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MANOEL MIRANDA NETO

INFLUÊNCIA DO POLIMORFISMO NO GENE DA CYP1A2 SOBRE OS EFEITOS DA SUPLEMENTAÇÃO COM O ÓLEO ESSENCIAL DE HORTELÃ-PIMENTA (*Mentha piperita* L.) NO DESEMPENHO FÍSICO DE CORREDORES RECREACIONAIS

JOÃO PESSOA
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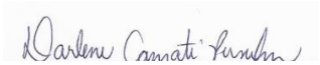

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RESUMO

Diversos alimentos têm sido propostos para melhorar a performance física. O óleo essencial de hortelã-pimenta (OEHP) está entre esses alimentos, mas ainda não existem dados na literatura do seu uso em atletas e em provas de longa duração, além de os mecanismos envolvidos ainda não se mostrarem claros. Enquanto isso, uma variabilidade individual na resposta ergogênica para o tempo até a exaustão (TAE) tem sido observada, e uma possível explicação pode ser a presença de polimorfismos genéticos. O objetivo deste estudo foi investigar a influência da suplementação com o OEHP no TAE de corredores e se o polimorfismo no gene da CYP1A2 influencia as respostas desta suplementação. Quarenta corredores recreacionais homens (36,5±2,0 anos; 24,3±0,6 kg/m^2; 52,4±1,2 ml.kg.min) realizaram dois procedimentos em modelo *cross over*, sendo um experimental (500ml de água adicionada de 0,05ml do OEHP) e outro placebo isovolumétrico adicionado de 0,05ml da essência de hortelã. Trinta minutos depois, realizaram uma corrida até a exaustão em esteira a 70% do $VO_{2máx}$, mantendo a ingestão de 100 ml da bebida experimental ou placebo a cada 10 minutos durante os primeiros 40 minutos da corrida. Antes e a cada 10 minutos durante o exercício foram registradas a temperatura corporal e a frequência cardíaca. Medidas de sensação térmica, conforto térmico e percepção subjetiva de esforço foram realizadas a cada 10 minutos durante o teste físico. Coleta da urina foi feita antes e após o exercício para registro de volume e densidade urinária. A taxa de sudorese foi calculada. Coletas sanguíneas foram feitas em repouso, imediatamente após o fim do exercício e 2 horas pós exercício, para dosagem de Malondialdeído e Capacidade Oxidante Total. Coleta da mucosa oral foi feita para genotipagem do gene da CYP1A2. Os primeiros 14 corredores correram 109,9 ± 7,4 minutos após a ingestão do OEHP e 98,5 ± 6,7 minutos no procedimento placebo, o que foi equivalente a uma melhora significativa de 11,6 ± 5,5 % (p=0,009) (artigo original 1). Para a amostra total de 40 corredores (artigo original 2), os atletas correram 104,0 ± 5,1 minutos após a ingestão do OEHP e 95,5 ± 3,9 minutos no procedimento placebo, o que foi equivalente a uma melhora significativa de 9,6 ± 3,6 % (p=0,011). Após a divisão genotípica, não foi observada diferença estatística quando comparados os procedimentos experimental e placebo para os atletas com o genótipo AA (95,6 ± 6,6 minutos; 88,6 ± 4,3 minutos, respectivamente; p=0,374). Para o genótipo AC+CC, o TAE foi significativamente maior após a ingestão do OEHP em comparação ao placebo (113,2 ± 7,4 minutos; 103,1 ± 6,4 minutos, respectivamente; p=0,026). Atletas com genótipos AC+CC apresentaram maior percentual de aumento no TAE quando comparados com o genótipo AA (14,9 ± 2,9 % vs 2,3 ± 4,3 % respectivamente) (p=0.008). Não foram observadas alterações significativas nas variáveis termorregulatórias, de hidratação e sanguíneas (p>0,05), mesmo após a divisão por genótipo (p>0,05). Concluímos que o OEHP aumenta o TAE de corredores, mas apenas nos atletas que possuem a presença do alelo C para o gene da CYP1A2.

Palavras-chave: Alimento Ergogênico. Exercício Aeróbio. Hortelã-pimenta. Óleo Essencial. Polimorfismo genético.

ABSTRACT

Several foods have been proposed to improve physical performance. Peppermint essential oil (PEO) is among these foods, but there is still no data in the literature on its use in athletes and long-term events, and the mechanisms involved are still not clear. Meanwhile, individual variability in the ergogenic response to time to exhaustion (TTE) has been observed, and a possible explanation may be the presence of genetic polymorphisms. The objective of this study was to investigate the influence of PEO supplementation on the TTE of runners and whether the polymorphism in the CYP1A2 gene influences the responses to this supplementation. Forty male recreational runners (36.5±2.0 years; 24.3±0.6 kg/m^2; 52.4±1.2 ml.kg.min) performed two procedures in a cross over model, one of which was experimental (500ml of water added with 0.05ml of PEO) and another isovolumetric placebo added with 0.05ml of mint essence. Thirty minutes later, they ran until exhaustion on a treadmill at 70% of VO_{2max}, maintaining the intake of 100 ml of the experimental drink or placebo every 10 minutes during the first 40 minutes of the run. Before and every 10 minutes during exercise, body temperature and heart rate were recorded. Measurements of thermal sensation, thermal comfort and subjective perception of exertion were taken every 10 minutes during the physical test. Urine collection was done before and after exercise to record urinary volume and density. The sweating rate was calculated. Blood samples were taken at rest, immediately after the end of exercise and 2 hours after exercise, to measure Malondialdehyde and Total Oxidizing Capacity. The oral mucosa was collected for genotyping of the CYP1A2 gene. The first 14 runners ran 109.9 ± 7.4 minutes after ingesting the PEO and 98.5 ± 6.7 minutes in the placebo procedure, which was equivalent to a significant improvement of 11.6 ± 5.5% (p =0.009) (original article 1). For the total sample of 40 runners (original article 2), athletes ran 104.0 ± 5.1 minutes after ingesting PEO and 95.5 ± 3.9 minutes in the placebo procedure, which was equivalent to a significant improvement of 9.6 ± 3.6% (p=0.011). After genotypic division, no statistical difference was observed when comparing the experimental and placebo procedures for athletes with the AA genotype (95.6 ± 6.6 minutes; 88.6 ± 4.3 minutes, respectively; p=0.374). For the AC+CC genotype, TTE was significantly higher after ingestion of PEO compared to placebo (113.2 ± 7.4 minutes; 103.1 ± 6.4 minutes, respectively; p=0.026). Athletes with AC+CC genotypes showed a higher percentage of increase in TTE when compared to the AA genotype (14.9 ± 2.9% vs 2.3 ± 4.3% respectively) (p=0.008). No significant changes were observed in thermoregulatory, hydration and blood variables (p>0.05), even after division by genotype (p>0.05). We conclude that PEO increases the TTE of runners, but only in athletes who have the presence of the C allele for the CYP1A2 gene.

Keywords: Aerobic Exercise. Ergogenic Food. Essential oil. Genetic polymorphism. Peppermint.

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LISTA DE ABRVIATURAS E SIGLAS

CAT - Capacidade Antioxidante Total

DRI's - Dietary Reference Intakes

FC - Frequência Cardíaca

IMC - Índice de Massa Corporal

IOM - Institute of Medicine

MDA - Malondialdeído

PSE - Percepção Subjetiva de Esforço

TCLE - Termo de Consentimento Livre e Esclarecido

UFPB - Universidade Federal da Paraíba

$VO_{2máx}$ - Consumo Máximo de Oxigênio

SUMÁRIO

1 INTRODUÇÃO

Uma das principais demandas da pesquisa com nutrição esportiva é a busca pelo uso de nutrientes como recurso ergogênico, definido este termo como procedimentos extra treino que buscam melhorar o desempenho físico de atletas, seja acelerando a recuperação pós exercício, aumentando o tempo até a exaustão ou reduzindo o tempo de prova nos treinamentos e durante as provas (Petróczi; Naughton, 2007). Dentro desse contexto, a literatura tem evidenciado que alimentos possuem efeito ergogênico, como mostrado por Costa *et al.* (2020) em revisão sistemática que mostrou que 26 alimentos investigados apresentaram algum efeito ergogênico, e destes, onze estudos verificaram os efeitos no aumento do tempo até a exaustão em testes de corrida e ciclismo. Dentre esses, oito estudos (81,8%) confirmaram a hipótese desse efeito ergogênico no tempo até a exaustão após a suplementação de algum alimento. O efeito antioxidante desses alimentos tem sido descrito pelos pesquisadores para justificar seus efeitos ergogênicos, como enfatizado por Doma; Gahreman; Connor (2020) em metanálise que avaliou o consumo de frutas como alternativa ergogênica para atletas.

Enquanto isso, a literatura tem proposto o óleo essencial de hortelã-pimenta (*Mentha piperita* L.) como possíveis candidatos a novo alimento ergogênico, uma vez que estudos têm apontado para a melhoria do rendimento físico de estudantes fisicamente ativos, devido ao aumento do tempo até a exaustão, trabalho total e força (Meamarbashi; Rajabi, 2013) e aumento da capacidade anaeróbia (Meamarbashi, 2014). A hortelã-pimenta é uma espécie de planta do gênero *Mentha* e seu óleo essencial é composto de Mentol e Mentona (Camele; Grulová; Elshafie, 2021), ácido caféico, polifenóis polimerizados, carotenos, tocoferóis, betaína, colina, taninos e flavonoides cítricos, reconhecidos antioxidantes (Karuza; Blasevic; Soljic, 1996; Sokovic *et al.*, 2009). Apesar de ainda não ter sido avaliada, esta composição antioxidante poderia explicar os efeitos ergogênicos já demonstrados na literatura para o óleo essencial de hortelã-pimenta (OEHP), assim como para os demais alimentos já estudados.

No entanto, existe a possibilidade de que o composto ativo mentol, presente na hortelã-pimenta, promova efeito ergogênico por uma via diferente da atividade antioxidante, o que seria um diferencial para este alimento. Sabe-se que uma maior produção de calor ou menor dissipação desse calor durante o exercício físico aumenta a desidratação e antecipa a fadiga (Hue, 2011). Por outro lado, a literatura tem indicado que o mentol tem uma capacidade resfriante que minimiza o aumento da temperatura corporal durante o exercício, como foi demonstrado por Riera *et al.* (2014) e Tran Trong *et al.* (2015), além de melhorar a sensação e o conforto térmico de atletas (Barwood *et al.*, 2015; Stevens *et al.*, 2016).

A despeito desses relevantes achados quanto à melhoria do desempenho físico, nos estudos de Meamarbashi; Rajabi (2013) e Meamarbashi (2014) não foi determinado se a melhoria da performance física foi associada à participação do mentol no controle do aumento da temperatura corporal. Enquanto isso, embora Riera *et al.* (2014) e Tran Trong *et al.* (2015) tenham mostrado menor aumento de temperatura corporal em exercício de longa duração, foi realizado utilizando o mentol de forma isolada e não o seu óleo essencial, que contém outros constituintes, inclusive antioxidantes, que podem contribuir para a melhora do desempenho físico. Além disso, nenhum dos estudos prévios que utilizaram o óleo essencial de hortelã-pimenta avaliaram o efeito da sua suplementação em atletas (estudos feitos com estudantes), assim como não avaliaram o status de hidratação nem a influência genética nos resultados.

Em estudos prévios com outros alimentos, tem sido notado que alguns polimorfismos genéticos têm mostrado influenciar as respostas ergogênicas de compostos nutricionais, como o polimorfismo no gene da CYP1A2 (rs762551) que foi associado com maior aumento no número de repetições em exercício resistido após a suplementação com cafeína em atletas homozigotos para o alelo A, em comparação com portadores de alelo C (Rahimi, 2019). O óleo de pequi, rico em carotenoides, reduziu a peroxidação lipídica plasmática e o dano ao DNA em corredores, mas indivíduos heterozigotos MnSOD – Val/Ala responderam à ingestão de pequi com menor dano ao DNA e menor peroxidação lipídica em reposta ao exercício quando comparados a indivíduos homozigotos MnSOD – Val/Val (Miranda-Vilela *et al.*, 2009). Complementando estes dados, em estudo realizado por nosso laboratório, embora o suco de uva tenha aumentado o tempo até a exaustão, 16 corredores não foram responsivos, e dentre os responsivos (trinta e um), a magnitude de melhoria variou muito, entre 2% e 40%. Esta variabilidade na resposta foi explicada uma vez que portadores do alelo G do gene da SOD3 eram mais responsivos à suplementação com o suco de uva, ou seja, tinham um melhor desempenho físico quando comparados aos portadores do alelo C (Sousa *et al.*, 2022).

Nenhum dos estudos que avaliaram a ingestão do OEHP no desempenho físico fizeram esta abordagem genética. Entretanto, sabe-se que o citocromo P-450 1A2 (CYP1A2) é uma enzima envolvida no metabolismo de vários nutrientes (Gougis *et al.*, 2021) e polimorfismos no gene da CYP1A2 (rs762551) podem levar a variações na atividade desta enzima. Gougis *et al.* (2021) mostraram em uma revisão da literatura que compostos nutricionais se mostraram capazes de influenciar a atividade da enzima CYP1A2, inclusive o chá de hortelã-pimenta (Maliakal; Wanwimolruk, 2001) e também o seu principal composto ativo, o mentol isolado (Feng *et al.*, 2019), ambos inibindo de forma moderada sua atividade. Esta inibição pode gerar prejuízos no metabolismo de nutrientes que atuam como substratos para a enzima CYP1A2,

uma vez que o mentol pode interagir com essas substâncias, como de fato já foi demonstrado que uma única dose oral de mentol atrasou a absorção de cafeína, resultando em diminuição da depuração e níveis plasmáticos mais elevados de cafeína, principal substância metabolizada pela enzima CYP1A2 (Gelal *et al.*, 2003). A participação da CYP1A2 no metabolismo do mentol ainda precisa ser melhor investigada, mas evidências apontam para um possível envolvimento, uma vez que Sakuma *et al.* (1999) demonstraram que o fenobarbital ativa a síntese e atividade da CYP1A2 em modelo animal e a análise do metabolismo do mentol mostrou estar maior em animais tratados com fenobarbital (Madyastha; Srivatsan, 1988). Portanto, devido a interação da CYP1A2 com compostos nutricionais, inclusive hortelã-pimenta e mentol, e um possível envolvimento desta enzima no metabolismo do mentol, este gene se torna um candidato promissor para se verificar a influência genética sobre os efeitos do OEHP após uma sessão de exercício.

Considerando a ausência de estudos relacionando a influência do OEHP no desempenho físico de atletas corredores de longas distâncias e no estresse oxidativo, temperatura corporal, percepção térmica e status de hidratação desses atletas, torna-se importante pesquisas que avaliem suas propriedades bioativas e seu efeito ergogênico, possibilitando adicionar um novo alimento ergogênico a dieta dos atletas. Ao mesmo tempo, visto que alguns nutrientes podem interagir com o genoma humano ou este genoma interagir com alguns nutrientes, torna-se importante essa avaliação da interação nutrigenômica, pois ainda não há dados na literatura desta interação após a suplementação do OEHP antes e durante um protocolo de exercício físico com atletas corredores recreacionais. Assim, estudos que avaliem esta interação podem contribuir para futuras intervenções dietéticas baseadas no conhecimento do requerimento nutricional, do estado nutricional e do genótipo.

Portanto, o objetivo deste estudo foi investigar a influência da suplementação com o OEHP no desempenho físico, na termorregulação durante o exercício, além da influência nos marcadores sanguíneos de estresse oxidativo e no estado de hidratação em atletas corredores de longa distância e de nível recreacional, e se o polimorfismo no gene da CYP1A2 influencia as respostas desta suplementação. Como objetivos específicos, verificou-se o efeito do OEHP no tempo de corrida até a exaustão em corredores, na percepção subjetiva de esforço, na temperatura corporal, na sensação térmica e conforto térmico em exercício de corrida até a exaustão. Além disso, foi observado a influência desta suplementação no *status* antioxidante e pró oxidante desses corredores. Por fim, foi determinada a presença ou não do polimorfismo no gene da CYP1A2 nestes corredores e se estes polimorfismos influenciam os resultados.

2 REFERENCIAL TEÓRICO

2.1 ALIMENTOS ERGOGÊNICOS

A ingestão de suplementos alimentares comerciais no mundo vem crescendo a cada ano, e estudos estimam a prevalência do uso destes suplementos entre 40% e 100% entre os atletas, dependendo de vários fatores, incluindo o nível de competição, o tipo de esporte e a definição do uso de suplementos dietéticos (Garthe; Maughan, 2018). Apesar desse intenso consumo de suplementos comerciais, esta prática sempre foi alvo de discussões, debates e controvérsias. De um lado, há quem defenda seu uso alegando efeitos ergogênicos, como o ganho de um aporte nutricional adequado às competições e treinos, melhor biodisponibilidade de nutrientes, consumo suficiente de nutrientes que dificilmente seriam atingidos pela alimentação, além da praticidade do consumo no cotidiano, de forma a suprir possíveis carências da alimentação convencional (Rodriguez; Dimarco; Langley, 2009). Do outro lado, existem questionamentos do real efeito destes suplementos comerciais e de suas substâncias bioativas como sendo controversos e duvidosos (Kreider *et al.*, 2010). Dados de consumo abusivo destes suplementos alimentares comerciais existem e mostram contaminação de cerca de 25% destes suplementos com substâncias proibidas (Petróczi; Taylor; Naughton, 2011), além de efedrina e cafeína em concentrações elevadas (Maughan, 2005) e efeitos colaterais associados ao uso destes suplementos comerciais (Silva *et al.*, 2014).

Paralela e independente a estes questionamentos, tem surgido uma relevante e promissora linha de pesquisa, que traz diversos alimentos que tem se mostrado eficazes em melhorar a performance física (alimentos ergogênicos). São mais de 70 estudos na base de dados *Pubmed/Medline*, dos quais destacam-se o suco de caju, sendo observado que sua suplementação aumentou a oxidação das gorduras e melhorou o consumo máximo de oxigênio ($VO_{2máx}$) de ciclistas de elite homens (Prasertsri *et al.*, 2013). Destaca-se também a proteína do arroz que após oito semanas de consumo melhorou o desempenho de homens treinados através da diminuição da gordura corporal, aumento da massa magra e aumento da força de pico em exercícios de força isométrica (Joy *et al.*, 2013). Tem sido mostrado também que o suco de uva melhorou o tempo até a exaustão de corredores recreacionais após um teste até a exaustão a 80% do $VO_{2máx}$ (Toscano *et al.*, 2015). O mais investigado até o momento, justamente por ter expressivo efeito ergogênico, é a beterraba. Já existem estudos de revisão sobre o assunto (Clements; Lee; Bloomer, 2014; Domínguez *et al.*, 2017), todos eles atestando para uma melhora da resistência cardiorrespiratória em atletas, aumento da eficiência respiratória,

melhora do desempenho físico em longas distâncias, aumento do tempo de exaustão em intensidades submáximas e melhora do desempenho cardiorrespiratório em intensidades de limiar anaeróbico e $VO_{2máx}$ após a ingestão do suco de beterraba. Com isso, os alimentos ergogênicos tem mostrado melhora do desempenho aeróbio (mais evidente) e alguns efeitos benéficos no desempenho anaeróbio.

Quanto ao efeito ergogênico associado à hidratação dos atletas, a literatura tem mostrado evidências apontando que alimentos possuem a capacidade de potencializar o desempenho físico através da reposição hídrica, com destaque para a água de coco que promoveu a hidratação de atletas jovens saudáveis que se desidrataram durante o exercício e mostrou ser efetiva em apoiar o exercício subsequente através da maior redução dos níveis de creatina kinase, quando comparado com bebida esportiva (Kalman *et al.*, 2012). Já o caldo de cana se mostrou assemelhar-se a água e a bebida esportiva na hidratação de atletas durante o exercício, além de aumentar o glicogênio muscular de atletas homens jovens pós treino contribuindo assim com a melhor recuperação após sessões de treinamento quando comparado com bebida esportiva e água (Kalpana *et al.*, 2013) e o achocolatado que mostrou ser capaz de gerar recuperação e dano muscular pós treino de ciclistas treinados semelhante a bebida esportiva (Pritchett *et al.*, 2011), tudo isso sem os riscos e controvérsias já discutidos para os suplementos comerciais (Silva *et al.*, 2014).

2.2 HORTELÃ-PIMENTA

A menta (*Mentha* ssp.) é um vegetal com capacidade de promover refrescância ao ser consumida. Esta peculiaridade é proporcionada por uma rica composição das substâncias mentol e mentona (Camele; Grulová; Elshafie, 2021). A etnofarmacologia mostra que os povos de diversos períodos da história têm usado diversas espécies de menta para tratamento de distúrbios gastrointestinais, desordens respiratórias, ações analgésicas dentre outros, desde centenas de anos atrás nas tradicionais medicinas chinesas e persa até os dias de hoje (Sadati *et al.*, 2016). Enquanto isso, estudos têm confirmado o efeito benéfico gastrointestinal (Scarpellini *et al.*, 2023), no fígado e rins (Zhao *et al.*, 2022), potencial quimioprotetivo (Samarth; Kumar, 2003), antialergênico (Inoue *et al.*, 2002) e anti-inflamatório (Radovanović; Gavarić; Aćimović; 2023), além de efeitos benéficos também no trato respiratório (Afzal *et al.*, 2021).

A hortelã-pimenta (*Mecntha piperita* L.) é uma espécie do gênero menta (*Mentha* ssp.), que possuí altas concentrações de mentol e mentona (Figura 1). É uma erva perene de origem

européia e cultivada por todo o mundo. É resultado de um híbrido composto de *Mentha spicata* L. e *Mentha aquatica* L., crescendo particularmente bem em áreas com alta umidade relativa do ar, conhecida por suas propriedades de sabor e fragrância, as folhas de hortelã-pimenta (fresca e seca) e seu óleo essencial extraído destas folhas são usadas em muitos alimentos, cosméticos e produtos farmacêuticos (Mckay; Blumberg, 2006)

Estudos de cromatografia tem revelado que o óleo essencial de hortelã-pimenta possui cerca de 33% a 60% de mentol e 15% a 32% de mentona, a depender da maturidade da planta, do solo, da localização geográfica, da variedade e condições de cultivo e processamento (Camele; Grulová; Elshafie, 2021). Além destes dois principais compostos, fazem parte da constituição da hortelã-pimenta o ácido caféico, polifenóis polimerizados, carotenos, tocoferóis, betaína, colina, taninos e flavonoides cítricos (Eriocitrina, luteolina e hesperidina), vários destes reconhecidos antioxidantes naturais (Karuza; Blasevic; Soljic, 1996; Sokovic *et al.*, 2009).

