the human mouth: Effects on exercise performance and brain activity. *J. Physiol.* **2009**, *587*, 1779–1794.
2. Carter, J.M.; Jeukendrup, A.E.; Jones, D.A. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med. Sci. Sports Exerc.* **2004**, *36*, 2107–2111.
3. Carter, J.M.; Jeukendrup, A.E.; Mann, C.H.; Jones, D.A. The effect of glucose infusion on glucose kinetics during a 1-h time trial. *Med. Sci. Sports Exerc.* **2004**, *36*, 1543–1550.
4. Jeukendrup, A.; Brouns, F.; Wagenmakers, A.J.; Saris, W.H. Carbohydrate-electrolyte feedings improve 1 h time trial cycling performance. *Int. J. Sports Med.* **1997**, *18*, 125–129.
5. Peters, H.P.; van Schelven, F.W.; Verstappen, P.A.; de Boer, R.W.; Bol, E.; Erich, W.B.; van der Togt, C.R.; de Vries, W.R. Gastrointestinal problems as a function of carbohydrate supplements and mode of exercise. *Med. Sci. Sports Exerc.* **1993**, *25*, 1211–1224.
6. Peters, H.P.; Wiersma, J.W.; Koerselman, J.; Akkermans, L.M.; Bol, E.; Mosterd, W.L.; de Vries, W.R. The effect of a sports drink on gastroesophageal reflux during a run-bike-run test. *Int. J. Sports Med.* **2000**, *21*, 65–70.
7. Rollo, I.; Williams, C.; Gant, N.; Nute, M. The influence of carbohydrate mouth rinse on self-selected speeds during a 30-min treadmill run. *Int. J. Sport Nutr. Exerc. Metab.* **2008**, *18*, 585–600.
8. Pottier, A.; Bouckaert, J.; Gilis, W.; Roels, T.; Derave, W. Mouth rinse but not ingestion of a carbohydrate solution improves 1-h cycle time trial performance. *Scand. J. Med. Sci. Sports* **2010**, *20*, 105–111.
9. Gant, N.; Stinear, C.M.; Byblow, W.D. Carbohydrate in the mouth immediately facilitates motor output. *Brain Res.* **2010**, *1350*, 151–158.
10. Backhouse, S.H.; Ali, A.; Biddle, S.J.; Williams, C. Carbohydrate ingestion during prolonged high-intensity intermittent exercise: Impact on affect and perceived exertion. *Scand. J. Med. Sci. Sports* **2007**, *17*, 605–610.
11. Backhouse, S.H.; Bishop, N.C.; Biddle, S.J.; Williams, C. Effect of carbohydrate and prolonged exercise on affect and perceived exertion. *Med. Sci. Sports Exerc.* **2005**, *37*, 1768–1773.

12. Beelen, M.; Berghuis, J.; Bonaparte, B.; Ballak, S.B.; Jeukendrup, A.E.; van Loon, L.J. Carbohydrate mouth rinsing in the fed state: Lack of enhancement of time-trial performance. *Int. J. Sport Nutr. Exerc. Metab.* **2009**, *19*, 400–409.
13. Sinclair, J.; Bottoms, L.; Flynn, C.; Bradley, E.; Alexander, G.; McCullagh, S.; Finn, T.; Hurst, H.T. The effect of different durations of carbohydrate mouth rinse on cycling performance. *Eur. J. Sport Sci.* **2013**, Epub ahead of print, doi:10.1080/17461391.2013.785599.
14. Fares, E.J.; Kayser, B. Carbohydrate mouth rinse effects on exercise capacity in pre- and postprandial States. *J. Nutr. Metab.* **2011**, *2011*, 385962.
15. Rollo, I.; Williams, C.; Nevill, M. Influence of ingesting versus mouth rinsing a carbohydrate solution during a 1-h run. *Med. Sci. Sports Exerc.* **2011**, *43*, 468–475.
16. Whitham, M.; McKinney, J. Effect of a carbohydrate mouthwash on running time-trial performance. *J. Sports Sci.* **2007**, *25*, 1385–1392.
17. Rollo, I.; Cole, M.; Miller, R.; Williams, C. Influence of mouth rinsing a carbohydrate solution on 1-h running performance. *Med. Sci. Sports Exerc.* **2010**, *42*, 798–804.
18. Lane, S.C.; Bird, S.R.; Burke, L.M.; Hawley, J.A. Effect of a carbohydrate mouth rinse on simulated cycling time-trial performance commenced in a fed or fasted state. *Appl. Physiol. Nutr. Metab.* **2013**, *38*, 134–139.
19. Lima-Silva, A.E.; Silva-Cavalcante, M.D.; Pires, F.O.; Bertuzzi, R.; Oliveira, R.S.; Bishop, D. Listening to music in the first, but not the last 1.5 km of a 5-km running trial alters pacing strategy and improves performance. *Int. J. Sports Med.* **2012**, *33*, 813–818.
20. Craig, A.D. How do you feel? Interoception: The sense of the physiological condition of the body. *Nat. Rev. Neurosci.* **2002**, *3*, 655–666.
21. Gam, S.; Guelfi, K.J.; Fournier, P.A. Opposition of carbohydrate in a mouth-rinse solution to the detrimental effect of mouth rinsing during cycling time trials. *Int. J. Sport Nutr. Exerc. Metab.* **2013**, *23*, 48–56.