Figura 1 – Folhas de Hortelã-pimenta (*Mentha piperita* L.)

Fonte: https://www.google.com.br/imagens/hortelapimenta

Juntamente com estes efeitos antioxidantes e anti-inflamatórios atribuídos a hortelã-pimenta (Karuza; Blasevic; Soljic, 1996; Sokovic *et al.*, 2009), o efeito resfriante do mentol seria um diferencial deste alimento, podendo promover, além dos efeitos terapêuticos investigados, efeito ergogênico para atletas. Sabe-se que uma menor dissipação do calor ou maior produção de calor durante o exercício antecipa a exaustão e que exercícios realizados em ambientes quentes e úmidos geram maior desidratação, diminuição do débito cardíaco e

consequentemente menor aporte de oxigênio para os músculos (Tucker *et al.*, 2017). De fato, já foi demonstrado menor aumento de temperatura corporal durante o exercício feito em ambiente ameno com consequente melhoria do desempenho físico (Hue, 2011). Existem dados mostrando esta capacidade resfriante do mentol e comprovando esta diminuição do aumento da temperatura corporal durante exercício de longa duração (Riera *et al.*, 2014; Tran Trong *et al.*, 2015).

Os mecanismos pelos quais esses efeitos acontecem ainda são incertos e estão sendo debatidos, mas a literatura tem postulado a hipótese de que o mentol provoca sensação de resfriamento por diferentes estímulos subsequentes (ar inspirado, água com mentol consumida ou através de enxágue bucal sem ingestão) melhorando o conforto térmico, a sensação térmica e a percepção subjetiva de esforço (Eccles, 2000). Também tem sido hipotetizado que, se o mentol realmente intensifica o fluxo do ar, ele poderia prevenir hipocapnia (dióxido de carbono reduzido no sangue) induzida pelo calor e a redução do fluxo sanguíneo cerebral geralmente tem um efeito positivo na fadiga central (Nybo, 2008). Na verdade, há algumas evidências de que o estresse térmico resulta em hiperventilação, o que diminui a tensão do dióxido de carbono e consequentemente reduz o fluxo sanguíneo cerebral (Nybo, 2008), induzindo a fadiga cerebral. Diminuindo essa hiperventilação, devido à sua interação com os receptores frios das vias aéreas (Eccles, 2000), o mentol pode, portanto, reduzir da hipocapnia e, por extensão, a fadiga cerebral.

Riera *et al.* (2014) observaram melhora no desempenho físico aeróbio após a ingestão de uma bebida fria contendo mentol em exercício de ciclismo de 20 km com triatletas homens, mas sem alterações significativas na sensação térmica e conforto térmico dos atletas. Resultados similares foram demonstrados por Tran Trong *et al.* (2015), que verificaram melhora da performance de ciclistas em ambiente quente ao ar livre percorrendo cinco blocos de 4 km de ciclismo e 1,5 km de corrida cada bloco, sendo que esta melhora foi acompanhada de menor aumento da temperatura corporal, mas sem alterações significativas na sensação térmica e conforto térmico dos atletas. Em estudo mais recente, Flood *et al.* (2017) investigaram o efeito do l-menthol no exercício em um ambiente quente. Oito participantes masculinos completaram dois testes aeróbios de alta intensidade. O tempo de exercício foi maior e a potência média aumentou após o enxágue oral com l-menthol. A sensação térmica foi menor na condição de l-mentol, apesar de nenhuma alteração na temperatura da pele ou central ter sido observada. Segundo os autores, estes resultados mostram que um resfriamento não térmico com enxágue da boca reduziu a sensação térmica, resultando em taxa de trabalho elevada, o que prolongou o tempo de exercício no calor, enquanto que nos estudos de Riera *et al.* (2014) e Tran Trong *et*

al. (2015), apesar de melhora do desempenho físico, não foi observada melhora nos parâmetros térmicos referidos. Curiosamente, estes dois estudos, diferentemente dos demais, utilizaram a ingestão do mentol e não o enxágue bucal. Talvez, os receptores sensitivos de frio na cavidade oral expliquem essa diferença encontrada nos resultados.

Quando analisamos a hortelã-pimenta, que é um alimento natural e integral, que além de mentol, possui outros constituintes químicos em sua composição (Karuza; Blasevic; Soljic, 1996; Sokovic *et al.*, 2009), apenas quatro estudos avaliaram sua influência no exercício físico, mas sem avaliar atletas e variáveis de termorregulação, hidratação, sanguíneas ou genéticas. O primeiro deles foi realizado por Sönmez *et al.* (2010), onde determinaram os efeitos do extrato de menta na dor muscular e nos níveis de lactato no sangue após uma corrida de 400m (curta duração). Para isso, 16 estudantes de educação física ingeriram extrato de menta (5 ml/kg de massa corporal) e o grupo placebo recebeu chá não açucarado (5 ml/kg) passando por um *cross-over* uma hora antes de um teste de corrida de 400 m. Os sujeitos do grupo controle não foram tratados. A administração oral de extrato de menta diminuiu significativamente as concentrações de lactato sanguíneo, mas os níveis de dor muscular permaneceram inalterados em todos os grupos. Os autores explicaram os resultados pela possibilidade de que a administração oral de extrato de hortelã pode ter um efeito benéfico na depuração do lactato no sangue e, portanto, pode aumentar o desempenho esportivo.

Enquanto isso, Meamarbashi; Rajabi (2013) avaliaram doze estudantes masculinos saudáveis que consumiam diariamente por dez dias 500ml de água mineral, contendo 0,05 ml de óleo essencial de hortelã-pimenta e realizaram um teste ergométrico incremental em esteira (protocolo Bruce). A capacidade vital forçada (4,57 ± 0,90 vs. 4,79 ± 0,84), a taxa máxima de fluxo expiratório (8,50 ± 0,94 vs. 8,87 ± 0,92) e o fluxo inspiratório máximo (5,71 ± 1,16 vs. 6,58 ± 1,08) melhoraram significativamente após dez dias de suplementação. O desempenho do exercício avaliado pelo tempo até a exaustão (664,5 ± 114,2 vs. 830,2 ± 129,8 s), trabalho (78,34 ± 32,84 vs. 118,7 ± 47,38 KJ) e potência (114,3 ± 24,24 vs. 139,4 ± 27,80 KW) aumentaram significativamente. Além disso, os resultados da análise de gases respiratórios apresentaram aumentos significativos no $VO_{2máx}$ (2,74 ± 0,40 versus 3,03 ± 0,351 L / min) e $VCO_{2máx}$ (3,08 ± 0,47 vs. 3,73 ± 0,518 L / min). De acordo com os autores, os resultados do experimento mostram a eficácia do óleo essencial de hortelã-pimenta na performance física, análise de gases, parâmetros de espirometria, pressão arterial e frequência respiratória nos jovens estudantes do sexo masculino. O relaxamento dos músculos lisos brônquicos, o aumento da ventilação, a concentração de oxigênio cerebral e a diminuição do nível de lactato no sangue são as explicações mais plausíveis levantadas pelos autores.

O mesmo Meamarbashi (2014) fez outro estudo com o objetivo de investigar os efeitos da ingestão do óleo essencial de hortelã-pimenta nos parâmetros fisiológicos e de desempenho físico no exercício após 5 min e 1 h. Trinta estudantes universitários saudáveis foram avaliados após a administração oral de dose única de óleo essencial de hortelã-pimenta (50µl). Os resultados revelaram melhora significativa em todas as variáveis após administração oral de óleo essencial de hortelã-pimenta. O grupo experimental em comparação com o grupo de controle mostrou aumento significativo na força isométrica máxima (36,1%), salto vertical (7,0%) e salto horizontal (6,4%). Os dados obtidos do grupo experimental após cinco minutos mostraram um aumento significativo na capacidade vital forçada (35,1%), fluxo inspiratório máximo (66,4%) e fluxo expiratório máximo (65,1%), enquanto que após uma hora, apenas o fluxo inspiratório máximo mostrou um aumento significativo quando comparado com o basal e o grupo controle. Em ambos os momentos, os tempos de reação visual e de áudio diminuíram significativamente. Um aumento considerável na força isométrica e parâmetros espirométricos foram os achados mais importantes deste estudo. Os autores enfatizaram que esta melhora nas medidas espirométricas pode ser devido aos efeitos da hortelã-pimenta na tonicidade do músculo liso brônquico com ou sem afetar o surfactante pulmonar. No entanto, não existem evidências científicas sobre o aprimoramento da força isométrica neste estudo.

O quarto e mais recente estudo foi realizado por Shepherd; Peart (2017), onde avaliaram os efeitos do óleo essencial de hortelã-pimenta na capacidade aeróbia (tempo até a exaustão) e diferente dos demais estudos não observaram melhora na performance física. Neste trabalho, sete participantes saudáveis fisicamente ativos realizaram um teste de exercício máximo graduado em ciclo ergômetro após 10 dias da ingestão do óleo essencial de hortelã-pimenta ou um controle. Não houve diferença significativa entre os ensaios de controle e hortelã-pimenta para as variáveis de gás expiradas (pico de O2 3,54 versus 3,52 L / min) ou medidas de desempenho físico (tempo de exaustão 583,33 vs. 587,04 segundos). Da mesma forma, as medidas cardio-pulmonares em repouso também permaneceram inalteradas entre os testes.

Enquanto isso, diferentemente destes únicos quatro estudos prévios que avaliaram a ingestão do óleo essencial de hortelã-pimenta no exercício, o presente trabalho pretende avaliar um protocolo de corrida até a exaustão de longa distância com ingestão aguda da mesma quantidade deste óleo usada nos estudos prévios e em atletas recreacionais. De qualquer maneira, apesar de cada um destes estudos prévios terem realizado diferentes protocolos de exercício físico, todos demonstraram efeito ergogênico, com exceção para Shepherd; Peart (2017). A melhora no teste incremental no estudo de Meamarbashi; Rajabi (2013) é o que mais se assemelha ao nosso protocolo. Testes incrementais avaliam o $VO_{2máx}$, uma capacidade

aumentada em atletas corredores de longa distância (Ferri *et al.*, 2012). No entanto, dados consolidados na área do treinamento desportivo indicam que o limiar anaeróbio é o melhor preditor da performance em corridas do que o $VO_{2máx}$ (Faude *et al.*, 2009), por isso, pesquisas que pretendem avaliar o efeito ergogênico em corredores adotam protocolos até a exaustão, como foi o adotado por nós no presente estudo.

2.3 TERMORREGULAÇÃO E EXERCÍCIO FÍSICO

A produção de calor acontece em todas as células do corpo humano, a partir da conversão da energia metabólica em energia mecânica e térmica. Na atividade física e no exercício, a maior parte da energia liberada durante a contração muscular se perde na forma energia térmica (Gonzalez-Alonso *et al.*, 2000; Krustrup *et al.*, 2001, 2003). Seja em uma caminhada rápida ou na corrida de longas distâncias, por exemplo, no máximo 40% da energia química vinda da oxidação dos nutrientes costumam se transformar em energia mecânica, responsável pelo movimento e o restante é transformado imediatamente em energia térmica. Posteriormente, inclusive até mesmo essa energia mecânica, que proporcionou o movimento, também é transformada em energia térmica (Carvalho; Mara, 2010).

Esse calor produzido eleva a temperatura corporal, de modo que deve ser dissipado. Sem a dissipação, o organismo entraria em colapso devido ao superaquecimento em questão de poucos minutos de exercício físico (Carvalho; Mara, 2010). Já é bem documentado que o exercício físico aumenta a taxa de produção de calor metabólico e, portanto, especialmente quando realizado em ambiente quente, pode causar hipertermia e até mesmo insolação fatal (Epstein *et al.*, 1999). Por exemplo, uma temperatura corporal acima de 42 °C, levaria a uma citotoxicidade, desnaturação protéica e prejuízo da síntese de DNA, resultando em falência de órgãos e comprometimento neuronal (Lepock, 2003). Por outro lado, se a temperatura do corpo cair abaixo de 27 °C (hipotermia grave), os efeitos neuromusculares, cardiovasculares, alterações hematológicas e respiratórias poderiam igualmente serem fatais (Mallet, 2002).

Apesar da necessidade de regulação rigorosa da temperatura central, os seres humanos podem sobreviver nos mais inóspitos ambientes e podem desafiar sua capacidade termorreguladora nos locais mais extremos. Mesmo diante desta alta capacidade que o ser humano tem de suportar ambientes com temperaturas muito altas ou muito baixas, há a necessidade de um sistema termorregulador eficiente para a manutenção da temperatura corporal, principalmente diante destas situações extremas. Para prevenir a hipertermia ou a hipotermia, o equilíbrio entre ganho de calor (metabólico e ambiental) e dissipação de calor

deve ser mantido através deste sistema termorregulador, que é baseado principalmente nas funções cardiovascular e sudomotora (sudorese) (Gleeson *et al.*, 1998).

A perda liquida no suor (sudorese) e a evaporação subsequente são os mais importantes mecanismos de termorregulação durante a atividade física, principalmente em ambientes quentes (Gonzalez-Alonso, 2012; Sawka *et al.*, 2012). De fato, resfriamento evaporativo é o único mecanismo de perda de calor quando a temperatura ambiente excede a temperatura do corpo (Tansey; Johnson, 2015). Exposição a um ambiente quente, ou ao exercício, eleva as temperaturas do núcleo e da pele, ambos contribuem para o aumento da taxa de suor. O limiar de transpiração normalmente excede o limiar para vasoconstrição em 0,2 °C, no entanto, sabe-se que a transpiração começa dentro de segundos do início do exercício, antes de qualquer alteração mensurável na temperatura interna (Van Beaumont; Bullard, 1963). Acredita-se que isso seja mediado por uma combinação de ordens do comando central e do reflexo pressor do exercício (Shibasaki; Crandall, 2010).

O suor é liberado pelas glândulas écrinas, que são distribuídas em grandes quantidades (1,6 a 4 milhões) em toda a superfície do corpo, com distribuições por região de acordo com suas densidades. O ato de suar é mediado pela ativação de fibras colinérgicas simpáticas (Shibasaki *et al.*, 2006). A evaporação do suor permite que o calor seja transferido para o ambiente em forma de vapor de água pelas passagens respiratórias da superfície da pele. O principal fator limitante na capacidade de um humano manter a temperatura corporal diante de um estresse térmico é a disponibilidade de água corporal para a produção de suor. Altos volumes de suor podem ser produzidos se uma pessoa se torna aclimatada ao calor, em torno de 2L a 3L por hora (Armstrong *et al.*, 1986), comparado com 1L por hora em indivíduos não aclimatados (Bates; Miller, 2008).

A aclimatação ao calor aumenta o mecanismo de transpiração e foi anteriormente associado a uma redistribuição de secreção de suor para os membros (Höfler, 1968). Isso poderia potencialmente ser desejável, pois os membros têm uma área superficial relativamente grande. Uma elevação na sudorese e evaporação nestes locais poderiam, portanto, melhorar a homeostase térmica, contudo, evidências mais recentes sugerem que uma redistribuição da transpiração do tronco aos membros não ocorre como se imaginava (Taylor; Machado-Moreira, 2013). Com a aclimatação ao calor, há um limiar de temperatura corporal mais baixo para a transpiração, sensibilidade e capacidade de evolução da glândula sudorípara, por isso, para uma determinada temperatura central, a taxa de suor aumenta (Lee *et al.*, 2014). Um aumento na sudorese altera a composição do suor e está particularmente associada à depleção na concentração de sódio e cloreto plasmático, contudo, a aclimatização mostrou atenuar essa

redução. É provável que isso esteja relacionado ao aumento nos níveis de renina e aldosterona que foram encontrados em indivíduos aclimatados produzindo uma menor concentração de sódio no suor na população estudada (Nielsen *et al.*, 1997).

Além da sudorese e da evaporação deste suor, existem outros mecanismos utilizados pelo corpo humano para controlar a homeostase interna e manter a temperatura corporal dentro dos valores normais fisiológicos. Estes mecanismos são a condução, a radiação e a convecção, que têm importância menor durante o exercício físico, principalmente os mais intensos e prolongados, mas ainda sim necessários para a termorregulação ideal. A condução é o movimento de calor do corpo diretamente para objetos em contato com o corpo. A Radiação é a radiação eletromagnética (calor) transferida para corpos que não estão em contato com o corpo. A convecção é a transferência de calor para um gás ou líquido em movimento. A transferência de calor sempre ocorre em um gradiente térmico (de quente para frio) através destes três processos citados. Como humanos muitas vezes são os objetos mais quentes em um determinado ambiente, a direção normal da transferência de calor é do corpo humano para o ambiente, no entanto, à medida que a temperatura central do corpo aumenta, a perda de calor através da evaporação do suor se torna o mecanismo primário de dissipação de calor (Tansey; Johnson, 2015).

2.4 HIDRATAÇÃO E EXERCÍCIO FÍSICO

Como já dito, o principal fator limitante na capacidade de um humano manter a temperatura corporal diante de um estresse térmico é a disponibilidade de água corporal para a produção de suor. O resultado deste estresse térmico é a grande perda de água e desidratação durante o exercício, podendo atingir valores que variam conforme as condições ambientais, duração, intensidade e tipo do exercício físico realizado (Shirreffs *et al.*, 2005).

Dados da literatura mostram perdas em torno de 2,48% a 5,13% do peso corporal para atletas corredores e entre 1,68% a 3,66% para os ativos não atletas que realizaram 80 minutos de exercício em esteira entre 75% e 85% do $VO_{2máx}$ e conduzido em temperatura ambiente média de 21,9 °C e umidade relativa do ar em torno de 89,2% (Cheuvront; Kenefick, 2014). Shirreffs *et al.* (2005) mostraram que jogadores profissionais de futebol jogando a 34,98 °C e 35,4% de umidade relativa do ar perderam 2,5±0,88 kg de massa corporal, com desidratação de 3,38±1,11%, apresentando comprometimento do desempenho físico. Este fato é comprovado por Cheuvront; Kenefick (2014) que mostraram, após uma vasta revisão da literatura, que um limiar de desidratação $\geq$ 2% já compromete o desempenho físico, podendo levar também em

casos extremos a situações críticas como hipertermia, síncope ou internação. A importância da hidratação durante o exercício é evidenciada na medida que o aumento da temperatura central e mesmo pequenas reduções de água corporal em torno de 2% já comprometem o desempenho físico, pois uma maior produção de calor ou menor dissipação do calor durante o exercício antecipa a fadiga e exercícios realizados em ambientes quentes e úmidos provem maior desidratação, diminuição do débito cardíaco e consequentemente menor aporte de oxigênio para os músculos (Casa *et al.*, 2005).

As grandes perdas de água corporal na forma de suor tornam evidente a importância da hidratação durante o exercício, principalmente quando existe a exigência da performance em alto nível, como acontece com atletas profissionais (Casa *et al.*, 2005). Cheuvront *et al.* (2005) mostraram que atletas de endurance que foram hipohidratados antes do treino tiveram 7,6±5,9 % de redução no trabalho total realizado em 30 minutos de cicloergômetro a 50 % do $VO_{2máx}$ quando comparado aos atletas que se hidrataram de forma adequada. Corroborando com estes resultados, McCartney *et al.* (2017) realizaram uma meta-análise e revisão sistemática da literatura para mostrar os efeitos da ingestão de líquidos no desempenho físico de atletas hidratados e desidratados, e concluíram que o consumo de líquidos após a desidratação melhora a performance do exercício contínuo sob condições de estresse térmico, mesmo quando o déficit hídrico do corpo é modesto e a ingestão de líquidos é inadequada para a reidratação completa. A manutenção de um volume hídrico relativamente constante e de uma composição estável dos solutos dos líquidos corporais é essencial para a homeostasia do organismo, manutenção da temperatura corporal (Gonzalez-Alonso, 2012) e para garantir um ótimo desempenho no exercício (Lowe *et al.*, 2016).

A necessidade de se hidratar durante o exercício físico é influenciada por condições ambientais, principalmente temperatura e umidade relativa do ar (Burke, 2010), além de características da atividade física, como duração da sessão, intensidade do exercício e necessidade de vestimentas que interferem na termorregulação (ACSM, 2007). Para garantir que o indivíduo inicie o exercício euhidratado, recomenda-se que ele beba cerca de 250 a 500ml de água duas horas antes do exercício. Durante o exercício recomenda-se iniciar a ingestão já nos primeiros 15 minutos e continuar bebendo a cada 15 a 20 minutos. O volume a ser ingerido varia conforme as taxas de sudorese, geralmente entre 500 e 2.000ml/hora. Após o exercício, deve-se continuar ingerindo líquidos para compensar as perdas adicionais de água pela diurese e sudorese (SBME, 2009). Estes dados são confirmados pelo American College Sports Medicine que ainda trazem uma necessidade de reposição de líquidos em torno de 1,25L a 1,5L para cada 1kg de peso corporal perdido (ACSM, 2007).

A despeito destes protocolos para a hidratação serem sugeridos por conceituadas diretrizes, existem estudos experimentais na literatura que percorrem por uma outra linha de raciocínio. Hidratar-se de acordo com a sede pode ser benéfico para a performance física? Esta pergunta tem sido feita pela comunidade científica e ainda são escassos os estudos que abordam essa temática, portanto ainda não existe um corpo de evidência para uma resposta mais precisa. Como já dito, Cheuvront; Kenefick (2014) em sua revisão de literatura mostraram que ≥ 2% de perda de massa corporal pela desidratação é o limiar de comprometimento do exercício de resistência. Uma provável explicação para o impacto da desidratação nestas provas seria uma redução no $VO_{2máx}$ durante o exercício associada a uma redução do volume do fluido extracelular, o que resulta em necessidade de um maior esforço relativo (% de $VO_{2máx}$) ao executar o mesmo trabalho. Os autores concluem que ainda não se sabe o mecanismo exato pelo qual a força e a performance no exercício são alteradas pela desidratação, mas já se sabe que o grau de perda da performance é pequeno, não tão expressivo como se imaginava, segundo estes estudos. O potencial de desidratação para prejudicar a cognição durante o exercício também parece ser pequeno e pode estar relacionado simplesmente com a distração ou desconforto durante uma prova de *endurance.*

Confirmando esta ideia de beber de acordo com a sede e indo contra o que propõem as principais diretrizes de hidratação no exercício, Kenefick (2018) mostrou em uma recente revisão da literatura que, como a prática de ad libitum/beber de acordo com a sede parece resultar em substituição de fluidos em cerca de metade das perdas de líquidos (Cheuvront *et al.*, 2010), esta estratégia parece ser bem-sucedida para prevenção da hiponatremia e também na manutenção do peso corporal. O autor deixa claro que independente da escolha da forma de consumo (de acordo com a sede ou predeterminado) é importante nunca consumir uma quantidade de líquido que aumente a massa corporal, que é a principal crítica às diretrizes atuais, já que realmente parece ser discutível as propostas destas diretrizes que suportam a ideia de altas doses de hidratação para a manutenção da performance, pois é provável que o ganho de massa corporal com essa reidratação não seja benéfico para o desempenho físico, principalmente em provas de longa duração como o atletismo. Apesar disso, é importante destacar que, como já dito, essa linha de pensamento ainda carece de um corpo de evidência robusto para que conclusões definitivas possam ser tomadas, portanto, seguir diretrizes nacionais ou internacionais parece ser uma atitude mais prudente a ser feita por atletas e treinadores.