APÊNDICE B – Termo de Consentimento Livre e Esclarecido (TCLE).

UNIVERSIDADE FEDERAL DE PERNAMBUCO

PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO

Termo de Consentimento Livre e Esclarecido (T.C.L.E.)

Convido o (a) Sr.(a) para participar, como voluntário (a), da pesquisa ("*EFEITO DO BOCHECHO DE CARBOIDRATO SOBRE AS RESPOSTAS PRÓ-INFLAMATÓRIAS, METABÓLICAS AO EXERCÍCIO E A ATIVIDADE CEREBRAL APÓS DIFERENTES TEMPOS DE JEJUM"*). Estudo 1: Efeito do bochecho de carboidrato sobre as respostas pró-inflamatórias, metabólicas ao exercício após diferentes tempos de jejum.

Após ser esclarecido (a) sobre as informações a seguir, no caso de aceitar fazer parte do estudo, assine ao final deste documento, que está em duas vias. Uma delas é sua e a outra é do pesquisador responsável. Em caso de recusa você não será penalizado (a) de forma alguma. Em caso de dúvida você pode procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da UFPE no endereço: (Avenida da Engenharia s/n – 1º Andar, Sla 4 - Cidade Universitária, Recife-PE, CEP: 50740- 600, Tel.: 2126.8588 – e-mail: cepccs@ufpe.br).

INFORMAÇÕES SOBRE A PESQUISA:

Título do Projeto: Efeito do bochecho de carboidrato sobre as respostas pró-inflamatórias, metabólicas ao exercício e a atividade cerebral após diferentes tempos de jejum.

Pesquisador Responsável: Thays de Ataide e Silva.

Endereço/Telefone/e-mail para contato (inclusive ligações a cobrar): Programa de Pós-Graduação em Nutrição. Departamento de Nutrição. Universidade Federal de Pernambuco. Av. Prof. Moraes Rego, 1235 – Cidade Universitária, Recife – PE. CEP: 50670-901. (81) 21268463 / (81) 85616313 / thays_de_ataide@hotmail.com.

Pesquisadores participantes: Carol Virgínia Góis Leandro; Adriano Eduardo Lima e Silva.

Telefones para contato: (81) 35233351

- Que o estudo se destina a entender como o bochecho de carboidrato e o tempo de jejum melhoram o desempenho no exercício físico;
- Que a importância deste estudo é investigar os efeitos do bochecho de CHO em diferentes tempos de jejum (2 e 12 horas), sobre o desempenho em uma prova de ciclismo contrarrelógio de 1 hora.

- Que esse estudo começará em Janeiro de 2013, com previsão de termino para Julho de 2013, mas eu estou ciente que a minha participação resume-se a oito visitas ao laboratório.
- Que o estudo será feito da seguinte maneira: Eu deverei visitar o laboratório de Aptidão Física, Desempenho e Saúde quatorze vezes. Na primeira visita eu irei realizar um teste onde os pesquisadores aumentam a intensidade do esforço a cada três minutos até eu não conseguir mais pedalar ou eu pedir para interromper o teste. Esse teste tem duração aproximada de 30 minutos. Na segunda visita será realizado um teste de familiarização para que eu conheça os procedimentos que serão adotados durante as próximas visitas. Nas visitas 3, 6, 9, 12 eu realizarei testes onde eu poderei variar a intensidade do esforço até o final. Esses testes tem duração de 60 minutos cada um. Eu compreendo que realizarei todos os testes no período da manhã, onde receberei duas soluções distintas para bochechar: 1) maltodextrina (64 g/1000mL de água destilada), sem sabor e sem cheiro, sendo portanto imperceptível e com improvável distinção e; 2) placebo (água destilada). Porém não serei informado qual solução estou recebendo em cada momento durante o estudo. Os procedimentos para os diferentes tempos de jejum se darão da seguinte forma: 1) Para jejum de 2 horas, eu deverei realizar a última refeição às 6 horas da manhã, mantendo-me em jejum até o experimento às 8 horas; 2) Para o jejum de 12 horas, iniciarei o jejum às 20 horas do dia anterior e realizarei o teste às 8 horas do dia seguinte. Eu fui informado que o teste poderá ser interrompido por decisão dos pesquisadores ou caso eu me sinta cansado e indisposto. Durante o teste serei submetido ao exame de eletromiografia de superfície, onde eu terei dois eletrodos fixados na superfície da pele da minha perna. Nas visitas 4, 5, 7, 8, 10 e 11, irei ao laboratório para coleta de 5 mL de sangue venoso.
- Que existe a possibilidade da utilização de placebo (sem substância) durante os testes.
- Que não existem outros meios conhecidos para se obter os mesmos resultados.
- Que os incômodos que poderei sentir com a minha participação são os seguintes: tonturas e mal estar.
- Que os possíveis riscos à minha saúde física e mental são: tonturas e mal estar que possam ocorrer durante o teste.
- Que deverei contar com a seguinte assistência: caso eu tenha algum problema, eu serei transportado de ambulância pública ou de automóvel particular para o hospital publico mais próximo da Universidade, sendo responsável pelo transporte a doutoranda Thays de Aatide e Silva e/ou o professor Dr. Adriano Eduardo Lima da Silva e/ou professora Dr.ª Carol Virgínia de Góis Leandro.
- Que os benefícios que deverei esperar com a minha participação, mesmo que não diretamente são: eu terei acesso a qualquer resultado referente ao meu teste e que poderei, a qualquer momento, esclarecer minhas dúvidas com o pesquisador responsável.

- Que a minha participação será acompanhada do seguinte modo: O professor responsável irá realizar o meu teste e eu estarei sendo monitorado por um monitor cardíaco.
- Que, sempre que desejar serão fornecidos esclarecimentos sobre cada uma das etapas do estudo.
- Que, a qualquer momento, eu poderei recusar a continuar participando do estudo e, também, que eu poderei retirar este meu consentimento, sem que isso me traga qualquer penalidade ou prejuízo.
- Que as informações conseguidas através da minha participação não permitirão a identificação da minha pessoa, exceto aos responsáveis pelo estudo, e que a divulgação das mencionadas informações só será feita entre os profissionais estudiosos do assunto.
- Nome e Assinatura do pesquisador _____

CONSENTIMENTO DA PARTICIPAÇÃO DA PESSOA COMO SUJEITO

Eu, _____, RG/ CPF/_____, abaixo assinado, concordo em participar do estudo _____, como sujeito. Fui devidamente informado(a) e esclarecido(a) pelo(a) pesquisador(a) _____ sobre a pesquisa, os procedimentos nela envolvidos, assim como os possíveis riscos e benefícios decorrentes de minha participação. Foi-me garantido que posso retirar meu consentimento a qualquer momento, sem que isto leve a qualquer penalidade ou interrupção de meu acompanhamento/ assistência/tratamento.

Local e data _____

Nome e Assinatura do sujeito: _____

Presenciamos a solicitação de consentimento, esclarecimentos sobre a pesquisa e aceite do sujeito em participar.

02 testemunhas (não ligadas à equipe de pesquisadores):

Nome: _____

Assinatura: _____

Nome: _____

Assinatura: _____

APÊNDICE C – Lista de alimentos que contêm cafeína.

UNIVERSIDADE FEDERAL DE PERNAMBUCO – UFPE
CENTRO ACADÊMICO DE VITÓRIA
GRUPO DE PESQUISA EM CIÊNCIA DO ESPORTE

LISTA DE ALIMENTOS/BEBIDAS QUE CONTÊM CAFEÍNA

Por favor, não consumir 24 horas antes de cada teste

CAFÉ

- Em pó
- Orgânico
- Espresso
- Instantâneo
- Descafeinado
- Extra-Forte
- Capuccino

CHÁ

- Chá verde
- Chá preto
- Chá Mate

CACAU

- Industrializado
- Cacau 50%
- Cacau 70%
- Cacau 80%

CHOCOLATE

- Chocolate Amargo
- Chocolate ao Leite
- Chocolate branco

REFRIGERANTES

- Coca Cola
- Coca Cola Zero
- Guaraná Antarctica
- Guaraná Zero
- Pepsi Cola
- Diet Pepsi
- Guaraná Black

MEDICAMENTOS*

- Excedrin
- Torsilax
- Sedalgina
- Neosaldina
- Tandrilax
- Benegrip
- Coristina D
- Engov
- Tylenol

BEBIDAS ENERGÉTICAS

- Redbull
- Burn
- Flying Horse
- Monster Energy Drink
- TNT

Fonte: Lista baseada no "*Caffeine Consumption Questionnaire*"

ANEXO A **–** Questionário para excluir indivíduos com riscos cardiovasculares (PAR-q).

NOME:.....

PAR-Q

A prática regular da atividade física é prazerosa e saudável. A cada dia que passa, torna-se maior o número de pessoas que se tornam, fisicamente mais ativas. Tornar-se fisicamente mais ativo é seguro para a grande maioria das pessoas, entretanto, algumas pessoas necessitam de exames médicos antes de submeter-se a esforço físico maior do que aquele ao qual está acostumado. Dessa forma, se você está planejando tornar-se, fisicamente, mais ativo do que é hoje, comece por responder as sete questões abaixo.