2.5 INFLUÊNCIA DE POLIMORFISMOS GENÉTICOS NA VARIABILIDADE INDIVIDUAL QUANTO A TERMORREGULAÇÃO DURANTE O EXERCÍCIO FÍSICO

Dentro desse contexto da termorregulação e reposição de líquidos, a literatura mostra que atletas respondem de maneira diferenciada em relação a resistência ao calor, principalmente em exercícios realizados em ambientes quentes. Verificou-se que, em uma resposta termorregulatória normal ao estresse por calor no exercício, há uma ampla gama de variabilidade (Frank *et al.*, 2001). Aqueles indivíduos que apresentam uma resposta de tensão mais severa ao exercício em ambientes quentes são definidos como menos tolerantes ao calor (Frank et al., 2001). A variabilidade na tolerância ao calor em humanos saudáveis tem se mostrado relacionada ao gênero (Sidman; Gallagher, 1995), aptidão física aeróbica (Havenith; Van Middendrop, 1990) e medidas antropométricas (Havenith *et al.*, 1995). No entanto, foi demonstrado que, mesmo após o ajuste desses fatores fenotípicos, as diferenças individuais na resposta ao estresse calórico existem (Havenith, 1997).

Esses indivíduos, especialmente entre a população jovem ativa, que são mais suscetíveis à hipertermia e correm o risco de serem acometidos por lesões causadas pelo calor. Quando as características fenotípicas não conseguem explicar a variabilidade na resposta ao estresse térmico, as características genotípicas tornam-se um candidato atraente (Heled *et al.*, 2004). Até onde se sabe, poucos são os estudos que avaliaram diretamente uma possível associação genotípica com a tolerância ao calor em modelos humanos ou animais. Apenas um estudo sugeriu que o polimorfismo ID da enzima conversora de angiotensina (ECA) humana está associado a diferenças individuais na tolerância ao calor durante o exercício (Heled *et al.*, 2004). Embora ainda controverso, foi relatado em uma série de artigos que o alelo de inserção (I) do polimorfismo ID no intron 16 do gene da ECA é associado a um melhor desempenho de endurance, enquanto o genótipo DD está associado a menor desempenho de endurance (Drozdovska *et al.*, 2013). O genótipo DD também foi associado com maiores níveis plasmáticos da ECA, enquanto que o polimorfismo II teve os níveis mais baixos de ECA (Ma *et al.*, 2013).

Também tem sido sugerido que o polimorfismo no gene da aquaporina 1 (AQP1), que são proteínas transmembranas que transportam água e glicerol através da membrana celular, influenciam a termorregulação corporal, mais especificamente o balanço de fluidos corporais. Rivera *et al.* (2011) mostraram que portadores do polimorfismo no gene da AQP1 tiveram um aumento significativamente maior na perda de fluidos corporais (3,7 ± 0,9 kg) do que os não-portadores deste polimorfismo (1,5 ± 1,1 kg). Os autores encontraram uma associação entre o

polimorfismo no gene da AQP1 e a perda aguda de fluidos corporais em corredores de longa distância.

Dando continuidade a esses polimorfismos genéticos propostos como influenciadores da termorregulação durante o exercício, também está sendo proposto o polimorfismo no gene da proteína de choque térmico, conhecida como *heat shock protein* 72 e 90 (Gibson *et al.*, 2016). Outro polimorfismo genético que tem sido sugerido como influenciador do sistema termorregulatório é o do gene do hormônio do crescimento (GH). Walpole *et al.* (2006) mostraram que temperaturas retais pós-corrida em corredores foram significativamente maior naqueles triatletas com um genótipo AA (37,7±0,8 °C) do que com aqueles com genótipo TT (37,2±0,8 °C). Com isso, os autores concluíram que temperaturas retais pós-corrida eram significativamente maior nos atletas de finalização mais rápida, que eram homozigotos para um genótipo GH associado com menor produção de hormônio de crescimento.

2.6 ATIVIDADE DA ENZIMA CYP1A2, POLIMORFISMO NO GENE DA CYP1A2 E INTERAÇÃO COM NUTRIENTES

A CYP1A2 é uma enzima encontrada em abundância no fígado. Pertence à família de enzimas do citocromo P450 e desempenha um papel crucial no processo de desintoxicação do corpo humano, metabolizando várias substâncias, incluindo medicamentos, toxinas e certos componentes de alimentos e bebidas, com destaque para a cafeína, principal nutriente metabolizado pela CYP1A2 (Gougis *et al.*, 2021). Ao participar do processo de metabolização destas substâncias, a CYP1A2 regula a velocidade com que estes compostos estarão disponíveis no sangue, portanto a atividade da CYP1A2 determina se os efeitos dos seus substratos no organismo serão curtos ou prolongados (Castorena-Torres *et al.*, 2005).

Acredita-se que a atividade da enzima CYP1A2 seja influenciada por vários fatores, incluindo estilo de vida, medicamentos e genética, como também sua biodisponibilidade está diretamente relacionada ao metabolismo destes compostos químicos (Gougis *et al.*, 2021). Além destes fatores influenciadores, a literatura tem mostrado que nutrientes possuem a capacidade de influenciar a atividade da enzima CYP1A2. A Tabela 1 traz estudos que relacionaram fitoterápicos e a enzima CYP1A2. Além disso, Gougis *et al.* (2021) mostraram a influência de 261 ervas, alimentos e suplementos dietéticos na atividade da CYP1A2, inclusive a hortelã-pimenta (Maliakal; Wanwimolruk, 2001) e o seu principal composto ativo, o mentol isolado (Feng *et al.*, 2019), ambos inibindo de forma moderada sua atividade em modelo animal. Esta inibição pode diminuir o metabolismo de nutrientes que atuam como substratos

para a enzima CYP1A2, uma vez que o mentol pode interagir com essas substâncias, como de fato já foi demonstrado que uma única dose oral de mentol diminuiu a depuração de cafeína no fígado gerando níveis plasmáticos mais elevados (Gelal *et al.*, 2003).

Álcool, flavonoides (quercetina) e vegetais apiáceos (cenoura, aipo, endro, salsa e nabo) também são inibidores da enzima CYP1A2 (Peterson *et al*., 2009; Yeh; Wu, 2006; Xiao *et al*., 2014; Gazzaz *et al.,* 2018). Enquanto isso, o aumento da atividade da CYP1A2 foi observado após a ingestão de cafeína (Djordjevic *et al.,* 2010) e vegetais crucíferos (brócolis) (Kall *et al.,* 1996; Lampe *et al.,* 2018). Esta interação de inibição e indução deixa clara a relação que os alimentos têm com a atividade desta enzima.

Nenhum estudo avaliou a interação do OEHP com a atividade da enzima CYP1A2, existindo apenas estudos com o mentol isolado (Gelal *et al.*, 2003; Feng *et al.*, 2019) e o chá de hortelã (Maliakal; Wanwimolruk, 2001; Begas *et al.*, 2017). Gelal *et al*. (2003) realizaram um trabalho para determinar se o mentol afeta o metabolismo da cafeína, um substrato do citocromo P450 1A2 (CYP1A2) e as respostas farmacológicas da cafeína nos indivíduos. Para isso, onze mulheres saudáveis participaram de um estudo que comparou a cinética e os efeitos de uma dose oral única de cafeína (200 mg) junto com uma dose oral única de mentol (100 mg) ou cápsulas de placebo. A coadministração de mentol resultou num aumento do tempo para atingir a concentração máxima de cafeína de 43,6±20,6 min para 76,4±28,0 min ($p<0,05$). Os valores de concentração máxima de cafeína foram menores na fase mentol do que na fase placebo, mas esse efeito não foi estatisticamente significativo ($p=0,06$). Os autores concluíram que uma dose oral única de mentol puro (100 mg) diminuiu a depuração de cafeína no fígado gerando níveis plasmáticos mais elevados desta substância. Sabe-se que mudanças na quantidade de cafeína plasmática refletem a atividade da CYP1A2 in vivo (Miners; Birkett, 1996), então, o fato de o mentol gerar níveis plasmáticos mais elevados de cafeína, pode impactar em maiores atividades da enzima CYP1A2, e a depender de qual genótipo para este gene o indivíduo possui, as repostas podem ser diferentes.

Enquanto isso, Feng *et al.* (2019) objetivaram investigar o comportamento farmacocinético do L-mentol após inalação (50 mg/kg) e injeção intravenosa (10 mg/kg), bem como sua influência nas atividades das enzimas do citocromo P450, dentre elas a CYP1A2. Concluíram que L-mentol teve inibições moderadas no CYP1A2, o que pode afetar a metabolização de nutrientes dependentes principalmente desta via. Begas *et al.* (2017) investigaram o efeito do chá de hortelã-pimenta nas atividades do CYP1A2, CYP2A6, Xantina Oxidase (XO), N-acetiltranferase-2 (NAT2) e UDP-glucuronosiltransferases-1A1/1A6 (UGT1A1/1A6) em indivíduos saudáveis. Quatro homens e cinco mulheres consumiram chá de

hortelã-pimenta (2 g de folhas secas/200 ml de água, duas vezes ao dia) durante seis dias. As atividades de CYP1A2, CYP2A6, XO, NAT2 e UGT1A1/1A6 foram determinadas antes e no final do período de estudo. Os índices de urina e saliva de CYP1A2 foram reduzidos, ainda que não significativamente, após o consumo do chá de hortelã-pimenta (urina: 3,17 ± 1,08 vs 2,91 ± 0,76, saliva: 0,56 ± 0,12 vs 0,50 ± 0,12; $p > 0,05$).

Maliakal e Wanwimolruk (2001) investigaram o efeito de chás de ervas (hortelã-pimenta, camomila e dente-de-leão) na atividade de enzimas metabolizadoras hepáticas de fase I e fase II. Ratas Wistar fêmeas foram divididas em seis grupos (n = 5 cada). Três grupos tiveram livre acesso a uma solução de chá (2%) enquanto o grupo controle teve água. Dois grupos receberam extrato de chá verde (0,1%) ou solução aquosa de cafeína (0,0625%). Após quatro semanas de pré-tratamento, diferentes isoformas do citocromo P450 (CYP) e atividades enzimáticas de fase II foram determinadas por incubação de microssomas hepáticos ou citosol com substratos apropriados. A atividade do CYP1A2 nos microssomas hepáticos de ratos que receberam chá de dente-de-leão, hortelã-pimenta ou camomila diminuiu significativamente ($p < 0,05$) para 15%, 24% e 39% do valor de controle, respectivamente. A atividade do CYP1A2 foi significativamente aumentada pelo pré-tratamento com solução de cafeína. Os resultados sugeriram que estes chás de ervas podem causar modulação das enzimas metabolizadoras de medicamentos de fase I e fase II.

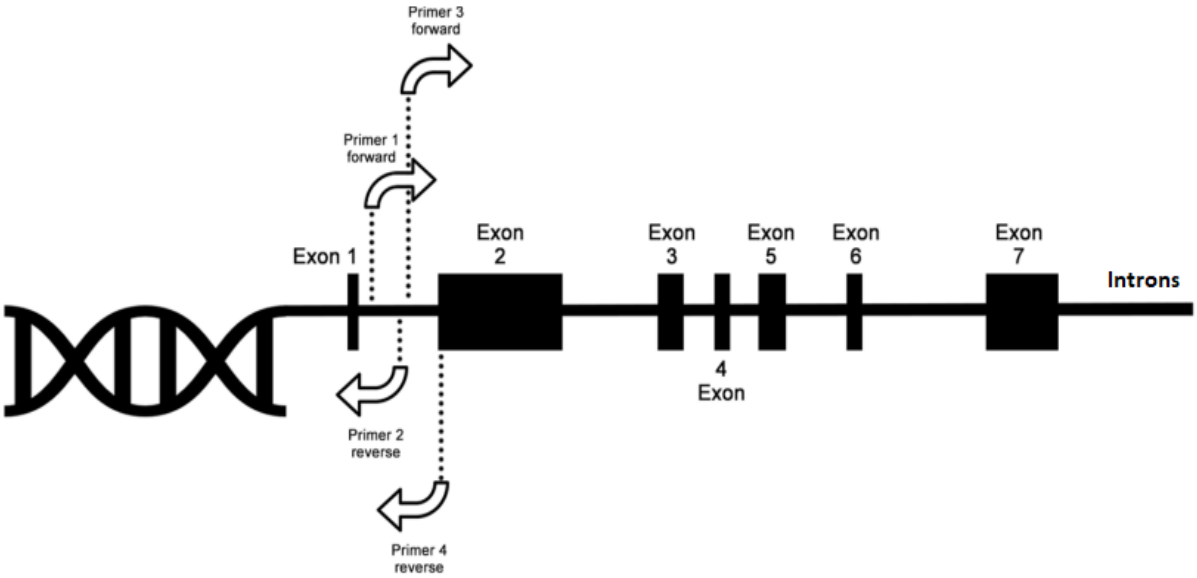
Tabela 1. Indução/inibição de fitoterápicos na expressão da enzima CYP1A2.

Nome da planta em Latim	Nome comum	CYP1A2
Mentha piperita	Mentol / Hortelã-pimenta	Inibição Feng *et al.* (2019) / Maliakal; Wanwimolruk (2001) Sem interação Gelal *et al.* (2003) / Begas *et al.* (2017)
Allium sativum	Alho	Sem interação Gurley *et al.* (2002) / Gurley *et al.* (2005)
Camellia sinensis	Chá verde	Sem interação Chow *et al.* (2006)
Citrus paradisi	Toranja	Inibição Fuhr *et al.* (1993) / Fuhr *et al.* (1995) Vande *et al.* (2000) / Lane *et al.* (2001)
Crataegus monogyna	Espinheiro	Inibição Bi *et al.* (2013)
Curcuma longa	Cúrcuma	Inibição Chen *et al.* (2010)
Ginkgo Biloba	Ginkgo	Sem interação Gurley *et al.* (2002) / Gurley *et al.* (2005) Reed *et al.* (2007) / Zadoyan *et al.* (2012)
Glycine max	Soja	Inibição Peng *et al.* (2003) / Chen *et al.* (2011)

Hypericum perforatum	Erva de São João	Sem interação Gurley *et al.* (2002) / Gurley *et al.* (2005) Morimoto *et al.* (2004)
Lycium barbarum	Goji	Inibição Eck; Shah (2009)
Matricaria recutita	Camomila (alemã)	Inibição Maliakal *et al.* (2001) / Ganzera *et al.* (2006)
Morinda citrifolia	Noni	Inibição Eck; Shah (2009)
Panax ginseng	Ginseng (asiático)	Sem interação Gurley *et al.* (2002) / Gurley *et al.* (2005) Kim *et al.* (2016)
Piper methysticum	Kava kava	Sem interação Gurley *et al.* (2005)
Stevia rebaudiana	Estévia	Indução Dusek *et al.* (2017)
Vitis vinifera	Uva/vinho	Indução Xiao Dong *et al.* (1999)
Zingiber officinale	Gengibre	Indução/ Inibição (Dados conflitantes) Brandin *et al.* (2007) / Kim *et al.* (2017) Kim *et al.* (2012) / Pandit *et al.* (2012) Mukkavilli *et al.* (2014)

A atividade da enzima CYP1A2 em humanos também pode ser influenciada pela variação genética. Evidências acumularam-se nos últimos anos implicando um polimorfismo do gene da CYP1A2 nas alterações da atividade desta enzima, levando a diferentes respostas fenotípicas. Este polimorfismo de nucleotídeo único (rs762551, -163A>C) ocorrendo na 734ª posição do primeiro íntron do gene CYP1A2 (Figura 2), uma região não codificadora, influencia a expressão de CYP1A2, criando três genótipos principais: AA homozigoto, AC heterozigoto e CC homozigoto (Sachse *et al.*, 1999). Tem sido mostrado que os homozigotos AA metabolizam a cafeína mais rapidamente que os portadores do alelo C (AC/CC), com os homozigotos CC metabolizando a uma taxa mais lenta que os heterozigotos AC (Sachse *et al.*, 1999; Castorena-Torres *et al.*, 2005). Assim, podemos categorizar os indivíduos como metabolizadores rápidos (AA), intermediários (AC) e lentos (CC) da cafeína, por exemplo, que é a principal substância envolvida na atividade da enzima CYP1A2.

Figura 2. Gene da CYP1A2 transcrito (mRNA). Os íntrons são representados como linhas e os éxons são representados como linhas verticais e caixas. As setas são as posições dos *primers* para identificação da sequência.



Fonte: Barreto *et al.* (2021) modificado.

Não existem estudos avaliando o fenótipo do polimorfismo na metabolização da hortelã-pimenta ou do mentol isolado. Entretanto, estudos com cafeína são relativamente abundantes. O primeiro estudo a investigar a influência do polimorfismo da CYP1A2 (rs762551) nos efeitos ergogênicos da suplementação aguda de cafeína avaliou 35 ciclistas recreacionais homens em um contra-relógio de ciclismo de 40 km (Womack *et al.*, 2012). Houve uma maior melhora no tempo de prova com cafeína em homozigotos AA (+4,9%, p<0,001) em comparação com portadores do alelo C (+1,8%, p=0,04). No entanto, o desempenho também melhorou significativamente com a cafeína para portadores do alelo C (Womack *et al.*, 2012). Outros estudos mostraram um padrão semelhante, com maior vantagem para os homozigotos AA sobre os portadores do alelo C. Homozigotos AA melhoraram o desempenho em exercícios de resistência com uma dose de 6 mg/kg de cafeína, mas os portadores do alelo C não (Rahimi, 2019), enquanto o desempenho no contra-relógio de ciclismo de 10 km foi melhorado com 2 e 4 mg/kg de cafeína nos genótipos AA, mas não nos genótipos AC ou CC (Guest *et al.*, 2018).

No entanto, os dados atuais que apoiam a influência do polimorfismo de nucleotídeo único CYP1A2 nos efeitos ergogênicos da cafeína ainda não são consensuais, uma vez que estes resultados foram replicados em outros estudos que mostram que não há diferença na eficácia da suplementação de cafeína entre genótipos para o desempenho no basquete (Puente *et al.*, 2018), no tênis (Klein *et al.*, 2012), no ciclismo de 3 km (Giersch *et al.*, 2018) e um contra-relógio de 30 minutos (Glaister *et al.*, 2020) e no handebol (Muñoz *et al.*, 2020).

3 MATERIAIS E MÉTODOS

3.1 TIPO DE PESQUISA E SUJEITOS DO ESTUDO

O estudo é de tipo experimental, duplo-cego, controlado e *cross-over* com a ordem dos tratamentos de forma aleatória. Desenvolvido com protocolos de respostas aguda à intervenção nutricional com o óleo essencial de hortelã-pimenta, com 40 corredores recreacionais do sexo masculino, com idade entre 18 e 50 anos. Como critério de inclusão, os atletas deveriam ser recreacionais, ou seja, possuir $VO_{2máx}$ menor que os atletas de elite (Kusy; Zieliński, 2012), deveriam ter no mínimo um ano de treinamento, com frequência semanal de cinco treinos, dos quais no mínimo três deveriam ser de corrida, e estar treinando para alguma competição há pelo menos quatro meses ininterruptamente na temporada, além de treinarem um volume semanal de 50km percorridos e terem um tempo na prova dos 10km abaixo de 60 minutos. Não poderiam apresentar nenhuma doença crônico-degenerativa reconhecida, não deveriam ser tabagistas ou fazer uso contínuo de qualquer medicamento. Não deveriam ter hábito de consumir regularmente hortelã e seus derivados, assim como qualquer bebida alcoólica ou suplementos alimentares vitamínicos ou que contenham as substâncias ativas presentes na hortelã-pimenta (polifenois, flavonoides, carotenos, mentol e mentona). Seriam excluídos do estudo os voluntários que modificassem o padrão habitual de alimentação ou de treinamento físico ao longo dos experimentos, não consumissem a quantidade correta das bebidas fornecidas, apresentarem intolerância gastrointestinal e não participarem de todos os procedimentos experimentais.