Se você tem idade entre 15 e 69 anos, este questionário (PAR-Q) lhe dirá da necessidade de se submeter a uma consulta médica antes de se engajar em um programa de atividade física. Se você tem mais de 69 anos de idade e não é acostumado a fazer atividades físicas procure seu médico antes de iniciar.

O bom senso é o seu melhor guia quando você for responder estas questões. Por favor, leia com atenção cada uma das questões e responda honestamente a cada uma delas, preenchendo com um "X" a lacuna do SIM ou do NÃO.

1. Alguma vez um médico lhe disse que você possui um problema de coração e recomendou que só fizesse atividade física sob supervisão médica?

SIM NÃO

2. Você sente dor no peito quando pratica atividade física?

SIM NÃO

3. Você sentiu dor no peito, sem fazer esforço, no último mês?

SIM NÃO

4. Você tende a perder a consciência ou cair, como resultado de tonteira?

SIM NÃO

5. Você tem algum problema ósseo, muscular ou articular que poderia ser agravado com a prática de atividade física?

SIM NÃO

6. Algum médico já recomendou o uso de medicamentos para a sua pressão arterial ou condição cardiovascular (ex: diuréticos ou outros)?

SIM NÃO

7. Você tem consciência, através da sua própria experiência ou aconselhamento médico, de alguma outra razão física que impeça lhe impeça de praticar atividade física sem supervisão médica?

SIM NÃO

Se você respondeu afirmativamente a uma ou mais questões acima, entre em contato com seu médico antes de iniciar a se tornar mais ativo fisicamente. Fale com seu médico do PAR-Q e de qual questões você respondeu afirmativamente. As seguintes situações poderão ocorrer:

Observações:

1. **Este questionário só deve ser aplicado para aqueles com idades compreendidas entre 15 e 69 anos.**

2. **Se você está temporariamente doente, como por exemplo: gripado ou com febre, ou não está se sentindo bem neste momento, você deve adiar o início da prática da atividade física.**

3. **Se você é mulher e está grávida, aconselha-se a discutir o uso do "PAR-Q" com seu médico, antes de iniciar um programa de exercícios.**

4. **Se houver alguma mudança em seu estado, relativo às questões acima, por favor, traga esta informação ao conhecimento do seu professor/treinador.**

ANEXO B - Parecer consubstanciado do Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal de Pernambuco (UFPE).

UNIVERSIDADE FEDERAL DE PERNAMBUCO CENTRO DE CIÊNCIAS DA SAÚDE / UFPE-

Plataforma Brasil

PROJETO DE PESQUISA

Título: EFEITO DO BOCHECHO DE CARBOIDRATO SOBRE AS RESPOSTAS PRÓ-INFLAMATÓRIAS, METABÓLICAS AO EXERCÍCIO E A ATIVIDADE CEREBRAL APÓS DIFERENTES TEMPOS DE JEJUM

Área Temática:

Área 8. Pesquisa com cooperação estrangeira.

Versão: 2

CAAE: 06610112.5.0000.5208

Pesquisador: Thays de Atalde e Silva

Instituição: CENTRO DE CIÊNCIAS DA SAÚDE

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 103.919

Data da Relatoria: 18/09/2012

Apresentação do Projeto:

Trata-se de um estudo que será realizado em duas etapas:
Etapa 1: realizada no Laboratório em Vitória de Santo Antão
Etapa 2: realizada na Universidade de Victoria, onde as coletas do estudo 2 serão realizados durante o período do doutorado sanduíche previsto para 2014.
O estudo efantiza que o bochecho de carboidratos (CHO) tem efeitos positivos sobre o desempenho durante exercícios de curta duração e alta intensidade através de uma ação central, mediada por sinais aferentes provenientes de receptores na boca. Esses sinais podem ativar algumas áreas do cérebro relacionadas à sensação de recompensa e controle motor e causar alterações fisio-metabólicas, melhorando o desempenho físico. Seguindo essa temática, o presente projeto se propõe a verificar: (1) o efeito do bochecho de CHO após diferentes tempos de jejum (2 e 12 horas) sobre a resposta pró-inflamatória, metabólica e melhora no desempenho e; (2) se o tempo de jejum (2 e 12 horas) influencia nas áreas e na intensidade de ativação cerebral após o bochecho de CHO. A amostra será composta por 20 ciclistas, dos quais 10 farão parte do estudo 1 e os outros 10 do estudo 2. O desenho experimental do estudo 1 constituirá de oito visitas ao laboratório, sendo a primeira para realização do teste incremental, a segunda para uma sessão de familiarização e as visitas 3 e 6 para realização do teste experimental (contra-relógio) após 2 e 12 horas de jejum, respectivamente,em qual os sujeitos realizarão bochecho com soluções contendo maltodextrina (MALT) insípida ou água (PLA). Nas visitas 9 e 12 serão realizados os mesmos procedimentos da visita anterior, alterando apenas a solução fornecida aos sujeitos. A cada 12,5% da distância percorrida, serão verificadas a percepção subjetiva ao esforço, a escala de afetividade, a escala de pensamentos associativos e dissociativos, a frequência cardíaca, a potência gerada, atividade eletromiográfica, o tempo de esforço, o VO2, VCO2 e a razão de troca respiratória (RER), este último visando estimar o metabolismo de lipídios e carboidratos. Uma hora antes do teste (repouso), e nas visitas 4, 7, 10 e 13 serão coletados 10mL de sangue para análise da IL-1,IL-6, TNF e creatina cinse e nas visitas 5, 8, 11e 14 serão coletados 10mL de sangue para análise da IL-10 e creatina cinse. No desenho experimental do estudo 2, os sujeitos visitarão o laboratório em três ocasiões até a conclusão do estudo. Nas visitas 1 e 2 os indivíduos serão submetidos a um jejum de 2 e 12 horas, respectivamente, e receberão soluções para bochecho contendo maltodextrina (MALT) ou água (PLA). Posteriormente, a solução inversa a qual foi oferecida será bochechada. Durante o bochecho, os sujeitos serão submetidos ao exame de ressonância magnética funcional.