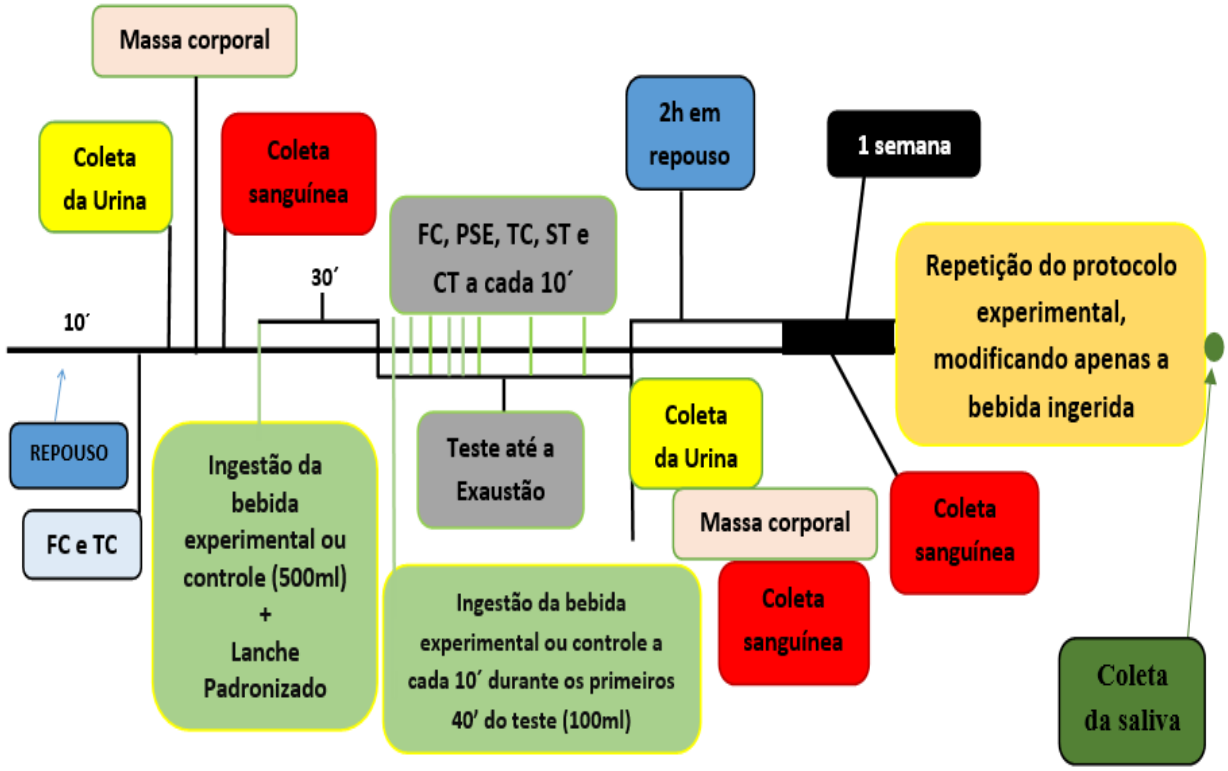
3.2 ASPECTOS ÉTICOS

Este projeto foi previamente submetido ao Comitê de Ética em Pesquisa com seres humanos do Centro de Ciências da Saúde da Universidade Federal da Paraíba, Brasil, sob protocolo nº CAAE 17130619.2.0000.5188. Também está publicado no Registro Brasileiro de Ensaios Clínicos (ReBEC) com o número RBR-75zt25z. Todos os participantes foram previamente esclarecidos quanto aos procedimentos e assinaram o termo de consentimento livre e esclarecido (TCLE) de acordo com resolução 466/12 do Conselho Nacional de Saúde (APÊNDICE E).

3.3 DESENHO DO ESTUDO

O OEHP foi testado em resposta a uma dose única em formato *cross-over*. Os voluntários participaram randomicamente (www.randomizer.org) de dois procedimentos, sendo um experimental com a ingestão de 0,05ml do OEHP diluído em 500ml de água (MINT) e como controle um placebo isovolumétrico de água com 0,05ml da essência de hortelã (PLACEBO). Como mostrado na Figura 3, os voluntários ingeriram randomicamente uma dose da bebida experimental ou controle, e, 30 minutos depois realizaram uma sessão de corrida até a exaustão a 70% do $VO_{2máx}$, mantendo a ingestão da bebida durante a realização do teste, 100ml a cada 10 minutos nos primeiros 40 minutos. Após um *washout* de pelo menos sete dias, eles repetiram o procedimento substituindo a bebida. O tempo até a exaustão foi registrado. Medidas de temperatura corporal (TC) e Frequência Cardíaca (FC) foram feitas antes (repouso) e a cada 10 minutos durante o teste até a exaustão, assim como imediatamente após o seu término. Medidas de sensação térmica (ST), conforto térmico (CT) e percepção subjetiva de esforço (PSE) foram realizadas durante o teste físico, também a cada 10 minutos. Coleta da urina e registro da massa corporal foram feitas antes e após o exercício. A taxa de sudorese (TS) foi calculada. Antes da ingestão da bebida experimental ou controle (repouso), imediatamente ao final e duas horas após o teste até a fadiga, coletas sanguíneas foram realizadas para dosagem de marcadores de estresse oxidativo [Malondialdeído (MDA) e Capacidade Antioxidante Total (CAT)]. Coleta de uma amostra da mucosa bucal para a análise genética foi feita após finalizados todos os protocolos experimentais.

Figura 3 – Desenho do Estudo



Fonte: Desenvolvido pelo pesquisador responsável do estudo.

3.4 AVALIAÇÃO FÍSICA E NUTRICIONAL

A avaliação física foi feita em dois momentos, em repouso imediatamente após a primeira coleta da urina e após o teste até a exaustão, mais especificamente após a coleta da urina pós exercício. Esta avaliação foi feita através de uma balança com analisador de composição corporal por bioimpedância tetrapolar InBody 570 (InBody Bldg. Co., Ltd. Seul, Coréia do Sul). Foi feito o registro da massa corporal total pré e pós exercício além dos percentuais de gordura, músculo, água e osso.

Para a avaliação nutricional, foi aplicado um recordatório alimentar de 24h (Gibson, *et al*. (1990) (APÊNDICE F) previamente a cada sessão experimental, visando quantificar todos os alimentos e bebidas ingeridos no dia anterior aos protocolos experimentais. Foi avaliada a quantidade do consumo de energia (kcal), macronutrientes e micronutrientes (sódio e potássio) na distribuição das refeições. Foram considerados como referência para a adequação do consumo dietético os limites propostos pelas Dietary Reference Intakes (DRI's) sugeridos pelo Institute of Medicine (IOM, 2000).

Os voluntários foram orientados a não ingerir qualquer suplemento esportivo, assim como não ingerir bebidas alcoólicas ao longo de todo o estudo. Foi também solicitado que os atletas realizassem um *washout* para alimentos que contenham compostos bioativos similares aos da hortelã-pimenta e a não utilizassem nenhum novo alimento rico em antioxidantes durante o estudo, a fim de assegurar a exclusão de quaisquer efeitos associados à alimentação prévia sobre o procedimento experimental. O jejum prévio aos protocolos experimentais foi usado para amenizar o impacto da dieta individual nos resultados. No período do estudo, foram orientados a manter e não modificar seus padrões alimentares habituais.

3.5 PROTOCOLO DE EXERCÍCIO (TESTE ATÉ A EXAUSTÃO)

Uma semana antes dos ensaios experimentais (testes até a exaustão), os corredores realizaram um teste de capacidade aeróbia máxima e limiar anaeróbio, determinados por meio do protocolo proposto por Weltman *et al.* (1987) para um teste de corrida de 3.200 metros em pista aberta de atletismo. Este teste prévio tinha como objetivo determinar de forma indireta o $VO_{2máx}$ dos atletas e a partir desta capacidade aeróbia máxima ser calculada a velocidade em que cada um dos atletas iriam correr na esteira nos dois protocolos experimentais.

Os testes até a exaustão foram realizados no Laboratório de estudos do treinamento físico aplicado ao desempenho e a saúde (LETFADS) da Universidade Federal da Paraíba, Brasil, como parte integrante das sessões experimentais. Os corredores foram orientados a abster-se de qualquer exercício físico durante as 48h que antecedessem um teste que determinou a capacidade aeróbia e o limiar anaeróbio, bem como antes das sessões experimentais. Ocorreram 30 minutos após a ingestão da bebida. Foi realizado com base no que foi descrito por Toscano *et al.* (2015), consistindo de uma corrida em esteira ergométrica à 70% do $VO_{2máx}$, com velocidade constante e determinada com base no teste prévio proposto por Weltman *et al.* (1989), com o objetivo de manter esta velocidade pelo maior tempo possível, ou seja, até a exaustão. O critério de interrupção do teste foi à incapacidade de manter-se na velocidade determinada, mesmo diante de estímulo verbal dos pesquisadores, além da confirmação verbal pelo corredor e uma referência entre 19 e 20 na Escala de Percepção Subjetiva de Esforço de Borg (1982) (ANEXO A). Para controle da intensidade do exercício, foi registrada a FC por um cardiofrequencímetro da marca Polar RS800CX (Polar Electro Oy, Kempele, Finland) e a PSE de acordo com a escala de Borg (1982) de 6 a 20. O resultado do teste até a exaustão foi expresso em minutos e segundos de corrida. O *crossover* ocorreu com pelo menos sete dias

depois. O exercício foi realizado em ambiente laboratorial com temperatura ambiente controlada e umidade relativa do ar registrada.

3.6 PROTOCOLO DE SUPLEMENTAÇÃO

O produto que foi ingerido pelos atletas neste estudo é o óleo essencial de uma planta (*Mentha piperita* L.) comestível e culturalmente utilizada pela população na forma in natura e em forma de chás e adicionada a saladas, sucos. Para o procedimento experimental, foi utilizado 0,05ml de óleo essencial puro de hortelã-pimenta comercial da marca Bio Essência (Jaú, São Paulo, Brasil) para cada dose líquida de 500 ml de água mineral (Figura 4). Para o placebo foi utilizado este mesmo volume de água mineral adicionado de 0,05 ml da essência líquida de hortelã comercial da marca Tenda da Alma (Paio Pires, Aldeia de Paio Pires, Portugal) (sem as propriedades bioativas do óleo essencial de hortelã-pimenta) (Figura 5).

A dose de hortelã-pimenta foi baseada em estudos prévios onde não foram observados efeitos tóxicos ou algum desconforto gástrico pelos voluntários após a ingestão do óleo essencial de hortelã-pimenta, como também não foram relatados desconforto quanto ao gosto ou odor intensos que impossibilitassem a ingestão (Mearmabashi; Rajabi, 2013; Mearmabashi, 2014).

No dia dos dois testes até a exaustão, os corredores chegaram ao laboratório e ingeriram 500 ml da bebida experimental ou controle 30 minutos antes da realização do exercício, assim como durante a realização do teste, 100ml a cada 10 minutos nos primeiros 40 minutos. Todos os corredores chegaram ao laboratório no início da manhã, estavam em jejum de 8h. No laboratório, foi fornecido desjejum padronizado composto de 2 torradas (20g de carboidratos, 6g de proteínas, 2g de gorduras totais, 3g de fibra alimentar e 140mg de sódio) e 250ml de suco artificial sabor laranja (25g de carboidratos, sem vitaminas e minerais e sem os compostos bioativos da hortelã-pimenta) sendo ingeridos 30 minutos antes do exercício.

Figura 4 – Produto comercial composto de Óleo Essencial de Hortelã-pimenta

Fonte: http://loja.bioessencia.com.br/pd-16b4b2-oleo-essencial-de-hortela-pimenta.html

Figura 5 – Produto comercial composto de essência de Hortelã-pimenta

Fonte:https://www.tendadaalma.com/pt/shop/essencias-e-aromaterapia/essencia-de-hortela-pimenta-detail

3.7 MEDIÇÃO DA TEMPERATURA CORPORAL, SENSAÇÃO TÉRMICA E CONFORTO TÉRMICO

Medidas de temperatura central foram realizadas através de termômetro timpânico digital da marca Incoterm Color Check Infravermelho Auricular (Modelo 29838, Radiant Innovation Inc/China; Registro ANVISA: nº 10343200015) (Figura 6), antes (sujeito sentado em repouso) e a cada 10 minutos durante o teste até a exaustão, assim como imediatamente após o seu término (sujeito sentado). A medição foi realizada orientando a ponta do termômetro dentro do canal auditivo, de tal forma que siga a anatomia natural do ouvido (Davis *et al.*, 2014). No momento da medição, os corredores não pararam de correr e durante a própria corrida eles mesmos mediam a sua temperatura corporal introduzindo o termômetro na cavidade auditiva. Foi realizada com a supervisão do pesquisador para minimizar erros de medição. Após esse

procedimento era registrada a temperatura corporal em aproximadamente 5 segundos, de modo que não atrapalhou o andamento do exercício**.**

As variáveis de sensação térmica e conforto térmico foram registradas a cada 10 minutos durante a realização do teste até a exaustão. Para a sensação térmica, foi utilizada a escala subjetiva de sete pontos baseada em Hodder; Parsons (2007), a qual é composta por uma escala que vai de 1 (ligeiramente frio) até 7 (extremamente quente) (ANEXO B). Para o conforto térmico, foi utilizada a escala subjetiva de quatro pontos baseada em Hodder; Parsons (2007), a qual é composta por uma escala que vai de 1 (sem desconforto) até 4 (muito desconfortável) (ANEXO C).

Figura 6 - Termômetro Timpânico usado no estudo

Fonte: https://www.google.com.br/imagens/termometrotimpanicoincoterm

3.8 TAXA DE SUDORESE

A taxa de sudorese (TS) foi calculada de acordo com a seguinte equação proposta por Murray (1996) para atletas:

$$\text{Taxa de sudorese } (mL.min^{-1}) = [(IM - FM) + FI - U] \div T \times 100$$

Em que: IM: massa corporal inicial em gramas; FM: massa corporal final em gramas; FI: volume de líquido ingerido em mililitros; U: volume de urina produzido em mililitros; T: tempo de duração do exercício em minutos. Após a obtenção da TS em mililitros/minuto ($mL.min^{-1}$), foi realizada a conversão para litros/hora ($L.h^{-1}$).

3.9 COLETA E ANÁLISE DA URINA

A urina foi coletada em repouso antes do teste de exaustão e imediatamente após este teste. Os participantes foram instruídos a esvaziar a bexiga em ambos os momentos. Eles receberam frascos plásticos estéreis com medidor de volume e foram orientados sobre os procedimentos adequados para coleta de urina, minimizando o risco de contaminação e perda de volume. Após a coleta, foi registrado o volume e feita a determinação da densidade da urina. Esta densidade foi determinada da seguinte forma: aproximadamente 40µl de cada amostra de urina foram analisados utilizando um refratômetro digital modelo RTP-20ATC (Instrutherm®, São Paulo, Brasil) para determinar a gravidade específica da urina (USG). Para classificação da hidratação, foram considerados os valores de referência descritos por Casa *et al.* (2005), conforme mostrado na Tabela 2.

Tabela 2. Parâmetros para determinar o estado de hidratação de acordo com a USG

Status de hidratação	**USG (UOsmol)**
Bem hidratado	< 1010
Desidratação mínima	1010 – 1020
Desidratação significativa	1021 – 1030
Desidratação severa	> 1030

Fonte: CASA et al. (2005) - *National Athletic Trainers' Association – NATA.*

3.10 COLETAS E ANÁLISES SANGUÍNEAS

Foram coletados 10 ml de sangue venoso de cada voluntário por uma enfermeira treinada e experiente. Os corredores deveriam abster-se de qualquer exercício físico durante as 48h que antecedem a coleta. As amostras foram centrifugadas a 3000rpm por 10 minutos e o sobrenadante (soro ou plasma) transferido para microtubos e refrigerado a -20°C até as análises. Amostras sanguíneas foram coletadas no momento basal agudo antes do início da suplementação, imediatamente após o teste até a fadiga e 2h após terminado esse teste sendo repetida essas coletas no segundo protocolo experimental.

3.10.1 ESTRESSE OXIDATIVO

3.10.1.1 Malondialdeído (MDA)

Os níveis de peroxidação lipídica foram quantificados no plasma, de acordo com Ohkawa *et al.* (1979). A atividade oxidante foi quantificada a partir da peroxidação lipídica do MDA, através da reação do ácido tiobarbitúrico (TBARS), com os produtos de decomposição dos hidroperóxidos. Em seguida, as amostras foram incubadas em banho maria a 37° durante 60 minutos. Posteriormente, o precipitado das amostras foi misturado com ácido perclórico (35%) e as amostras centrifugadas a 14000 rpm por 10 minutos à 4°C. O sobrenadante foi transferido para novas alíquotas e adicionado 400µl de ácido tiobarbitúrico (0,6%) e incubado a 100°C por 60 minutos. Após o resfriamento, o material final foi lido em espectrofotômetro (Bioespectro, modelo SP 22, Brasil), com um comprimento de onda de 532nm.

3.10.1.2 Capacidade Antioxidante Total (CAT)

A análise da CAT foi baseada em Brand-Williams *et al.* (1995). Uma alíquota de 1,25 mg de 2,2 diphenyl-1-picrylhydrasyl (DPPH) foi diluída em 100 ml de etanol (99,5%), mantida sob refrigeração e protegida da luz. Foram adicionados 3,9 ml da solução de DPPH a 100 µl de plasma em tubos, que em seguida foram agitados em vórtex e deixados em repouso por 30 minutos. Posteriormente, foram centrifugados a 10.000 rpm à 20ºC por 15 minutos e o sobrenadante utilizado para a realização da leitura em espectrofotômetro (Biospectro SP-22, Curitiba, Brasil), com um comprimento de onda de 515 nm. Os resultados foram expressos como percentual da atividade antioxidante (AOA): AOA=100–[DPPH•R]t/[DPPH•R]B100) onde, [DPPH•R]t e [DPPH•R]B correspondem as concentrações de DPPH• remanescente após 30 minutos, avaliadas na amostra (t) e no branco (B), o qual foi utilizada água destilada.

3.11 AVALIAÇÃO GENÉTICA

3.11.1 Coleta e extração de DNA da mucosa bucal

Amostras de células bucais foram coletadas por meio de bochecho, por 60 segundos, de 5 ml de solução de sacarose a 3%. O conteúdo resultante foi transferido para um tubo de 15 ml, sendo adicionado logo em seguida 3ml de uma solução de TNE (Tris-HCl 17 mM pH 8,0, NaCl

50 mM e EDTA 7mM), diluída em álcool 70% e água destilada autoclavada. As amostras eram armazenadas sob refrigeração até o processo de extração, em até 30 dias.

O procedimento de extração seguiu Aidar; Line (2007). Iniciou com a adição de TNE em cada tubo, até o equilíbrio dos volumes entre os tubos. Após este processo, os tubos foram centrifugados a 3000 rpm por 10min para descarte do sobrenadante e adicionado 1ml de TNE a cada amostra. Nova centrifugação foi realizada, a 3000 rpm durante 5min. O sobrenadante foi descartado e o precipitado de cada amostra foi ressuspendido em 1ml de solução de lise (Tris-HCl 10 mM pH 8, duodecil sulfato de sódio 0,5%, EDTA 5 Mm) e levado ao vórtex para soltar o precipitado. Foi feito um mix de 290µl solução de lise e 10 µl de proteinase K (Invitrogen, Carlsbad, CA, EUA), sendo o volume total do mix adicionado em cada amostra.

As amostras foram incubadas a 55 ºC e após 12 horas de incubação as amostras foram transferidas para microtubos de 2ml, acrescentando 500 µl de acetato de amônio 7,5 M em cada amostra. Após as amostras serem homogeneizadas em vórtex, eram levadas para centrifugação refrigerada por 10 min a 15000 rpm e 4 ºC. Após centrifugadas, as amostras eram separadas em duplicata de 900 µl cada e adicionadas em microtubos de 1,5ml sendo adicionado 540 µl de isopropanol em cada amostra para a precipitação do DNA. Os microtubos eram invertidos manualmente 20 vezes para homogeneização e logo após levados para centrifugação refrigerada por 5 min a 15000 rpm e 4 ºC. O sobrenadante foi desprezado e o precipitado de DNA foi lavado com 1ml de etanol 70% em cada amostra, e após novo processo de inversão por 20 vezes passavam novamente por centrifugação refrigerada por 5 min a 15000 rpm e 4 ºC. O sobrenadante era novamente descartado e após retirado todo o líquido do microtubo, eram colocados para secar por 1 hora e meia. Após seco, eram adicionados 40 µl de solução tampão de TE (TE Buffer Tris-EDTA). Após isso, era batido o micro tubo com o dedo até o DNA desprender da parede do micro tubo. Finalizado o processo de extração, as amostras eram armazenadas congeladas a -20 °C até o processo de genotipagem.

3.11.2 Genotipagem do polimorfismo do gene da CYP1A2

Após isolado, o DNA foi submetido à amplificação através da reação em cadeia da polimerase (PCR) com os iniciadores 5'- GGAAGGTATCAGCAGAAAGCC-3´ e 5'-GGCTCATCCTTGACAGTGCC3'. Para a realização da PCR, as amostras foram preparadas e colocadas no termociclador nas seguintes condições: desnaturação de 10 minutos à 95 ºC, seguida de 35 ciclos de 30 segundos à 95 ºC, 66 ºC por 30 segundos e 72 ºC por 30 segundos. Etapa final de extensão de 72 ºC por 10 minutos foi programada e seguida de incubação a 4 ºC.

O produto gerado foi digerido (Anza™ 32 ApaI) seguindo recomendações do fabricante (Invitrogen by Thermo Fisher Scientific, Made in Lithuania), utilizando banho seco para controlar temperatura (37 ºC) e tempo (3 horas). Como resultado da digestão, o alelo A produz fragmento de 626 pb e o alelo C 181 e 445 pb. Para heterozigotos, as três bandas estão presentes (Christiansen *et al.,* 2000). Foi realizada eletroforese em poliacrilamida à 10%, com as amostras adicionadas de corante blue juice e gel red. A corrida ocorreu a 80 v por 120 minutos e o gel foi revelado a partir da visualização por transiluminador e coloração (nitrato de prata).

3.12 ANÁLISE ESTATÍSTICA

Os dados estão expressos como média e erro padrão da média. Inicialmente, foram testadas a normalidade e homogeneidade por meio dos testes de Shapiro-Wilk e Levene respectivamente. As análises foram feitas dividindo-se de acordo com o genótipo, considerando a presença ou ausência do alelo com a característica do polimorfismo. Os atletas foram divididos com base na presença do alelo C (genótipos AC+CC) ou genótipo AA para o polimorfismo da CYP1A2. Inicialmente, foi realizado o teste T pareado para comparar os dados basais dos corredores e o tempo até a exaustão geral (n=40), além disso, após subdivisão por genótipos, teste T independente foi usado para comparar os dados basais dos voluntários e o percentual de variação no tempo até a exaustão. Para a análise das medidas de SPE, delta da temperatura corporal, sensação térmica, conforto térmico, taxa de sudorese e os momentos pré e pós para volume e densidade urinária e massa corporal total perdida, adotou-se ANOVA de duas vias para medidas repetidas, com *post-hoc* de Bonferroni, considerando a esfericidade dos dados. Quando necessário, foi utilizado correspondente não paramétrico para os dados que não apresentaram distribuição normal, assim como os *outliers* foram excluídos. Além disso, foi realizada uma análise individual do tempo até a exaustão para identificar quantos atletas melhoraram seu desempenho físico. Teste Qui-quadrado foi feito para comparar a presença ou ausência dos alelos e a melhora ou não da performance física. *Effect size* foi calculado usando o teste d de Cohen para amostras dependentes e classificado de acordo com Cook *et al.* (2018) em pequeno ($d < 0,40$), médio ($d = 0,40 - 0,79$), grande ($d = 0,80 - 1,29$) ou muito grande ($d \geq 1,30$). Eta quadrado (η^2) foi usado como medida do tamanho do efeito para ANOVA, classificado em pequeno ($\eta^2 = 0,02 - 0,15$), médio ($\eta^2 = 0,15 - 0,35$) e grande ($\eta^2 > 0, 35$) (Cook *et al.,* 2018). Todas essas análises foram realizadas por meio do software Jamovi [The jamovi Project (2021), version 1.6, Sydney, Australia], adotando significância de $p<0,05$.