Objetivo da Pesquisa:

Objetivo Primário:
Realizar dois estudos, investigando: (1) o efeito do bochecho de CHO após diferentes tempos de

Endereço: Av. da Engenharia s/nº - 1º andar, sala 4, Prédio do CCS
Bairro: Cidade Universitária **CEP:** 50.740-600
UF: PE **Município:** RECIFE
Telefone: (81)2126-8588 **Fax:** (81)2126-8588 **E-mail:** cepccs@ufpe.br

jejum (2 e 12 horas) sobre a resposta proinflamatória e desempenho e; (2) se o tempo de jejum (2 e 12 horas)influencia nas áreas e intensidade de ativação cerebral após o bochecho de CHO.
Objetivo Secundário:
- Analisar as concentrações séricas de cortisol, IL-1, IL-6, TNF e creatina cina em hora antes, quatro horas e vinte e quatro horas após e IL-10 e creatina cinase quarenta e oito horas após o exercício sob o bochecho de CHO após diferentes tempos de jejum;
- Avaliar a Atividade Eletromiográfica (EMG) durante a prova entre os diferentes tempos de jejum (2 e 12 horas)e em condição de bochecho;
- Determinar quais áreas cerebrais e em qual intensidade elas são ativadas nos diferentes tempos de jejum (2 e 12 horas) após o bochecho de CHO.

Avaliação dos Riscos e Benefícios:

Riscos:
Estudo 1. O presente estudo poderá provocar aos voluntários tonturas e mal estar durante os testes, bem como o incômodo em responder os questionários específicos. Porém, caso isto ocorra, os mesmos contarão com assistência necessária, ou seja, serão transportados de ambulância pública ou de automóvel particular para o hospital público mais próximo da Universidade, sendo responsá¬vel pelo transporte a pesquisadora Thays de Ataide e Silva e/ou a professora Drª Carol Virginia Góis Leandro e/ou o professor Dr. Adriano Eduardo Lima da Silva. É importante ressaltar que a eletromiografia de superfície não oferece nenhum risco potencial ao indivíduo estudado.
Estudo 2. O estudo 2 prevê o exame de ressonância magnética funcional, é um exame muito importante para auxiliar o diagnóstico de várias doenças. Como não utiliza elementos radioativos, como o raio-X, não é prejudicial à saúde, porém ela pode ter algumas contra indicações ou situações que precisam de cuidados:Alguns tipos de cirurgia recente (nos últimos seis meses); implante metálico (dispositivo intra-uterino DIU,válvula cardíaca, placa, pino, parafuso, stent, clip de aneurisma cerebral, estilhaço metálico no corpo, piercing,prótese metálica, aparelho ortodôntico); implante eletrônico (marca-passo cardíaco,neuro-estimulador, implante coclear); Suspeita de gravidez; Alergia (devido à sedação, se necessária); Claustrofobia (medo de lugares fechados); Maquiagem definitiva ou tatuagem recente (nos últimos três meses). Portanto, os sujeitos serão questionados quanto às situações acima descritas, antes de qualquer procedimento, visando eliminar riscos de contraindicações ao exame.
Benefícios:
Estudo 1 e 2. A presente proposta se destina a entender como o bochecho de carboidrato e o tempo de jejum melhoram o desempenho no exercício físico, investigando os efeitos do bochecho de CHO em diferentes tempos de jejum (2 e 12 horas). Espera-se que o desenvolvimento deste projeto, proporcione benefícios aos voluntários, que terão acesso a qualquer resultado referente aos seus testes e o esclarecimento a qualquer momento de
dúvidas com o pesquisador responsável. Uma vez que todos os participantes serão atletas, será interessante para eles saber qual estratégia de suplementação melhora a sua performance, uma vez que isso poderá ser utilizado durante uma competição real, melhorando seu desempenho. Além disso, o presente estudo é inédito no que se refere à resposta inflamatória decorrente do exercício em relação ao jejum e o bochecho, roduzindo um conhecimento inovador para à pratica clínica de nutricionista do esporte.