4 RESULTADOS

Os resultados desta tese são apresentados na forma de dois artigos originais e um artigo de revisão sistemática.

O primeiro artigo original apresentado no APÊNDICE A [PEPPERMINT ESSENTIAL OIL (Mentha piperita L.) INCREASES TIME TO EXHAUSTION IN RUNNERS] está publicado no periódico European Journal of Nutrition, estrato A1 e fator de impacto 5.0. Este artigo avaliou se o óleo essencial de hortelã-pimenta é capaz de promover melhora do desempenho físico de atletas corredores recreacionais e se a temperatura corporal, a sensação térmica, o conforto térmico e status de hidratação destes corredores influenciam neste processo. Foi observado que a suplementação com o óleo essencial de hortelã-pimenta foi capaz de aumentar de forma significativa o tempo até a exaustão de corredores recreacionais adultos jovens que realizaram uma corrida em esteira a 70% do $VO_{2máx}$. Este efeito ergogênico não foi acompanhado de significativo menor aumento da temperatura corporal, melhora da sensação térmica e do conforto térmico, além de melhora do status de hidratação.

O segundo artigo original, intitulado ERGOGENIC EFFECT OF PEPPERMINT ESSENTIAL OIL (*MENTHA PIPERITA* L.) ON TIME TO EXHAUSTION OF RUNNERS IS GENOTYPE DEPENDENT: A DOUBLE-BLIND, RANDOMIZED AND CONTROLLED STUDY, está apresentado no APÊNDICE B desta tese e está submetido no periódico internacional Sports Medicine, estrato A1 e fator de impacto 9.8. Este artigo objetivou investigar a influência da suplementação com o óleo essencial de hortelã-pimenta no desempenho físico de corredores recreacionais e se o polimorfismo no gene da CYP1A2 influencia as respostas à esta suplementação. Como principal conclusão, observamos que o óleo essencial de hortelã-pimenta aumentou o tempo até a exaustão de corredores, mas este efeito só aconteceu nos atletas que possuem a presença do alelo C, não ocorrendo nos atletas com o genótipo AA para o gene da CYP1A2.

Adicionalmente, um terceiro artigo está apresentado no APÊNDICE C desta tese, um artigo de revisão sistemática intitulado INFLUENCE OF THE ADMINISTRATION FORM OF MENTHOL IN PHYSICAL PERFORMANCE IN ENDURANCE EXERCISE: A SYSTEMATIC REVIEW. Este artigo está publicado no periódico Science & Sports, estrato B1 e fator de impacto 1.1. O objetivo foi realizar uma revisão sistemática da literatura e apresentar os resultados de ensaios clínicos randomizados apontando em quais condições e formas de administração o mentol é realmente capaz de melhorar o desempenho físico no exercício de endurance. As evidências disponíveis sugeriram que praticantes de exercício físico que fazem

uso de mentol por via oral parecem ser capazes de ter vantagem esportiva, mas esses resultados precisam ser ponderados devido ao nível de evidência ser considerado de baixa qualidade devido ao pequeno tamanho amostral, diferentes protocolos de suplementação e exercício, além do baixo volume de estudos.

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APÊNDICES

APÊNDICE A – ARTIGO ORIGINAL 1

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ORIGINAL CONTRIBUTION

Peppermint essential oil (*Mentha piperita* L.) increases time to exhaustion in runners

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Abstract

Purpose This study aimed to evaluate the capacity of peppermint essential oil to improve the physical performance of runners in running protocol until exhaustion.

Methods In a clinical, randomized, double-blind, cross-over and controlled study, fourteen male recreational runners (37.1 ± 2.0 years; 24 ± 1.1 kg/m^2; 53.1 ± 1.7 mL kg min) performed two runs to exhaustion at 70% of VO_{2max}, after intake of 500 mL of water added with 0.05 mL of peppermint essential oil (PEO) or placebo (PLA), plus 400 mL of the drink during the initial part of the exercise. Records were made of body temperature (BT), thermal sensation (TS), thermal comfort (TC), subjective perception of effort (SPE), sweat rate (SR), and urine volume and density.

Results Time to exhaustion was 109.9 ± 6.9 min in PEO and 98.5 ± 6.2 min in PLA ($p = 0.009$; effect size: 0.826). No significant changes were observed in the values of BT, TS, TC, SPE, SR, lost body mass, and urine volume and density ($p > 0.05$).

Conclusion Peppermint essential oil added to water before and during a race significantly increases the time to exhaustion of recreational runners but without altering BT, TS, TC, or hydration status, so the mechanisms involved were not clarified in this study.

Brazilian registry of clinical trials (ReBEC) RBR-75zt25z.

Keywords Body temperature · Exercise · Menthol · Mint · Thermoregulation

Introduction

During exercise, most of the energy released during muscle contraction is in the form of heat [1]. To dissipate heat, sweating is the most important thermoregulatory mechanism [2]. Water volume reductions during long-term continuous exercises in hot environments reach between 1.3 and 4.2% of body mass in athletes [3, 4]. Dehydration influence thermoregulation, generating loss of physical performance, so water replacement is one of the most effective nutritional ergogenic resources for exercise [5, 6].

Meanwhile, in addition to this rehydration, Riera et al. [7] and Stevens et al. [8] demonstrated that fatigue was delayed by minimizing the increase in body temperature during exercise by adding menthol to water served to elite cyclists and runners during continuous exercise in a hot environment, with menthol being more cooling than ice water only. An improvement in thermal sensation and thermal comfort was also observed after the use of menthol in elite cyclists who performed a long-term time trial in a hot environment [9, 10].

Within this context, the use of menthol in its isolated form appears as an ergogenic nutritional alternative to improve physical performance. Some justifications are used to explain the improvement of this physical performance,

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such as the minimization of the increase in body temperature and perceptual cooling capacity [10], ventilatory effect [11], analgesic effect [12], and excitatory effect [13]. Meanwhile, peppermint essential oil, extracted from peppermint leaves (*Mentha piperita* L.), naturally contains menthol in its composition (about 50%) [14], so it could provide these same benefits, which could still be potentiated because, in addition to menthol, this oil also contains other components that may be beneficial for exercise, such as menthone (about 30% of its composition) [14], caffeic acid, polymerized polyphenols, carotenes, tocopherols, betaine, choline, tannins, and citric flavonoids (eriocitrin, luteolin, and hesperidin), recognized antioxidants [15, 16]. Antioxidant compounds have been shown to have ergogenic power to improve the physical performance of runners when ingested in other foods [17–19].

This peppermint essential oil has already had its ergogenic power tested, being shown to improve the physical performance of physically active students who consumed this oil for 10 days and performed an incremental exercise test on a treadmill (Bruce protocol) [20], as well as a single dose was tested in another study, showing improvement in strength parameters [21]. A third study showed no improvement in the physical performance of physically active subjects who ingested the oil for 10 days and performed a ramped exercise protocol that started at 0 watts and increased by 30 watts every 60 s (1 W to every 2 s) until voluntary exhaustion [22].

Despite these results on physical performance, none of them used runner athletes, nor used long-term exercise protocols, or investigated possible mechanisms involved (body temperature, thermal sensation, thermal comfort, or hydration status). These variables were evaluated only in studies that used menthol isolated. Another gap is that there are already reviews and meta-analyses on the isolated compound menthol, but these reviews selected a limited number of studies (between 11 and 17 articles) [23–25], with only two articles with runners and no articles with selected peppermint essential oil. Despite the studies concluded to be a promising resource, the level of evidence is limited by the small volume of studies, mainly involving this oil and the population of runner athletes, so increasing the volume of information on this subject is still necessary, as well as to bring data that address possible mechanisms of action after ingestion of this oil and to propose to the literature a new low-cost ergogenic food that can help improve the physical performance of athletes.

In this study, it was hypothesized that peppermint essential oil added to water is capable of promoting fatigue delay and that this ergogenic effect occurs due to a lower increase in body temperature, lower thermal sensation, greater thermal comfort, and improvement in the hydration status of recreational runner athletes after a running session to exhaustion.

Therefore, this study aims to verify if peppermint essential oil can improve the physical performance of recreational runners and if body temperature, thermal sensation, thermal comfort, and hydration status influence this process.

Materials and methods

Type of research, study subjects, and ethical issues

Experimental, randomized, cross-over, double-blind and controlled study developed with an acute response protocol to nutritional intervention with peppermint essential oil. The sample size was determined from previous data from a study that showed an improvement in the running performance of physically active students who ingested a drink based on peppermint essential oil and improved the time to exhaustion after an incremental treadmill exercise test [20], which resulted in an effect size of 1.3. Adopting an alpha error of 0.95 and beta sampling power of 95%, it was estimated that the minimum sample size necessary for the present study would be 8 athletes. A total of 16 athletes started this study and two did not complete the experimental protocols, so this study was concluded with 14 male recreational runners, aged between 18 and 50 years (age: 37.1 ± 2.0 years; body mass index: 24 ± 1.1 kg/m^2; VO_{2max}: 53.1 ± 1.7 mL kg min).

As an inclusion criterion, athletes should be regular runners, have at least one year of training, with a weekly frequency of at least five training sessions, of which at least three should be running and be training for a competition for at least four months uninterrupted in the season. They could not have any recognized chronic degenerative disease, could not be smokers, or make continuous use of any medication. They should not be in the habit of regularly consuming mint and its derivatives, as well as any alcoholic beverage or food supplements that contain the active substances present in peppermint. Would be excluded from this study volunteers who changed their usual pattern of eating or physical training throughout the experiments, did not consume the correct amount of the drinks provided, had gastrointestinal intolerance, and did not participate in all experimental procedures.

This project was approved by the ethics committee in research with human beings of the Health Sciences Center, Federal University of Paraíba, under protocol n° CAAE 17130619.2.0000.5188 and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This study is also published in the Brazilian Registry of Clinical Trials (ReBEC) under the number RBR-75zt25z. All participants were previously informed about the procedures and signed an informed consent form in accordance with resolution 466/12 of the National Health Council.

Study design

The athletes performed two sessions of running to exhaustion in a randomly determined order (www.randomizer.org): one after the ingestion of 0.05 mL of peppermint essential oil diluted in 500 mL of water (PEO) and the other after an isovolumetric placebo drink. As shown in Fig. 1, this volume of drink was ingested 30 min before exercise until exhaustion at 70% of VO_{2max}. During the exercise, the athletes ingested another 400 mL of the drink, 100 mL every 10 min for the first 40 min. After a *washout* of at least seven days, they repeated the experimental procedure, replacing just the drink. Body temperature (BT) and Heart Rate (HR) measurements were taken before (rest) and every 10 min during the test until exhaustion, as well as immediately after its completion. Measures of thermal sensation (TS), thermal comfort (TC), and subjective perception of effort (SPE) were performed during the physical test, also every 10 min. Urine collection and bioimpedance assessment were performed before and after exercise. The sweat rate (SR) was calculated.

Chemical analysis of peppermint essential oil

Qualitative and quantitative analyses characterized the volatile compounds of essential oil isolated from the species Mentha piperita L. being performed by gas chromatography together with mass spectrometry [26] (Agilent Technologies model 7890A, Santa Clara, CA, USA) equipped with an HP-5MS capillary and column (phenyl-methyl-siloxane 30 m × 250 µm × 0.25 µm, Agilent 19091S-433). The oil sample (1/100 in n-hexane, v/v) was injected in a volume of 1 µL, in split mode. The flow rate was 16 mL min and the separation ratio was 1:15. The oven temperature was linearly programmed from 60 to 240 °C (at a rate of 3 °C/min) and then held for 10 min at the last temperature. The carrier gas was helium with a head pressure of 8.2317 psi. [27]. The volatile compounds analyzed were menthol, menthone, eucalyptol, nonan-3-one, pentan-2-one, acetoacetate, and hexan-2-ol.

The antioxidant activity of the oil was determined using the methodology described by Brand-Willams et al. [28], through the ability of the antioxidants present in the samples to scavenge the stable radical DPPH. In triplicate, sample aliquots (5 mg mL^{-1}) were mixed with ethanol and 2.700 µL of the DPPH solution. After 30 min in a bath, protected from light and at room temperature, the reading was performed at 517 nm, in a Shimadzu UV–Vis spectrophotometer, model UV-2550. A control curve was prepared using Trolox (0.5–4 µg mL^{-1}) as a standard. Results are expressed as µmol Trolox/mL oil.

The total phenolic content of the essential oil was determined according to the Folin–Ciocalteu method [29] with some modifications. A 300 µL aliquot of the extract (5 mg mL in ethanol) was transferred to a test tube containing 60 µL of Folin–Ciocalteu reagent and 2.61 µL of distilled water. The mixture was stirred and after 1 min 180 µL of Na_2CO_3 (15%) was added. After incubating the solutions for 2 h at room temperature, the absorbance readings of the mixtures were measured at 760 nm. Gallic acid was used in the standard curve (0.001–0.020 mg mL in ethanol) and the results were expressed in terms of gallic acid equivalent (mg EAG/g).

Physical and nutritional assessment

The physical assessment (body mass and percentages of fat, muscle, water, and bone) was performed in two moments, at rest immediately after the first urine collection and after the test until exhaustion, more specifically after the collection of post-exercise urine. This evaluation was performed using a balance with an InBody 570 tetrapolar bioimpedance body composition analyzer (InBody Bldg. Co., Ltd. Seoul, South Korea).

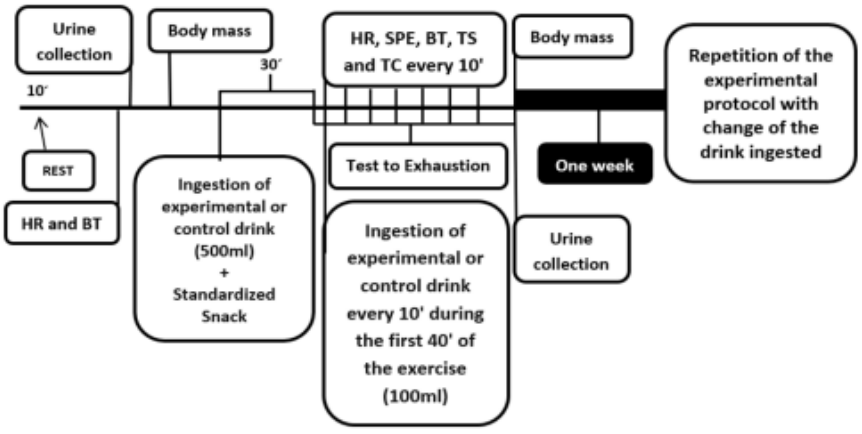

Fig. 1 Experimental study design. HR Heart Rate, BT Body Temperature, SPE Subjective Perceived Effort, TS Thermal Sensation, TC Thermal Comfort

A 24 h food recall [30] was applied before each experimental session, aiming to quantify all foods and beverages ingested the day before the experimental protocols. The amount of energy consumption (kcal), macronutrients, and micronutrients (sodium and potassium) in the distribution of meals were evaluated. The limits proposed by the Dietary Reference Intakes (DRIs) suggested by the Institute of Medicine [31] were considered as a reference for the adequacy of dietary consumption.

Fasting before the experimental protocols was used to mitigate the impact of the individual diet on the results. During the study period, they were instructed to maintain their usual eating patterns.

Supplementation protocol

For the experimental procedure, 0.05 mL of commercial peppermint pure essential oil from the brand Bio Essência (Jaú, São Paulo, Brazil) was used for each liquid dose of 500 mL of mineral water. For the placebo, the same volume of mineral water was used plus 0.05 mL of commercial mint liquid essence from the brand Tenda da Alma (Paio Pires, Aldeia de Paio Pires, Portugal). This placebo has a taste, color, and odor compatible with the experimental drink, but without the bioactive properties of peppermint essential oil. In a sensory test based on Santos et al. [32] with 50 subjects of both sexes, it was shown that more than 90% of these people who drank both drinks were unable to differentiate the placebo from the experimental drink. The peppermint dose was based on previous studies where no toxic effects or gastric discomfort was observed by the volunteers after the ingestion of peppermint essential oil, as well as no discomfort related to the intense taste or odor that made ingestion impossible [20, 21].

On the day of the two tests until exhaustion, the runners arrived at the laboratory and ingested 500 mL of the experimental or control drink 30 min before the exercise, as well as during the test, 100 mL every 10 min for the first 40 min. All runners arrived at the laboratory early in the morning, after fasting for 8 h. In the laboratory, a standardized breakfast consisting of 2 pieces of toast (20 g of carbohydrates, 6 g of protein, 2 g of total fat, 3 g of dietary fiber, and 140 mg of sodium) and 250 mL of artificial orange-flavored juice (25 g of carbohydrates, without vitamins and minerals, was provided and without the bioactive compounds of peppermint) being ingested 30 min before exercise and before breakfast.

Preliminary tests and exercise protocol

The runners were instructed to abstain from any physical exercise during the 48 h before a test that determined their aerobic capacity, as well as before the experimental sessions. Maximum aerobic capacity was determined using the protocol proposed by Weltman et al. [33] for a test run of 3200 m being done on the open athletics track and carried out one week before the start of the experimental sessions.

The first exhaustion test took place one week after the determination of the anaerobic threshold and aerobic capacity (previously estimated data). It was performed based on what was described by Toscano et al. [17], consisting of a run on a treadmill at 70% of VO_{2max}, with constant speed and determined based on the previous test [33], to maintain this speed for as long as possible. The test interruption criterion was the inability to maintain the determined speed, even in the face of verbal stimulus from the researchers, in addition to verbal confirmation by the runner and a reference between 19 and 20 on the Subjective Perception of Effort (SPE) Scale proposed by Borg [34]. Heart rate was recorded using a Polar RS800CX heart rate monitor (Polar Electro Oy, Kempele, Finland). The exercises were performed in a laboratory environment with controlled room temperature (26 ± 0.2 °C/26 ± 0.2 °C) and recorded relative humidity ($52 \pm 0.8\%$/$51.9 \pm 0.7\%$) for the PEO and PLA procedures, respectively ($p > 0.05$). The test result to exhaustion was expressed in minutes and seconds of running. The crossover took place seven days later.

Measurement of body temperature, thermal sensation, and thermal comfort

Core temperature measurements were performed using a digital tympanic thermometer branded Incoterm Color Check Infrared Auricular (Model 29838, Radiant Innovation Inc/China; ANVISA Registration: nº 10343200015), before (subject seated at rest) and every 10 min during the test until exhaustion, and in addition, immediately after its completion (subject seated), the measurement is performed by orienting the thermometer tip inside the ear canal, in such a way that it follows the natural anatomy of the ear [35]. At the time of measurement, the runners did not stop running and during the race, and they took their body temperature by introducing the thermometer into the auditory cavity. This procedure was performed under the supervision of the researcher to minimize measurement errors. After this procedure, the body temperature was recorded by the device in approximately 5 s, so that it did not interfere with the progress of the exercise.

The thermal sensation and thermal comfort variables were recorded every 10 min during the test until exhaustion. For thermal sensation, the subjective scale of seven points [36] was used, which is composed of a scale that goes from 1 (slightly cold) to 7 (extremely hot). For thermal comfort, a four-point subjective scale [36] was used, which is composed of a scale ranging from 1 (no discomfort) to 4 (very uncomfortable).

Sweat rate

The sweat rate (SR) was calculated according to the following equation proposed by Murray [37] for athletes:

$$\text{Sweat rate}\left(\text{mL min}^{-1}\right) = [(\text{IM} - \text{FM}) + \text{FI} - U] \div T \times 100,$$

where IM is the initial body mass in grams; FM is the final body mass in grams; FI is the volume of liquid ingested in milliliters; U is the volume of urine produced in milliliters; and T is the duration of the exercise in minutes. After obtaining the SR in milliliters/minute (mL min^{-1}), the conversion to liters/hour (L h^{-1}) was performed.

Urine collection and analysis

Urine was collected at rest before the exhaustion test and immediately after this test. Participants were instructed to empty their bladders at both times. They received plastic bottles with a volume meter and were instructed on the proper procedures for urine collection, minimizing the risk of contamination and loss of volume. After collection, the volume was recorded and the urine density was determined. This density was determined as follows: approximately 40 µL of each urine sample was analyzed using a digital refractometer model RTP-20ATC (Instrutherm®, São Paulo, Brazil) to determine the urine specific gravity (USG). To classify hydration, the reference values were described by Casa et al. [38], as shown in Table 1.

Statistical analysis

Data are expressed as mean and standard error of the mean. After testing the data for normality and homogeneity using the Shapiro–Wilk and Levene tests, respectively, the independent t test was performed to compare the volunteers' baseline data, and the paired t test compared the times to exhaustion of the two exercise sessions, in addition to sweat rate and delta for body temperature. For the analysis of measurements of SPE, thermal sensation, and thermal comfort, and pre- and post-variables of urine volume and density and total body mass, the two-way ANOVA test for repeated measures was adopted, with Bonferroni post hoc, considering the sphericity of the data. In addition, an individual analysis (single subject analysis) was performed on the variable time to exhaustion to identify how many athletes improved their physical performance. When using the paired t test the effect size was calculated using Cohen's d test for dependent samples and then classified according to Cook et al. [39] in small ($d = 0.00$–0.40), medium ($d = 0.50$–0.70), large ($d = 0.80$–1.00), or very large ($d \geq 1.30$). Eta squared (η^2) was used as a measure of the ANOVA effect size, classified in small ($\eta^2 = 0.02$–0.15), medium ($\eta^2 = 0.15$–0.35), and large ($\eta^2 > 0.35$) [39]. All these analyses were performed using the Jamovi software [The Jamovi Project (2021), version 1.6, Sydney, Australia], adopting a significance of $p < 0.05$.

Table 1 Parameters to determine hydration status according to USG

Hydration status	USG (UOsmol)
Well hydrated	< 1010
Minimal dehydration	1010–1020
Significant dehydration	1021–1030
Severe dehydration	> 1030

Source: Casa et al. [38]—National Athletic Trainers' Association–NATA

Results

Chemical analysis of peppermint essential oil

The GC–MS analysis showed the presence of seven chemical compounds: menthol (44.12%), menthone (37.63%), eucalyptol (6.41%), nonan-3-one (5.57%), pentan-2-one (3.37%), acetoacetate (1.50%), and hexan-2-ol (1.40%).