Comentários e Considerações sobre a Pesquisa:

Trata-se de projeto de pesquisa de doutoramento que será desenvolvido no Centro Acadêmico de Vitória de Santo Antão/UFPE em colaboração com o School of Biomedical and Health Sciences ¿ Victoria University,Austrália, onde está previsto o estágio de doutorado sanduíche para 2013. Apresnta Carta referente ao estágio de doutorado sanduíche na School of Biomedical and Health Sciences ¿ Victoria University, Melbourne,Austrália, sob a supervisão do Professor Drª Christos Stathis.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos obrigatórios foram apresentados.

UNIVERSIDADE FEDERAL DE PERNAMBUCO CENTRO DE CIÊNCIAS DA SAÚDE / UFPE-

Recomendações:

Não se aplica.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O colegiado aprova o presente parecer e o pesquisador está autorizado a iniciar a coleta de dados.
A APROVAÇÃO definitiva da pesquisa será efetuada, através de ofício, após a entrega do relatorio final.

RECIFE, 21 de Setembro de 2012

Assinado por:
GERALDO BOSCO LINDOSO COUTO

Endereço: Av. de Engenharia s/nº - 1º andar, sala 4, Prédio do CCS
Bairro: Cidade Universitária **CEP:** 50.740-600
UF: PE **Município:** RECIFE
Telefone: (81)2126-8588 **Fax:** (81)2126-8588 **E-mail:** cepccs@ufpe.br

ANEXO C – Percepção Subjetiva ao Esforço (PSE).

Tabela 1: Percepção Subjetiva ao Esforço (PSE).

6	
7	MUITO FÁCIL
8	
9	FÁCIL
10	
11	RELATIVAMENTE FÁCIL
12	
13	LIGEIRAMENTE CANSATIVO
14	
15	CANSATIVO
16	
17	MUITO CANSATIVO
18	
19	EXAUSTIVO
20	

ANEXO D – Comprovante de submissão artigo científico original.

Zimbra **adrianosilva@usp.br**

MSSE Submission Confirmation

De : Medicine & Science in Sports & Exercise <em@editorialmanager.com> Ter, 29 de Dez de 2015 02:16

Remetente : em msse 0 48261b 93e0155d <em.msse.0.48261b.93e0155d@editorialmanager.com>

Assunto : MSSE Submission Confirmation

Para : Adriano Eduardo Lima-Silva <adrianosilva@usp.br>

Responder para : Medicine & Science in Sports & Exercise <msse@acsm.org>

Dr Lima-Silva,

Your submission, "CHO mouth rinse ameliorates neuromuscular response with lower endogenous CHO stores," has been received by the Medicine & Science in Sports & Exercise Editorial Office.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an Author. Additionally, you may view the Additional Information questions to obtain the copyright information by clicking here: 6. Adriano Eduardo Lima-Silva, PhD

Question: RETAINED RIGHTS: Except for copyright, other proprietary rights related to the Work (e.g., patent or other rights to any process or procedure) shall be retained by the author. To reproduce any text, figures, tables, or illustrations from this Work in future works of their own, the author must obtain written permission from Wolters Kluwer Health, Inc. ("WKH").

ORIGINALITY: Each author warrants that his or her submission to the Work is original, does not infringe upon, violate, or misappropriate any copyright or other intellectual property rights, or any other proprietary right, contract or other right or interest of any third party, and that he or she has full power to enter into this agreement. Neither this Work nor a similar work has been published nor shall be submitted for publication elsewhere while under consideration by this Publication.

AUTHORSHIP RESPONSIBILITY: Each author warrants that he or she has participated sufficiently in the intellectual content, the analysis of data, if applicable, and the writing of the Work to take public responsibility for it. Each has reviewed the final version of the Work, believes it represents valid work, and approves it for publication. Moreover, should the editors of the Publication request the data upon which the work is based, they shall produce it.

PREPRINTS: Upon acceptance of the article for publication, each author warrants that he/she will promptly remove any prior versions of this Work (normally a preprint) that may have been posted to an electronic server.

DISCLAIMER: Each author warrants that this Work contains no libelous or unlawful statements and does not infringe or violate the publicity or privacy rights of any third party, libel or slander any third party, contain any scandalous, obscene, or negligently prepared information, or infringe or violate any other personal or proprietary right of others. Each author warrants that the Work does not contain any fraudulent, plagiarized or incorrectly attributed material. Each author warrants that all statements contained in the Work purporting to be facts are true, and any formula or instruction contained in the

Work will not, if followed accurately, cause any injury, illness, or damage to the user. If excerpts (e.g., text, figures, tables, illustrations, or audio/video files) from copyrighted works are included, a written release will be secured by the author prior to submission, and credit to the original publication will be properly acknowledged. Each author further warrants
that he or she has obtained, prior to submission, written releases from patients whose names or likenesses are submitted as part of the Work. Should the Editor or WKH request copies of such written releases, the author shall provide them in a timely manner.