The total phenolics in this oil were quantified at 30.73 µg/g. The antioxidant capacity was 0.75 µmol/g.

Characterization of volunteers

As shown in Table 2, the runners were young adults, eutrophic, and with a fat percentage within the recommended range for health according to the Brazilian Society of Nutrology [40], but above that considered for high-performance athletes' level according to the Brazilian Society of Sports Medicine [41]. They had good hydration status, using data from Casa et al. [38]. Aerobic capacity was classified as good for health purposes (American College of Sports Medicine) [42], but lower than that of high-level athletes [43], characterizing the athletes as recreational level.

In the moments before the two experimental procedures, the athletes had similar values for all outcome variables (body composition, resting body temperature, and resting heart rate) (Table 2) ($p > 0.05$). Likewise, the athletes showed similar nutritional intake in the 24 h that preceded the two experimental sessions for total calories, macronutrients, and the micronutrients sodium and potassium (Table 2) ($p > 0.05$).

Time to exhaustion

As shown in Fig. 2 (Panel A), the athletes ran 11.4 ± 3.5 min longer in the experimental procedure, which was equivalent

Table 2 Baseline conditions of runners in experimental procedures

				Mean ± standard error
Age (years)				37.1 ± 2.0
Height (meters)				1.77 ± 0.02
VO_{2max} (mL kg min)				53.1 ± 1.7
Anaerobic threshold (L/min^{-1})				13.5 ± 0.5
	Experimental protocols			
	PEO	PLA	*p*	Reference values
Body mass (Kg)	74.5 ± 2.8	75.0 ± 3.0	0.262	X
BMI (kg/m^2)	24.0 ± 1.1	24.2 ± 1.1	0.274	18.5–24.9 kg/m^2
Body fat (%)	17.2 ± 2.0	15.3 ± 2.1	0.179	10–20%
Skeletal muscle mass (kg)	34.6 ± 0.9	34.7 ± 0.8	0.968	X
Total body water (L)	44.9 ± 1.0	44.9 ± 1.1	0.973	34.5–42.1 L
HR_R (bpm)	53.2 ± 2.7	54.2 ± 2.9	0.961	50–90 bpm
BT_R (°C)	34.7 ± 0.1	34.8 ± 0.1	0.689	34–42 °C
Total calories (Kcal)	2285.9 ± 114.5	2439.2 ± 129.1	0.348	40–70 kcal/kg/d
Carbohydrates (g)	310.9 ± 19.8	312.1 ± 15.9	0.960	5–8 g/kg/d
Lipids (g)	73.7 ± 5.9	83.4 ± 8.2	0.329	0.5–1 g/kg/d
Proteins (g)	101.8 ± 7.8	114.2 ± 11.0	0.249	0.8–1 g/kg/d
Sodium (mg)	1783.5 ± 375.4	1959.1 ± 294.5	0.760	500 mg/d
Potassium (mg)	2458.0 ± 275.7	2562.0 ± 183.7	0.165	2000 mg/d

Data expressed as mean ± standard error. *BMI* Body Mass Index, VO_{2max} Maximum Oxygen Consumption, *PEO* procedure with mineral water + peppermint essential oil, *PLA* procedure with mineral water + mint essence, HR_R Resting Heart Rate, BT_R Resting Body Temperature. No statistical difference between the procedures ($p > 0.05$). Kerksick et al. [44] used as a reference for total calories, macro- and micronutrients for athletes who perform moderate amounts of intense training (2–3 h/day of intense exercise performed 5–6 times/week). For body composition, the reference provided by the manufacturer (InBody 370) was used. BT_R was used the reference provided by the manufacturer (Incoterm Color Check Infrared Auricular). Reference for HR_R according to Nanchen [45]

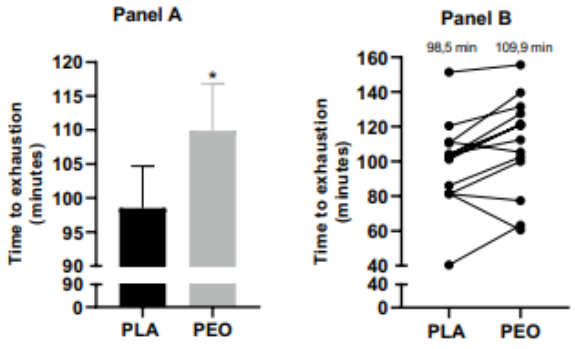

Fig. 2 Panel **A** Time to exhaustion in the PLA and PEO procedures; Panel **B** Time to exhaustion of each athlete in each of the procedures (the values inside the graph represent the averages of each procedure). Data are expressed as mean ± standard error. *Statistical difference between the procedures ($p = 0.009$; effect size: 0.826, classified as large). Paired *t* test was adopted

to a significant improvement of 11.6 ± 5.5% ($p = 0.009$; effect size: 0.826, classified as large) in the procedure with the ingestion of peppermint essential oil. Still in Fig. 2 (Panel B), the single subject analysis of the sample can be observed, showing that 11 of the 14 runners increased the time to exhaustion in the PEO procedure.

Subjective perception of effort

Athletes reported increasing perception of effort throughout the physical test and the last values were close to 18–20 points, indicating that they really reached maximum effort during the exercise (Fig. 3). Peppermint essential oil was not able to improve the subjective perception of effort when compared to PLA at any of the evaluated moments ($p = 0.701$; effect size: 0.003, classified as small), so the improvement in physical performance was not accompanied by less effort reported by athletes.

Body temperature, thermal sensation, and thermal comfort

As shown in Fig. 4 (Panel A), exercise naturally promoted an increase in body temperature in relation to rest in the two exercise procedures. When the interaction time × procedure was performed, it was noted that there was no significant variation in the behavior of body temperature

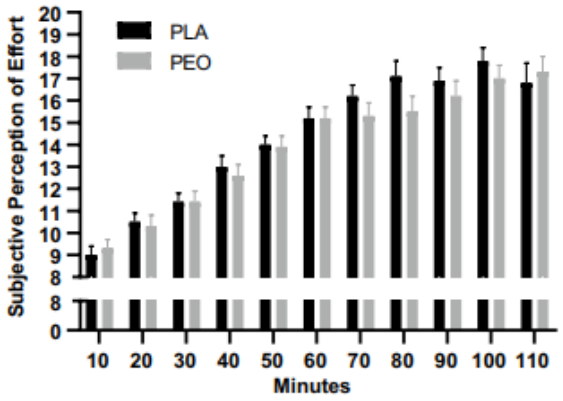

Fig. 3 Subjective perceived effort in PLA and PEO procedures. Data are expressed as mean ± standard error. There was no statistical difference between the procedures for all moments ($p = 0.701$; effect size: 0.003). The two-way ANOVA test for repeated measures was adopted, with Bonferroni's post hoc

(Δ) when comparing PEO and PLA ($p = 0.854$; effect size: 0.052, classified as small).

The thermal sensation behavior is shown in Fig. 4 (Panel B). No statistical differences were observed in the thermal sensation reported during exercise at any of the evaluated moments ($p = 0.103$; effect size: 0.105, classified as small). There were also no statistical differences in the referred thermal comfort during exercise at any of the evaluated moments ($p = 0.871$; effect size: 0.001, classified as small) (Panel C—Fig. 4).

Hydration status

The hydration status of runners is represented by total body mass lost, sweat rate, as well as urine volume and density (Fig. 5). In both procedures, there was a significant loss of body mass (Panel A) (effect size: 0.865, classified as large) after exercise when compared to rest, 1.5 kg in the PEO procedure ($p < 0.001$) and 1.4 kg in the PLA procedure ($p < 0.001$). The intergroup analysis showed no difference between the procedures both at rest and after exhaustion ($p = 0.902$; effect size: 0.001, classified as small). As for the sweating rate (Panel B), no difference was observed between this rate when comparing the experimental and placebo procedures ($p = 0.784$; effect size: 0.074, classified as small).

Athletes produced a greater volume of urine (Panel C) after testing to exhaustion in both the PEO and PLA procedures when compared to rest, but with no statistical difference ($p = 0.868$; effect size: 0.001, classified as small). The intergroup analysis showed no difference between the procedures both at rest and after exhaustion ($p = 0.749$; effect size: 0.004, classified as small). Meanwhile, there was a significant reduction in urinary density (Panel D) (effect size: 0.480, closer to being classified as medium than small) between pre- and post-exercise moments in both PEO ($p = 0.013$) and PLA ($p = 0.007$); however, the intergroup analysis showed no difference between the procedures both

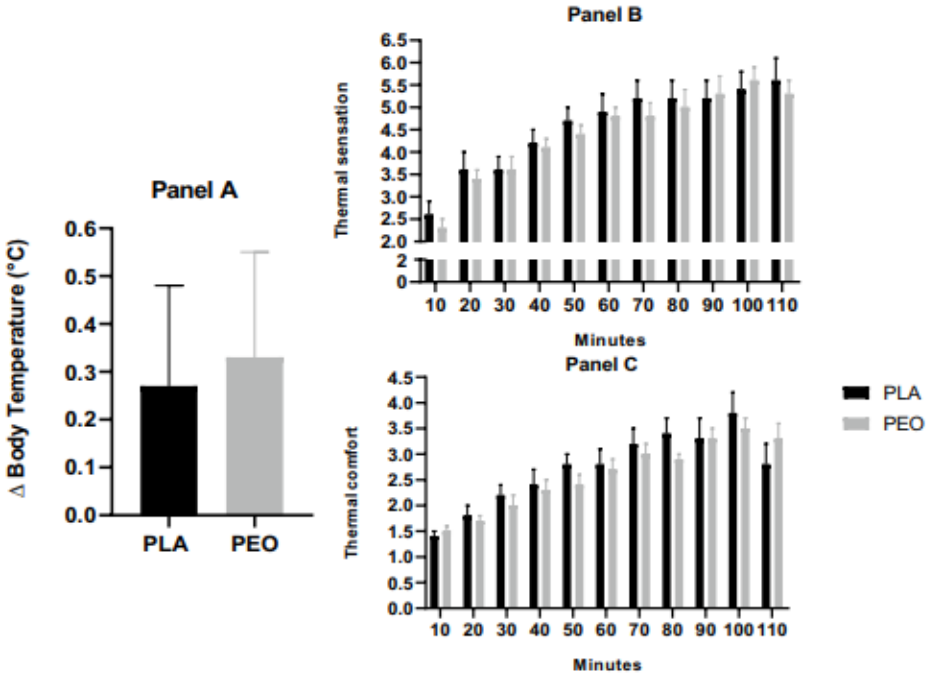

Fig. 4 Panel **A** Body temperature variation; Panel **B** Thermal Sensation; Panel **C** Thermal comfort. Data are expressed as mean ± standard error. There was no statistical difference between the procedures for all moments ($p > 0.05$). Paired *t* test was adopted for the analysis of body temperature variation; Two-way ANOVA test for repeated measures with Bonferroni post hoc was adopted for the analysis of thermal sensation and thermal comfort

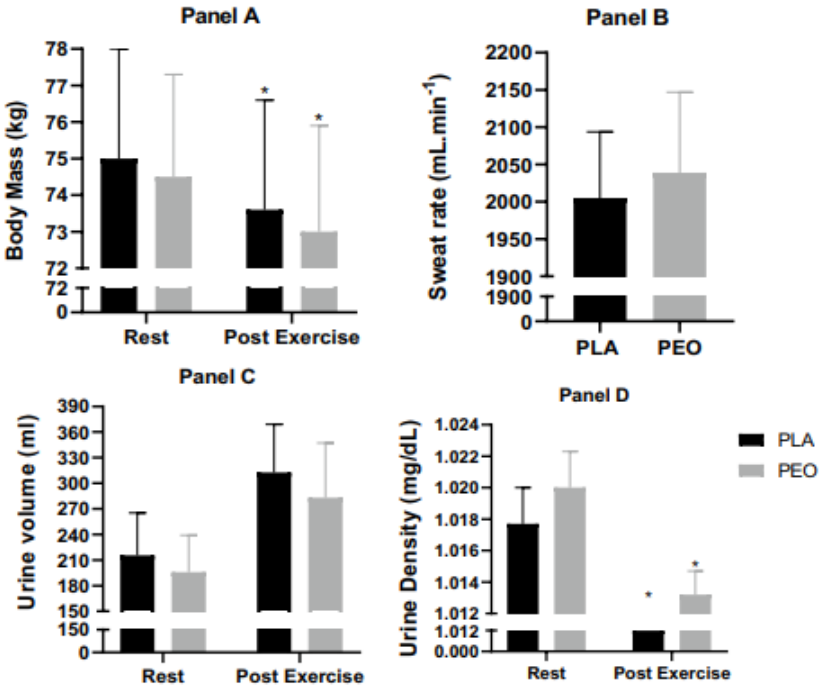

Fig. 5 Panel **A** Total body mass lost; Panel **B** Sweat rate; Panel **C** Urine volume; Panel **D** Urine density. Data are expressed as mean ± standard error. *Intragroup statistical difference ($p < 0.05$). Paired t test was adopted for the analysis of sweat rate; Two-way ANOVA test for repeated measures with Bonferroni post hoc was adopted for the analysis of body mass, and volume and density of urine

pre-exercise and post-exercise ($p = 0.305$; effect size: 0.040, classified as small).

Discussion

This study demonstrated that adding 0.05 mL of peppermint essential oil to hydration with 500 mL of water before an exercise session, followed by an additional 400 mL divided into four doses every 10 min during the first 40 min of exercise, significantly delays the fatigue of recreational runners in a running protocol to exhaustion at 70% of VO_{2max}, with an effect size classified as large. None of the mechanisms tested (body temperature, thermal sensation, thermal comfort, and hydration status) were revealed to have been an explanatory factor for this improvement in physical performance.

The power of the effect found for the experimental drink was considered large, and the 11% increase in time to exhaustion is very expressive in the context of sports training. These encouraging data should be weighed against the fact that the athletes are at a recreational level, so they have an open trainability window for interventions, which does not happen with very well-trained athletes. Furthermore, although the increase in physical performance has been shown to be significant in 11 of the 14 subjects, it is shown in Fig. 2 (Panel B) that the increase was very expressive in two athletes, one of them being the one who presented the lowest physical performance in the test with ingestion of the placebo drink. In addition, this same figure shows great individual variability in responses to supplementation.

The present study was carried out in an environment with a mild temperature (26 °C) and a relative humidity of 52%. Previous studies have developed their protocols in environments that are warmer than ours (between 29 and 35 °C) and with very different relative humidity (between 27 and 78%) [24]. This is an important difference between our study to previous studies, as the ambient temperature of our study was not so high and even so an ergogenic effect was observed.

While the present study was done with peppermint essential oil, which is a whole food and contains menthol in its composition, most of the previous studies were done with isolated menthol, so there are already systematic reviews and meta-analyses that involved between 11 and 17 studies that used this compound [23–25], nine of which were with topical application, six with mouthwash without ingestion, and two were with oral ingestion, all attesting to an ergogenic capacity of this substance. For the two studies that used oral ingestion as the method of administration, this ergogenic effect has been found with equal volumes, but with different concentrations of menthol [190 mL of a solution containing 0.05 mL of menthol ingested at seven times in the experimental session (total of 1330 mL) [46] or 0.02 mL of menthol for the same volume of solution [7]].

In the present study, we used peppermint essential oil, which contains about 0.02 mL of menthol in 0.05 mL of this oil, an amount similar to studies that used menthol in isolation and also similar to that found in studies previous studies that also used this essential oil [20–22], in which the ergogenic effect was also observed. Therefore, our data corroborate data from four previous studies that used similar doses of menthol, either alone [7, 46] or in the whole compound (peppermint essential oil) [20, 21].

The difference between isolated menthol and peppermint essential oil is that it contains other substances that could contribute to the ergogenic effect, such as menthone, caffeic acid, polymerized polyphenols, carotenes, tocopherols, betaine, choline, tannins, and citrus flavonoids (eriocitrin, luteolin, and hesperidin) [15, 16]. It is important to highlight that several of these substances are antioxidants, and there is evidence that antioxidant compounds promote an ergogenic effect, especially in studies that investigated the effect of fruits [47, 48].

Despite previous studies having shown an ergogenic effect, it is still not possible to establish a body of evidence for the ergogenic action of peppermint essential oil. The reason for this is that the outcomes studied are very diverse. While Sönmez et al. [49] showed that acutely ingested mint extract (5 mL/kg) decreased blood lactate and did not alter muscle soreness levels or the speed of a 400 m sprint, Meamarbashi and Rajabi [20] showed that ten days of ingestion of 500 mL of water containing 0.05 mL of peppermint essential oil improved the time to exhaustion after an incremental treadmill exercise test (Bruce protocol). The same Meamarbashi [21] also tested a single dose of this same amount in another study, showing improvement in strength parameters. In a more recent study, Shepherd and Peart [22] showed that ten days of ingestion of this same amount of water and oil did not improve time to exhaustion after a graded cycle ergometer maximal exercise test. Meanwhile, unlike these previous studies, our study evaluated a protocol that simulates physical performance in long-distance races with acute ingestion of the same amount of this oil. Despite that, each of these studies showed an ergogenic effect for these different physical and supplementation outcomes, except Shepherd and Peart [22], being the only study that showed no ergogenic effect. Despite this single study, the other articles show that there are several ergogenic effects for different outcomes, but there is still no confirmation of each of these effects in the studies because mechanisms have not been evaluated.

Despite the different exercise protocols of previous studies, most showed an ergogenic effect of PEO. The improvement in incremental testing in the study by Meamarbashi and Rajabi [20] is what most closely resembles our protocol. Incremental tests assess VO_{2max}, an increased capacity in long-distance running athletes [50]. However, despite being an indicator of ergogenic effect, consolidated data in the area of sports training indicate that the anaerobic threshold is the best predictor of performance in races than VO_{2max} [51]; therefore, studies that intend to evaluate the ergogenic effect in runners adopt protocols until exhaustion, because it is more directly associated with race performance, such as was the one adopted by us in the present study.

Shepherd and Peart [22] also did incremental physical testing and supplementation for 10 days in non-athletes, as did Meamarbashi and Rajabi [20], but as already mentioned, it was the only study with a negative result. A possible explanation for the contrasts in these results is that the authors did not report whether the last dose was given minutes before the physical test, unlike in our study, in which we offered supplementation before and during exercise. It is possible that the cooling power of menthol, which can generate an ergogenic effect, is only active when it is in contact with the oral mucosa. This aforementioned contrast raises questions about which would be the best form of administration and for how long. Empirical studies suggest not only that the perceptual effects of menthol are responsible for this improvement [10, 52], but also physiological explanations have been proposed to explain the cooling. Rinsing the mouth with menthol has been used because there is a transfer of fresh sensations to the brain, through the stimulation of the trigeminal nerve [53, 54] and in particular transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1) as Farco and Grundmann [55] showed in a literature review. This acute stimulation of cold receptors seems to show that single-dose supplementation may be more advantageous than chronically. The temporal distance between the last dose and the physical test from previous studies by Meamarbashi and Rajabi [20] and Shepherd and Peart [22] was not reported. Another interesting observation is that all four previous studies, that used students or physically active individuals and non-recreational athletes, were used in the present study.

It was expected that the ergogenic results observed after the use of peppermint essential oil would be explained by an improvement in hydration status, a lower increase in body temperature, as well as an improvement in thermal sensation, thermal comfort, and SPE during the exercise, but these hypotheses were not confirmed. There are still no studies in the literature that have investigated these mechanisms after the ingestion of peppermint essential oil (they only evaluated parameters of spirometry, blood pressure, HR, visual and audio reaction, blood lactate, and muscle pain), but studies that used isolated menthol has been shown to have a lower increase in body temperature [8], improvement in TS [8, 11, 56], SR [8], and SPE [56, 57]. All these studies used mouthwash without ingestion as an administration method, confirming our suspicion that contact with the oral mucosa and the mouth cold receptors generate cooling power and

may be responsible for the ergogenic effect in these studies. This thermal effect of menthol would also be accompanied by the ventilatory [11], analgesic [12], and excitatory [13] effects.

In studies that used topical administration of menthol, where these sensitive cold receptors also exist, these variables also improved, but there was no improvement in physical performance [9, 10, 58]. In the only two studies in which menthol was ingested, the improvement in physical performance was not accompanied by an improvement in any of these variables [7, 46], as was the case in the present study. Therefore, the cooling effect seems to be one of the explanations of the ergogenic effect, but only in specific places of the body (mouth) and this cooling effect does not seem to be part of the explaining mechanisms when there is ingestion, as confirmed by the present study, probably because there are no these cold-sensitive receptors internally in blood vessels.

Since we have not confirmed the thermal and hydration effect of peppermint essential oil as explaining the observed ergogenic effect, other possibilities should be investigated in future studies. A possible advantage of this oil is that, in addition to having menthol and its composition, it also has antioxidant substances [15, 16]. Therefore, the evaluation of this antioxidant effect to explain the mechanisms involved in the ergogenic effect of peppermint essential oil is relevant because previous studies, that evaluated the ingestion of isolated menthol or this oil, did not evaluate these blood variables; however, there are studies with other foods rich in antioxidants that showed that this nutritional property is valid in protocols with exercise [17–19].

Another possibility that could explain the ergogenic effect found would be the possible capacity of peppermint essential oil to induce vasodilation. Previous studies have discussed the possibility of this oil having the capacity to reduce arterial smooth muscle tone [20, 21]. It is well established that respiratory smooth muscle relaxation is triggered by two important mechanisms: activation of β-2 receptors [59] and release of epithelial-derived relaxing factors such as nitric oxide and prostaglandins [60]. In addition to the mechanisms suggested above, the participation of K^+ channels in the relaxing effect of peppermint oil was also speculated. Based on this, several in vitro and in vivo studies show that the opening of K^+ channels induces bronchodilation and hyperpolarization of airway smooth muscle cells [61]. It is important to clarify that these mechanisms have been speculated by previous studies and therefore need to be better understood and clarified in future studies.

In practical terms, the present study presents the proposal for a new ergogenic food for recreational athletes, which can be consumed in the form of a drink before and during exercise in environments with a lower temperature than previous studies had already demonstrated. This would be important in practice as street racing events around the world take place in the most diverse environments depending on the location of the competition. It is important to highlight that these previous studies were carried out with the use of isolated menthol, so all four previous studies that used peppermint essential oil or mint extract did not control room temperature or record relative humidity.

It should be noted that the present study was developed with recreational athletes and not with athletes at a professional level; therefore, we cannot extrapolate these results to elite athletes. Another limitation of our data is that body temperature, despite having been measured by a good and accurate technique (aural infrared) [35], is not considered the gold standard. As it is an important variable in sports performance, future studies should be carried out using the gold standard for measuring body temperature, which would be the rectal temperature, recommended by the National Athletic Trainers Association for measurement in resting conditions, during and after exercise, through the use of suppository type capsules between 8 and 10 cm or a sensor called thermistor between 10 and 15 cm [62]. Finally, this study was mostly conducted with young adults (11 athletes were between 26 and 41 years old), only two subjects were between 41 and 50 years old and one subject was younger than 26 years old. Although the sample calculation has shown that the sample size of 14 subjects was sufficient, we still believe that this sample size needs to be increased in order to allow for the possible influence of age to be verified in future studies.

As no changes were observed in the mechanisms tested, it seems that the explaining mechanism for the improvement in physical performance after the ingestion of peppermint essential oil does not depend directly on the control of body temperature nor on the improvement of thermal sensation or thermal comfort. Therefore, physiological mechanisms still need to be investigated. Animal model and human studies suggest that menthol may promote cooling vasodilation [63, 64], and this may improve blood flow to active muscles. Meanwhile, there is a protein that is sensitive to heat (Heat shock protein) [65]. It may be influenced by peppermint essential oil, but it has not yet been investigated in a human study.

It was concluded that supplementation with peppermint essential oil was able to increase the time to exhaustion of young adult recreational runners who performed a run at 70% of VO_{2max}. This ergogenic effect was not accompanied by a significantly lower increase in body temperature, improved thermal sensation and thermal comfort, and improved hydration status, so the mechanisms involved in this ergogenic effect remain undetermined.

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Data availability The database that supports the results and analyses presented in this article can be requested from the authors by email at any time.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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APÊNDICE B – ARTIGO ORIGINAL 2

Ergogenic Effect of Peppermint Essential Oil (*Mentha Piperita* L.) on Time to Exhaustion of Runners is Genotype Dependent: A Double-Blind, Randomized and Controlled Study

Running heading: Peppermint essential oil, physical performance and genetic polymorphism

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ABSTRACT

Objectives: to investigate the influence of supplementation with peppermint essential oil (PEO) on the physical performance and whether the polymorphism in the CYP1A2 gene influences responses to this supplementation. **Methods:** clinical, randomized, double-blind, cross-over and controlled study, in which 40 male recreational runners (36.5±2.0 years; 24.3±0.6 kg/m^2; 52.4±1.2 ml.kg.min) performed two running sessions until exhaustion at 70% of VO_{2max}, after ingesting 500ml of water plus 0.05ml of PEO or placebo, plus 100ml of the drink every 10 minutes during the first 40 minutes of the exercise. Thermal sensation, thermal comfort, sweating rate, urine volume and density were recorded. Oral mucosa sample was collected for genotyping of the CYP1A2 polymorphism. **Results:** athletes ran 104.0 ± 5.1 minutes after ingesting PEO and 95.5 ± 3.9 minutes with placebo (p=0.011). After the genotypic division, no statistical difference was observed when comparing the experimental and placebo procedures for athletes with the AA genotype (95.6 ± 6.6 minutes; 88.6 ± 4.3 minutes, respectively; p=0.374). For the AC+CC genotype, time to exhaustion (TTE) was significantly longer after ingestion of PEO compared to placebo (113.2 ± 7.4 minutes; 103.1 ± 6.4 minutes, respectively; p=0.026). Athletes with AC+CC genotypes showed a greater percentage increase in TTE when compared to the AA genotype (14.9 ± 2.9%; 2.3 ± 4.3% respectively; p=0.008). No significant changes were observed in thermoregulatory and hydration variables (p>0.05). **Conclusion:** PEO increases the TTE in runners, but this effect only occurs in athletes who have the presence of the C allele for the CYP1A2 gene.

INTRODUCTION

Menthol is an organic compound isolated from peppermint (Mentha piperita L.), and also consumed through its essential oil [1]. Studies have demonstrated the ergogenic capacity of menthol in improving time to exhaustion and race time in running and cycling, accompanied by relief from thermal tension associated with exercise in the heat, as demonstrated in reviews and meta-analyses [2-7]. Meanwhile, peppermint essential oil (PEO) is derived from fresh food, and for this form of food, three studies investigated the time until exhaustion in running, two of which showed the ergogenic effect of this oil [8,9] and another with no effect [10].

A characteristic of all these studies is that, regardless of the results found, individual variability in the time to exhaustion has been observed. This variability is evidenced by the high standard deviation observed in the experimental procedures of these works. In the study by Parton et al. [11], when menthol alone was administered, the time until exhaustion was 34.5 ± 10.3 minutes, so that the standard deviation represented almost a third of the mean (29.8%). When the PEO was used, the standard deviation corresponded to 33.5% of the average time to exhaustion (587.04 ± 196.53 seconds) [10]. A study carried out by our laboratory showed that, among athletes who showed an improvement in physical performance, there were those who improved by 23.4%, but also those who improved by only 7.9% [9].

A possible explanation for this individual variability may be the presence of genetic polymorphisms. MnSOD –Val/Ala heterozygous individuals responded to pequi oil intake with less DNA damage and lower lipid peroxidation in response to exercise when compared to MnSOD –Val/Val homozygous individuals [12]. In the study by Sousa et al. [13] this variability in response was explained by the fact that carriers of the G allele of the SOD3 gene were more responsive to grape juice supplementation when compared to athletes who had the C allele.

None of the studies that used peppermint essential oil took this genetic approach. Meanwhile, it is known that cytochrome P-450 1A2 (CYP1A2) is an enzyme involved in the metabolism of several nutrients [14] and polymorphisms in the CYP1A2 gene (rs762551) can lead to variations in activity levels of this enzyme. Gougis et al. [14] showed in a literature review that nutritional compounds were capable of influencing the activity of CYP1A2, including peppermint tea [15] and also its main active compound, isolated menthol [16], both inhibited its activity. This inhibition can reduce the metabolism of nutrients that act as substrates for the CYP1A2 enzyme, since menthol can interact with these substances, as in fact the interaction of menthol with caffeine has already been demonstrated [17], main substance metabolized by CYP1A2. However, no studies are available to date on the effect of peppermint

essential oil on CYP1A2 activity or its polymorphism. In fact, data on the effect of peppermint derivatives on enzymatic activity are limited, with only a few studies with peppermint tea, in an animal model, showing inhibition of CYP1A2 enzyme activity [15,18]. The participation of CYP1A2 in menthol metabolism still needs to be further investigated, but evidence points to a possible involvement, as Sakuma et al. (1999) [45] demonstrated that phenobarbital activates the synthesis and activity of CYP1A2 in an animal model and the analysis of menthol metabolism showed that it was higher in animals treated with phenobarbital [46].

Some research has also suggested that regular exercise may increase CYP1A2 activity, leading to faster caffeine metabolism and potentially influencing its effects, but only in those with the AA genotype, as caffeine had no effect in those with the AC genotypes and CC [19]. This influence of physical exercise and caffeine on CYP1A2 activity can be modified by the presence of menthol, since the ability of menthol to inhibit CYP1A2 in an animal model has already been demonstrated [16]. It is also known that the plasma clearance of caffeine reflects the activity of CYP1A2 in vivo [20] and it has been demonstrated that a single oral dose of menthol decreased caffeine clearance, resulting in higher plasma levels of caffeine [17]. Meanwhile, menthol has been shown to be beneficial for physical performance in the heat [4,5].

Therefore, the ability that physical exercise and menthol, the main chemical constituent of PEO, may have in influencing the activity of CYP1A2, and knowing that polymorphisms in the CYP1A2 gene can lead to variations in the activity levels of this enzyme, support the hypothesis that that the ergogenic effect of this oil on the time until exhaustion in recreational runners is influenced by the presence of the C allele, that is, individuals who are heterozygous AC or homozygous CC may not respond to supplementation with PEO compared to homozygous AA, as has been seen with caffeine.

Therefore, the objective of this study was to investigate the influence of supplementation with PEO on the physical performance of recreational runners, in sensation and thermal comfort during exercise, on the hydration status of these runners and whether the rs762551 polymorphism of the CYP1A2 gene influences these responses to supplementation.

MATERIALS AND METHODS

TYPE OF RESEARCH, STUDY SUBJECTS AND ETHICAL ISSUES

Type study experimental, double-blind, controlled and cross over with the order of treatments randomly, developed with an acute response protocol to nutritional intervention with PEO. The sample size was determined from previous data from a study that showed an improvement in the running performance of recreational runners who drank an PEO-based drink and improved time to exhaustion after a treadmill exhaustion test [9], which resulted in an effect size of 0.826. Adopting an alpha error of 0.95 and beta sampling power of 80%, it was estimated that the minimum sample size required for the present study would be 11 athletes (Software GPower 3.1, Franz Faul, Universitat Kiel, Germany). However, as the subjects in this study would be categorized by genotype, a larger number of participants was necessary to ensure that this minimum sample size occurred in each of the genotype subgroups. Considering that the expected frequency of CYP1A2 gene polymorphism is around 40% to 50% [21,22], the study was carried out with 40 male recreational runners, aged between 18 and 50 years old (age: 36.5±2.0 years; body mass index: 24.3±0.6 kg/m2; VO_{2max}: 52.4±1.2 ml.kg.min). After genotyping, the group with the smallest sample size (AC+CC) had 19 individuals. It is important to highlight that, from this sample of 40 runners used in the present study, data from the first 14 athletes who completed the experimental protocols had already been previously published [9].

As inclusion criteria, athletes should be regular practitioners of running training, train a weekly volume of at least 50km run, have a time in the 10km race under 60 minutes, have a VO_{2max} lower than elite athletes [23], have at least one year of training, with a weekly frequency of at least five training sessions, of which at least three must be running and have been training for a competition for at least four uninterrupted months of the season. They could not have any recognized chronic degenerative disease, could not be smokers or continually use any medication. They should not be in the habit of regularly consuming peppermint and its derivatives, as well as any alcoholic beverage or food supplements that contain the active substances present in peppermint. Volunteers would be excluded from the study if they changed their usual eating or physical training pattern throughout the experiments, did not consume the correct amount of drinks provided, had gastrointestinal intolerance and did not participate in all experimental procedures. Figure 1 shows the randomization, allocation and follow-up of subjects, according to the inclusion criteria.

The project was approved by the human research ethics committee of the Health Sciences Center of the Federal University of Paraíba, under protocol number CAAE 17130619.2.0000.5188 and, therefore, was carried out in accordance with the ethical standards established in the Declaration of Helsinki of 1964 and its subsequent amendments. This study

is also published in the Brazilian Clinical Trials Registry (ReBEC) under number RBR-75zt25z. All participants were previously informed about the procedures and signed the Informed Consent Form in accordance with resolution 466/12 of the National Health Council.

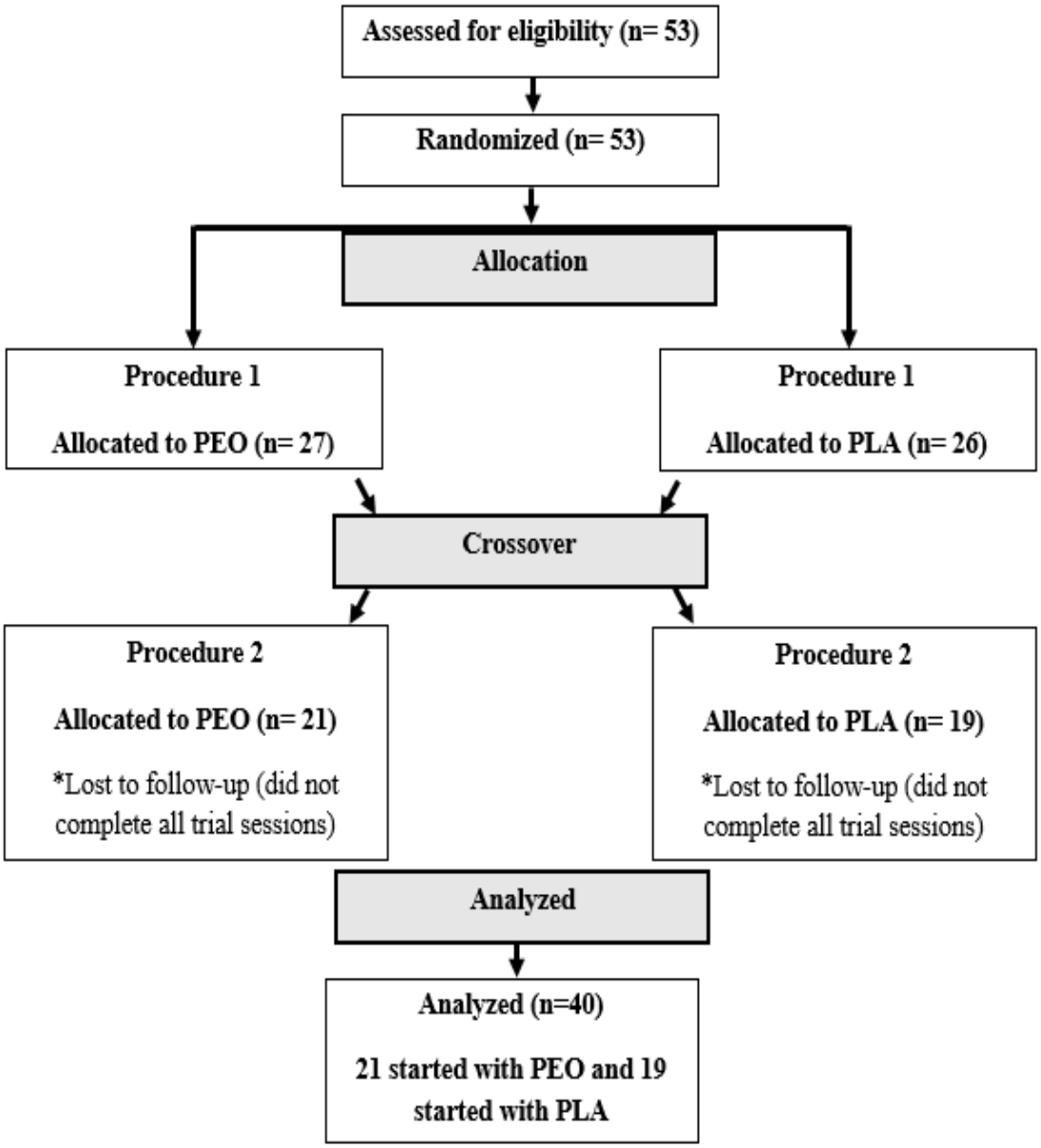

Fig 1 CONSORT flow diagram. PEO: Procedure with ingesting peppermint essential oil; PLA: Procedure with placebo ingestion

STUDY DESIGN

As can be seen in Figure 2, the athletes performed two sessions of running until exhaustion in a randomly determined order (www.randomizer.org) for a cross-over with an

interval of at least seven days, being: 1 - after ingestion 0.05 ml of PEO diluted in 500 ml of water (PEO); 2 - after an isovolumetric placebo drink (PLA). During the exercise, the athletes drank another 400ml of the drink, 100ml every 10 minutes in the first 40 minutes. Heart rate (HR) measurements were taken before (rest) and every 10 minutes during the tests until exhaustion, as well as immediately after their completion. Measurements of thermal sensation (TS), thermal comfort (TC) and subjective perception of effort (SPE) were performed during the physical test, also every 10 minutes. Urine collection and physical assessment by bioimpedance were performed before and after exercise. The sweating rate (SR) was calculated from physical, urinary and physical performance data. At the end of the last experimental protocol, a collection of the oral mucosa was carried out for DNA extraction.

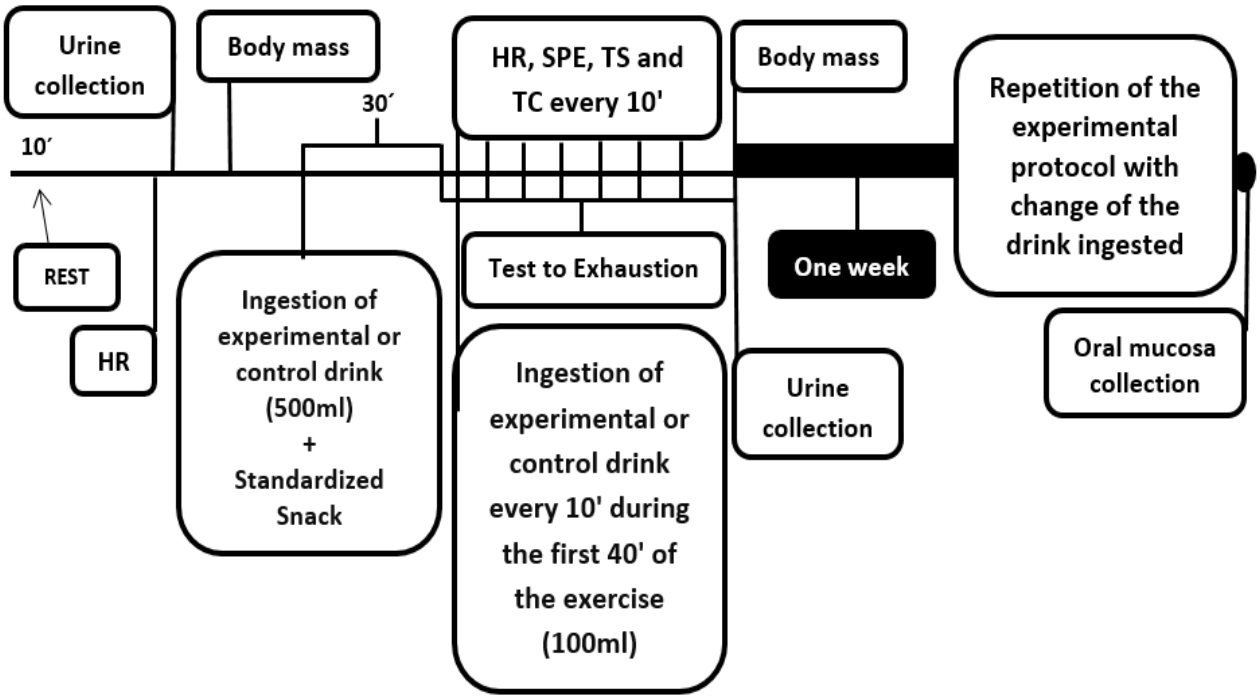

Fig 2 Experimental study design. HR = Heart Rate; SPE = Subjective Perceived of Effort; TS = Thermal Sensation; TC = Thermal Comfort

PHYSICAL AND NUTRITIONAL ASSESSMENT

The physical assessment (total body mass, muscle mass and fat percentage) was carried out in both the experimental procedure and the placebo, at two moments, at rest and after the test until exhaustion, to determine water loss according to body mass lost. This assessment was performed using a scale with an InBody 570 tetrapolar bioimpedance body composition analyzer (InBody Bldg. Co., Ltd. Seoul, South Korea).

A 24-hour dietary recall [24] was applied before each experimental session, with the aim of quantifying all foods and drinks ingested the day before the experimental protocols and ensuring that peppermint and its derivatives were not consumed. The amount of energy consumed (kcal), macronutrients and micronutrients (sodium and potassium) during meal distribution were evaluated. The limits proposed by the Dietary Reference Intakes (DRI's) suggested by the Institute of Medicine [25] were considered as a reference for the adequacy of dietary intake.

SUPPLEMENTATION PROTOCOL

For PEO, 0.05 ml of commercial PEO from the Bio Essência brand (Jaú, São Paulo, Brazil) was used for each 500 ml dose of mineral water. For PLA, the same volume of mineral water was used plus 0.05 ml of commercial liquid mint essence from the brand Tenda da Alma (Paio Pires, Aldeia de Paio Pires, Portugal) (without the bioactive properties of PEO). The dose of peppermint was based on previous studies where no toxic effects or gastric discomfort were observed by volunteers after ingesting PEO, as well as no discomfort related to the taste or intense odor that would preclude ingestion [8,9,26]. In a sensory test based on Santos et al. [27] in which it was used with 50 individuals of both sexes, it was demonstrated that more than 90% of these people who drank both drinks were unable to differentiate the placebo from the experimental drink. After randomization, 19 runners started the experimental protocol with PEO intake and 21 started with PLA intake.

On the days of the two tests until exhaustion, the runners ingested 500 ml of the experimental or control drink 30 minutes before exercise, as well as during the test (100 ml every 10 minutes in the first 40 minutes). All runners arrived at the laboratory early in the morning, after fasting for 8 hours. Fasting before experimental protocols was used to mitigate the impact of individual diet on results. During the study period, they were instructed to maintain their usual eating patterns. Before starting the exercise protocol, a standardized breakfast, without peppermint bioactive compounds, was provided after the physical assessment and before the experimental drinks. This meal consisted of 2 pieces of toast (20g of carbohydrates, 6g of protein, 2g of total fat, 3g of dietary fiber and 140mg of sodium) and 250ml of artificial orange juice (25g of carbohydrates, without vitamins and minerals).

PRELIMINARY TESTS AND EXERCISE PROTOCOL UNTIL EXHAUSTION

The runners were instructed to refrain from any physical exercise in the 48 hours before a test that determined their aerobic capacity, as well as before the two experimental sessions. Maximum aerobic capacity was determined using the protocol proposed by Weltman et al. [28], in which a 3200-meter running test was carried out on an open athletics track, one week before the start of the experimental sessions.

The first test until exhaustion occurred one week after determining the anaerobic threshold and aerobic capacity. The second test until exhaustion was carried out at least seven days after the first test. They were carried out based on the protocol described by Miranda Neto et al. [9], consisting of running until exhaustion on a treadmill at 70% of VO_{2max}, with constant speed and with the objective of maintaining this speed for as long as possible. The criterion for interrupting the test was the inability to maintain the determined speed, even in the face of verbal encouragement from the researchers, in addition to verbal confirmation from the runner and a reference between 19 and 20 on the SPE proposed by Borg [29]. HR was recorded using a Polar RS800CX heart rate monitor (Polar Electro Oy, Kempele, Finland). The exercises were carried out in a laboratory environment, with controlled room temperature (25.9±0.2 °C / 25.9±0.2 °C) and relative air humidity recorded (51.1±1.0 % / 51.4±0.8%) for the PEO and PLA procedures respectively ($p>0.05$). The result of the test to exhaustion was expressed in minutes and seconds of running.