DISCLOSURES/CONFLICT OF INTEREST
Each author must identify any financial interests or affiliations with institutions,
organizations, or companies relevant to the manuscript by completing the
form below. Additionally, any financial associations involving a spouse, partner or children must be disclosed as well.

Note: Some sections below come from the ICMJE Uniform
Disclosure Form for Potential Conflicts of Interest at http://www.icmje.org/downloads
/coi_disclosure.pdf (dated July 2010).
Response: I agree

Question: Did you or your institution at any time receive payment or support in kind for any aspect of the submitted work (including but not limited to grants, consulting fee or honorarium, support for travel to meetings for the study or other purposes, fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like, payment for writing or reviewing the manuscript, provision of writing assistance, medicines, equipment, or administrative support, etc...)?
Response: No

Question: Other: Did you or your institution at any time receive additional payments or support in kind for any aspect of the submitted work?
Response: No

Question: Please indicate whether you have financial relationships (regardless of amount of compensation) with entities. You should report relationships that were present during the 36 months prior to submission including board membership, consultancy, employment, expert testimony, grants/grants pending, payment for lectures including service on speakers bureaus, payment for manuscript preparation, patents (planned, pending or issued), royalties, payment for development
of educational presentations, stock/stock options, travel/accommodations/meeting expenses unrelated to activities listed (for example, if you report a consultancy above there is no need to report travel related to that consultancy), etc.
Response: No

Question: Other (err on the side of full disclosure): Please indicate whether you have any additional financial relationships (regardless of amount of compensation) with entities. You should report relationships that were present during the 36 months prior to submission.
Response: No

Question: Other Relationships
Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?
Response: No other relationships/conditions/circumstances that present potential conflict of interest

Question: AUTHOR'S OWN WORK: In consideration of WKH and American College of
Sports Medicine's publication of the Work, the author hereby transfers, assigns, and otherwise conveys all his/her copyright ownership worldwide, in all languages, and in all forms of media now or hereafter known, including electronic media such as CD-ROM, Internet, and Intranet, to American College of Sports Medicine. If American College of Sports Medicine should decide for any reason not to publish the Work, American College of Sports Medicine shall give prompt notice of its decision to the corresponding author,

this agreement shall terminate, and neither the author, WKH, nor American College of Sports Medicine shall be under any further liability or obligation. Each author grants WKH and American College of Sports Medicine the rights to use his or her name and biographical data (including professional affiliation) in the Work and in its or the journal's promotion. Notwithstanding the foregoing, this paragraph shall not apply, and any transfer made pursuant to this
paragraph shall be null and void if (i) the Work has been accepted by WKH for publication, and (ii) the author chooses to have the Work published by WKH as an open access publication.

WORK MADE FOR HIRE: If this Work or any element thereof has been commissioned by another person or organization, or if it has been written as part of the duties of an employee, an authorized representative of the commissioning organization or employer must also sign this form stating his or her title in the organization.

GOVERNMENT EMPLOYEES: If the Work or a portion of it has been created in the course of any author's employment by the United States Government, check the "Government" box at the end of this form. A work prepared by a government employee as part of his or her official duties is called a "work of the U.S. Government" and is not subject to copyright. If it is not prepared as part of the employee's official duties, it may be subject to copyright.

INSTITUTIONAL REVIEW BOARD/ANIMAL CARE COMMITTEE
APPROVAL: Each author warrants that his or her institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

WARRANTIES: Each author warranty made in this form is for the benefit of WKH, American College of Sports Medicine, and the Editor; each author agrees to defend, indemnify, and hold
harmless those parties for any breach of such warranties.
Response: I agree

Question: Medicine & Science in Sports & Exercise will permit the author(s) to deposit for display a "final peer-reviewed manuscript" (the final manuscript after peer-review and acceptance for publication but prior to the publisher's copyediting, design, formatting, and other services) 12 months after publication of the final article on the author's personal web site, university's institutional repository or employer's intranet, subject to the following:

* You may only deposit the final peer-reviewed manuscript.

* You may not update the final peer-reviewed manuscript text or replace it with a proof or with the final published version.

* You may not include the final peer-reviewed manuscript or any other version of the article on any commercial site or in any repository owned or operated by any third party. For authors of articles based on research funded by the National Institutes of Health ("NIH"), Welcome Trust, Howard Hughes Medical Institute ("HHMI"), or other funding agency, see below for the services
that WKH will provide on your behalf to comply with "Public Access Policy" guidelines.

* You may not display the final peer-reviewed manuscript until twelve months after publication of the final article.

* You must attach the following notice to the final peer-reviewed manuscript: "This is a non-final version of an article published in final form in (provide complete journal citation)".

* You shall provide a link in the final peer-reviewed manuscript to the Medicine & Science in Sports & Exercise website.
Response: I agree

Question: "Public Access Policy" Funding Disclosure
Please disclose below if you have received funding for research on which your article is based from any of the following organizations:

Response:

Question: Please select:
Response: Author's Own Work

Question: Any additional comments?
Response: No

Question: <u>Compliance with RCUK and Wellcome Trust Open Access Policies</u>
Both the Research Councils UK (RCUK) and the Wellcome Trust have
adopted policies regarding Open Access to articles that have been funded
by grants from the RCUK or the Wellcome Trust. If either "Wellcome
Trust" or "Research Councils UK (RCUK)" has been selected above, and
the authors of the applicable article choose to have the article published
as an open access publication, the following policies will apply:

* If the article is to be published pursuant to the "Gold" route of Open
Access, both the RCUK and the Wellcome Trust require that WKH make
the article freely available immediately pursuant to the Attribution 4.0
Creative Commons License, currently found at <a href=' http://creativecommons.org/licenses/by/4.0
/legalcode' target='_blank'>http://creativecommons.org/licenses/by/4.0/legalcode
(the "CC BY License"). The CC BY License is the most accommodating of the
Creative Commons licenses and allows others to distribute, remix,
tweak, and build upon the article, even commercially, as long as they
credit the authors for the original creation.

* If the article is to be published pursuant to the "Green" route of Open
Access, both the RCUK and the Wellcome Trust require that WKH make
the article freely available within six months pursuant to the Attribution-
NonCommerical 4.0 Creative Commons License, currently found at
<a href=' http://creativecommons.org/licenses/by-nc/4.0/legalcode'
target='_blank'>http://creativecommons.org/licenses/by-nc/4.0/legalcode (the "CC
BY-NC License"). The CC BY-NC License allows others to remix, tweak,
and build upon the article non-commercially, and although their new
works must also acknowledge the authors for the original creation and
be non-commercial, they don't have to license their derivative works on
the same terms.

As a service to our authors, WKH will identify the National Library of Medicine
(NLM) articles that require deposit pursuant to the RCUK and Wellcome Trust
policies described in this section. This Copyright Transfer Agreement provides the
mechanism for identifying such articles.

WKH will transmit the final peer-reviewed manuscript of an article based on
research funded in whole or in part by either RCUK or the Wellcome Trust to Pub
Med Central.

Upon NIH request, it remains the legal responsibility of the author to confirm with
NIH the provenance of his/her manuscript for purposes of deposit. Author will not
deposit articles him/herself. Author will not alter the final peer-reviewed manuscript
already transmitted to NIH.

With respect to the "Green" route of Open Access, author will not authorize the
display of the final peer-reviewed manuscript prior to 6 months following
publication of the final article.

Authors of articles that have been funded from grants from the RCUK or the
Wellcome Trust are required to sign the WKH Open Access License Agreement
prior to publication of the applicable article. Please contact the Editorial Office of
the applicable journal to receive the Open Access License Agreement that is to be
signed in connection with the publication of the article.
Response:

Question: I am the person in question for this submission or otherwise have approval to complete this agreement.
Response: I agree

Question: CME/CE Disclosure

Each author must identify and disclose any financial associations involving a spouse, partner or children by completing the Family Disclosure question below, and whether any off-label uses or
unapproved drugs or devices are discussed in his/her manuscript by completing the Off-Label Use/Unapproved Drugs or Products question below. In the event that the Work is published as a continuing education or continuing
medical education article, this information will be provided to the accrediting body and may be included in the published article. When applicable, articles accepted for
publication may need to comply with additional standards related to CME or CE accreditation. Please refer to guidelines for authors for details.
WKH and its affiliates reserve the right to publish the manuscript as a continuing education article.
Response: I agree

Question: Family Disclosure

Do your children or your spouse or partner have financial relationships with entities that have an interest in the content of the submitted work?

Response: No other relationships/conditions/circumstances that present potential conflict of interest

Question: Off-Label Use/Unapproved Drugs or Products

If your manuscript discusses an unlabeled use of a commercial product or device or an investigational use of a product or device not yet approved by the FDA for any
purpose, you must specifically disclose in the manuscript that the product is not labeled for the use under discussion or that the product is still investigational. Please check
the item below that applies to you
Response: I will not discuss unlabeled/investigational uses of any commercial product or device

.

http://msse.edmgr.com/

Your username is: adrianosilva
Your password is: available at this link http://msse.edmgr.com/Default.aspx?pg=accountFinder.aspx&firstname=Adriano&lastname=Lima-Silva&email_address=adrianosilva@usp.br;limasilvaae@hotmail.com

Your manuscript will be given a reference number once an Associate Editor has been assigned. You will receive notice of the acting Associate Editor when he/she accepts the assignment.

Thank you for submitting your work to this journal.

Regards,

Medicine & Science in Sports & Exercise

MSSE Editorial Office
401 West Michigan Street
Indianapolis, IN 46202-3233
USA
P (317) 634-8932
F (317) 634-8927
msse@acsm.org