MEASUREMENT OF THERMAL SENSATION AND THERMAL COMFORT

The TS and TC were recorded every 10 minutes during the test until exhaustion. For thermal sensation, the seven-point subjective scale was used [30], which is composed of a scale ranging from 1 (slightly cold) to 7 (extremely hot). For thermal comfort, a four-point subjective scale was used [30], which is composed of a scale ranging from 1 (no discomfort) to 4 (very uncomfortable). During the race, runners responded verbally to the scales shown.

SWEATING RATE

The SR was calculated according to the following equation proposed by Murray [31] for athletes:

$$\text{SR } (mL.min^{-1}) = [(IM - FM) + FI - U] \div T \times 100$$

Where: IM: initial body mass in grams; FM: final body mass in grams; FI: volume of liquid ingested in milliliters; U: volume of urine produced in milliliters; T: exercise duration in minutes. Once the SR was obtained in milliliters per minute ($mL.min^{-1}$), the conversion was performed to liters per hour ($L.h^{-1}$).

URINE VOLUME AND DENSITY

Urine was collected at rest before the exhaustion test and immediately after this test, always before bioimpedance assessments. Participants were instructed to completely empty their bladder at both times. They received a plastic bottle with a volume meter and were instructed on the appropriate procedures for collecting urine, minimizing the risk of contamination and volume loss. After collection, the volume was recorded and the urine density measured. This urinary density was determined as follows: approximately 40μL of each urine sample was analyzed using a digital refractometer model RTP-20ATC (Instrutherm®, São Paulo, Brazil) to determine urine specific gravity (USG). To classify hydration, the reference values described by Casa et al. [32], as shown in Table 1.

Table 1. Parameters to determine hydration status according to USG

Hydration status	**USG (UOsmol)**
Well hydrated	< 1010
Minimal dehydration	1010 – 1020
Significant dehydration	1021 – 1030
Severe dehydration	> 1030

Source: CASA et al. [32] - National Athletic Trainers' Association – NATA.

GENETIC EVALUATION

Collection and extraction of DNA from the oral mucosa

Buccal cell samples were collected by rinsing 5 ml of 3% sucrose solution for 60 seconds. The resulting mouthwash content was transferred to a 15 ml tube, and 3 ml of a TNE solution (17 mM Tris-HCl pH 8.0, 50 mM NaCl and 7 mM EDTA), diluted in 70% alcohol and autoclaved distilled water. After this procedure, the samples were stored under refrigeration

until the extraction process, within 30 days. The extraction procedure followed that proposed by Aidar and Line [33]. Once the extraction process was completed, the samples were stored frozen at -20 °C until the genotyping process.

Genotyping of the CYP1A2 gene polymorphism (rs762551)

Conventional polymerase chain reaction (PCR) was conducted in accordance with previous literature [34]. PCR occurred in the presence of the primers 5'-GGAAGGTATCAGCAGAAAGCC-3' and 5'-GCTCATCCTTGACAGTGCC-3' and under the following conditions: denaturation for 10 minutes at 95 ºC, followed by 35 cycles of 30 seconds at 95 ºC, 66 ºC for 30 seconds and 72 ºC for 30 seconds. A final extension step of 72°C for 10 minutes was programmed and followed by incubation at 4°C.

The product generated was digested with AnzaTM 32 ApaI following the manufacturer's recommendations (Invitrogen by Thermo Fisher Scientific, Made in Lithuania). As a result of digestion, the A allele produces a fragment of 626 bp and the C allele 181 and 445 bp. In the case of heterozygotes, all three bands are present [34].

STATISTICAL ANALYSIS

Data are expressed as mean and standard error of the mean. Initially, the data were tested for normality and homogeneity using the Shapiro-Wilk and Levene tests, respectively. The analyzes were carried out by subdividing according to the genotype, considering the presence or absence of the allele with the polymorphism characteristic. Athletes were divided based on the presence of the C allele (AC+CC genotypes) or AA genotype for the CYP1A2 polymorphism. The paired T test was performed to compare the baseline data of the runners and the time until general exhaustion (n=40), in addition, after subdivision by genotypes, an independent T test was used to compare the baseline data of the volunteers and the percentage of variation in time until exhaustion. To analyze the measurements of SPE, thermal sensation, thermal comfort, sweating rate and the pre and post moments for urinary volume and density and total body mass lost, two-way ANOVA for repeated measures was adopted, with Bonferroni post-hoc, considering the sphericity of the data. When necessary, non-parametric matching was used for data that did not present a normal distribution, and outliers were excluded. Chi-square test was performed to compare the presence or absence of alleles and the improvement or lack of physical performance. Furthermore, an individual analysis (single

subject analysis) of the time to exhaustion was carried out to identify how many athletes improved their physical performance. Effect size was calculated using Cohen's d test for dependent samples and then classified according to Cook et al. [35] in small ($d < 0.40$, medium ($d = 0.40 - 0.79$), large ($d = 0.80 - 1.29$) or very large ($d \geq 1.30$). Eta square ($\eta 2$) was used as a measure of effect size for ANOVA, classified as small ($\eta 2 = 0.02 - 0.15$), medium ($\eta 2 = 0.15 - 0.35$) and large ($\eta 2 > 0.35$) [35]. All of these analyzes were carried out using the Jamovi software [The Jamovi Project (2021), version 1.6, Sydney, Australia], adopting a significance of $p<0.05$.

RESULTS

Characterization of volunteers

Table 2 shows the baseline characteristics of the runners before the experimental procedures and after being categorized by genotypes. A balance was observed in the genotypic distribution between AA and AC, so that the AA genotype is present in 21 athletes (52.5%) and AC in 16 (40.0%). The CC genotype was observed in only 3 athletes (7.5%), therefore, as there was a very small sample of this genotype, the group was categorized according to the presence of the C allele (AC+CC), which totaled 19 athletes (47.5%).

Still in Table 2, it can be seen that the runners were young adults, well-nourished and with a fat percentage within the recommended range for health according to the Brazilian Society of Nutrology [36], but above that considered for high-performance athletes according to the Society of Medicine. Sports [37]. They presented a good state of hydration, according to data from Casa et al. [32]. Aerobic capacity was classified as good for health purposes [38], but lower than that of high-level athletes [23], characterizing athletes as recreational level.

In the moments before the two experimental procedures, the athletes presented similar values for all outcome variables (body composition and resting heart rate) (Table 2) ($p>0.05$). Likewise, the athletes had similar nutritional intake in the 24 hours preceding the two experimental sessions for total calories, macronutrients and micronutrients sodium and potassium (Table 2) ($p>0.05$). The genotypic groups were also similar in all variables (Table 2) ($p>0.05$).

Table 2. Baseline conditions of runners from the general procedures

	Mean ± standard error (n=40)			
Age (years)	36,5±2,0			
Height (meters)	1,75±0,02			
Anaerobic Threshold (L/min^{-1})	13,3±0,3			
	General		**CYP1A2 Genotypes**	
	PEO (n=40)	**PLA (n=40)**	**AA (n=21)**	**AC+CC (n=19)**
VO_{2max} (ml.kg.min)	52,4±1,2	52,4±1,2	54,4±1,9	50,3±1,3
Body Mass (Kg)	73,8±1,7	74,2±1,7	73,1±2,2	75,4±2,9
BMI (kg/m^2)	24,3±0,6	24,3±0,6	24,1±0,6	24,7±1,1
Body fat (%)	16,5±1,2	17,0±1,2	15,4±1,8	18,4±1,7
Skeletal Muscle Mass (kg)	34,6±0,6	34,5±0,6	34,6±0,9	34,2±0,9
Total Body Water (L)	44,9±1,1	44,9±1,1	44,2±1,5	45,2±1,4
HR_R (bpm)	58,1±1,6	56,4±1,3	56,5±2,5	58,8±1,9
Total Calories (Kcal)	2411,5±94,0	2350,1±79,0	2382,0±140,0	2349,0±93,5
Carbohydrates (g)	311,3±10,3	308,4±8,7	311,0±15,1	314,0±11,3
Lipids (g)	82,3±4,2	79,6±3,7	83,8±5,9	79,7±4,0
Proteins (g)	117,8±6,5	113,9±5,6	113,0±9,1	110,0±8,3
Sodium (mg)	1924,8±144,9	1840,1±150,1	2106,0±124,0	1607,0±95,5
Potassium (mg)	2590,3±123,3	2614,1±108,6	2515,0±184,0	2754,0±157,0

Data expressed as mean ± standard error. BMI – Body Mass Index. VO_{2max} – Maximum Oxygen Consumption. PEO – procedure with mineral water + peppermint essential oil; PLA – procedure with mineral water + mint essence; HR_R – Resting Heart Rate. No statistical difference between the procedures both for the general and subdivided by genotype ($p>0.05$). Kerksick et al. [39] used as a reference for total calories, macro and micronutrients for athletes who perform moderate amounts of intense training (2 to 3 hours/day of intense exercise performed 5 to 6 times/week). For body composition, the reference provided by the manufacturer (InBody 370) was used. Reference for HR_R according to Nanchen [40].

Physical Performance

As shown in Figure 3 (Panel A), athletes ran 104.0 ± 5.1 minutes after ingesting the PEO and 95.5 ± 3.9 minutes in the placebo procedure, which represented 8.5 ± 3.2 minutes more in the experimental procedure, equivalent to a significant improvement of 9.6 ± 3.6% ($p=0.011$; $d=0.421$, classified as medium). Still in Figure 3 (Panel B), one can observe the simple subject analysis of the sample, showing that 27 (67.5%) of the 40 runners increased the time to exhaustion in the PEO procedure, while only 13 (32.5%) runners did not respond to supplementation. Among those who improved their physical performance, there were those who improved 26.3% and those who improved only 0.8%.

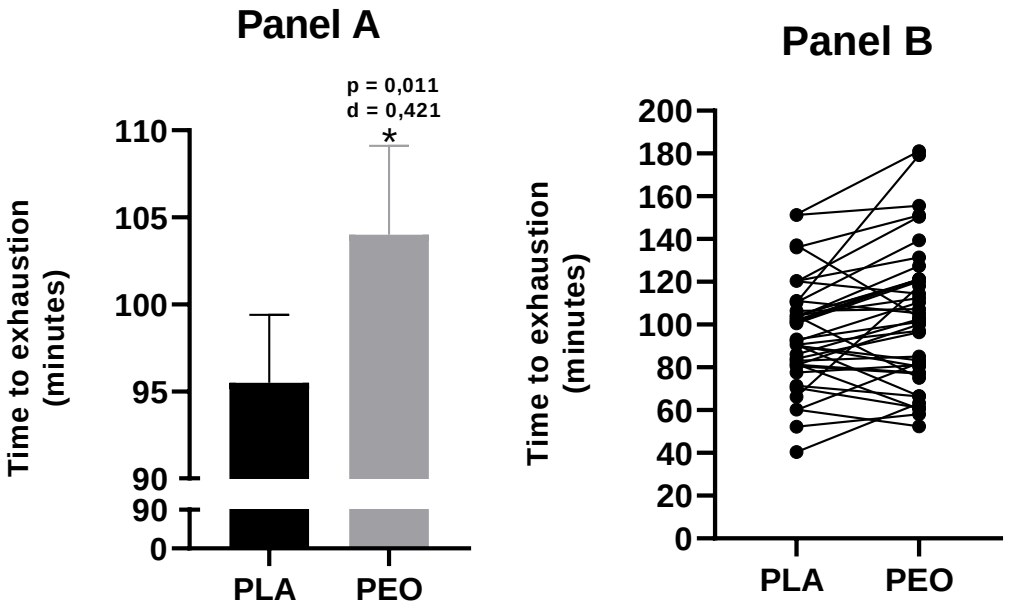

Fig 3 Panel A - Time to exhaustion in the PLA and PEO procedures; Panel B – Time to exhaustion of each athlete in each of the procedures. Data are expressed as mean ± standard error. *Statistical difference between the procedures (p=0.011; effect size: 0.421, classified as medium). Paired T-test was adopted

Data categorized according to genotypic groups are presented in Figure 4. Athletes with AC+CC genotypes showed a greater percentage increase in time to exhaustion (Δ%) when compared to the AA genotype (14.9 ± 2.9% vs 2.3 ± 4.3 % respectively) (p=0.008; d=0.80, classified as large) (Panel A). No statistical difference was observed when comparing the experimental and placebo procedures for athletes with the AA genotype (95.6 ± 6.6 minutes; 88.6 ± 4.3 minutes, respectively; p=0.374; d=0.261, classified as small). For the AC+CC genotype, time to exhaustion was significantly longer after ingestion of PEO compared to placebo (113.2 ± 7.4 minutes; 103.1 ± 6.4 minutes, respectively; p=0.026; d =0.330, classified as small). The individual analysis of each athlete's physical performance test subdivided by genotype is also presented in Figure 4 (panels B and C). It was found that 15 (88.2%) of the 17 runners analyzed with AC+CC genotypes (Panel C) were responsive to supplementation with PEO and improved time to exhaustion, while 2 (11.8%) did not respond to supplementation. However, only 10 (52.6%) of the 19 athletes analyzed with AA genotype (Panel B) responded to PEO and had a higher time to exhaustion, while 9 (47.4%) did not respond to supplementation (p=0.046). This time, when categorized by genotype, the variability between responders in the AA group showed that there were those who improved 19.7% and those who improved only

0.8%, while for the AC+CC group there were those who improved 26.3% and those improved by just 2.8%.

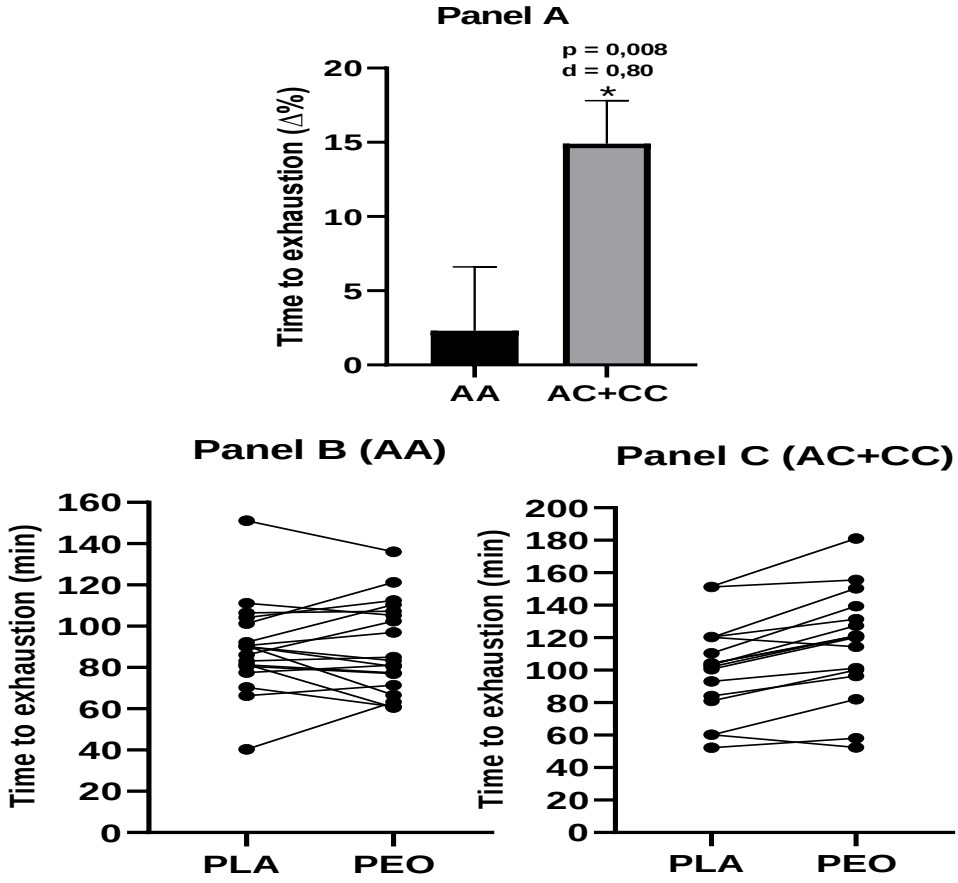

Fig 4 Panel A: Percent change in time to exhaustion of runners according to CYP1A2 genotypes. Panels B and C: Time to exhaustion for each athlete who has the AA (panel B) and AC+CC (panel C) genotypes for each procedure. Data expressed as mean ± standard error. *Statistical difference in percentage change in time to exhaustion between each genotype (p=0.008; effect size: 0.80, rated large). The independent t test was adopted

Thermal sensation and thermal comfort

The behavior of thermal sensation and thermal comfort can also be observed in Figure 5 (Panels A and B respectively). When comparing PEO and PLA, the thermal sensation did not differ between the procedures (p>0.05; d=0.024, classified as small). Regardless of the genotype analyzed, the thermal sensation reported during exercise did not vary significantly at all evaluated moments (p=0.06). Despite this, this division by genotype resulted in an effect size of 0.252 (classified as medium) in favor of the presence of the C allele. Regarding thermal comfort, when comparing PEO and PLA, the behavior of thermal comfort did not differ between the procedures. (p>0.05; d=0.022, classified as small). Regardless of the genotype

analyzed, there were also no statistical differences during exercise at all times evaluated (p=0.526; d=0.05, classified as small).

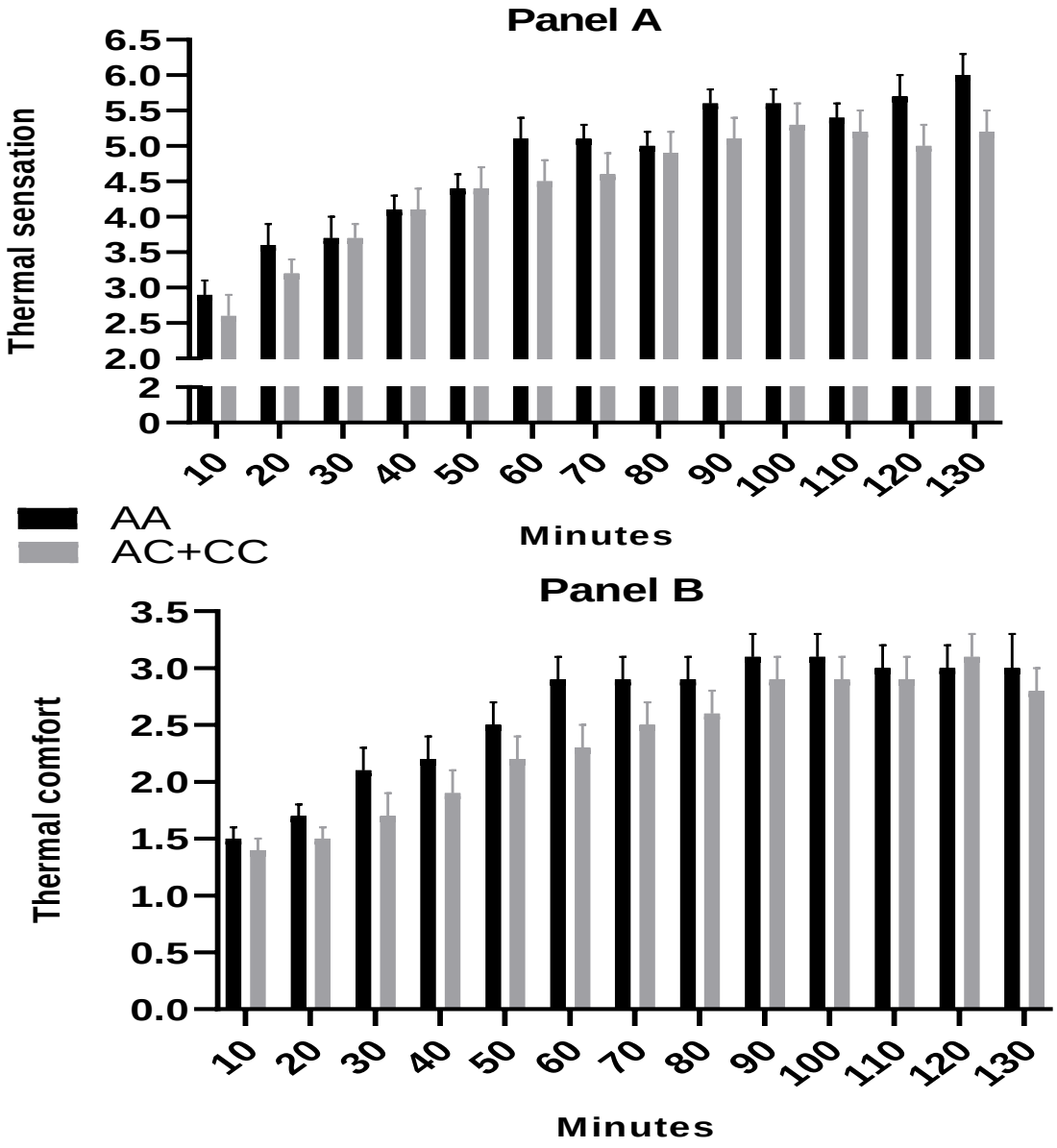

Fig 5 Panel A – Thermal Sensation; Panel B – Thermal Comfort. Data are expressed as mean ± standard error. There was no statistical difference between the procedures for all moments and for intra and intergroup analyzes (p>0.05). Two-way ANOVA test for repeated measures with Bonferroni posthoc was adopted

Hydration Status

The hydration status of the runners is represented in Figure 6 and is composed of the total body mass lost, the sweating rate, as well as the volume and density of urine. When comparing PEO and PLA, the total body mass lost did not differ between the procedures (Panel

A) (p=0.205; d=0.001, classified as small). As expected, the exercise protocol led to a loss of body mass in both procedures, when compared to rest, but without significant difference, even after subdivision by genotypes (p=0.925; d=0.001, classified as small). Regarding the sweating rate (Panel B), when comparing PEO and PLA, this rate did not differ between the procedures (p=0.850; d=0.001, classified as small). The same was observed when the analysis was carried out between the genotypic groups, that is, regardless of the genotype analyzed, the sweating rate was not significantly changed (p=0.192; d=0.031, classified as small).

When PEO and PLA were compared, urinary volume (Panel C) did not differ between procedures (p=0.852; d= 0.002, classified as small). The athletes produced a greater volume of urine after the test until exhaustion in the PEO and PLA procedures when compared to rest, but without statistical difference, even after subdivision by genotype (p=0.809; d=0.001, classified as small). Urinary density (Panel D) also showed no statistical difference when comparing PEO and PLA (p=0.344; d=0.010, classified as small). The analysis of the genotypic groups followed these results and showed no statistical difference between the resting and post-exhaustion moments, even after subdivision by genotype (p=0.776; d=0.001, classified as small).

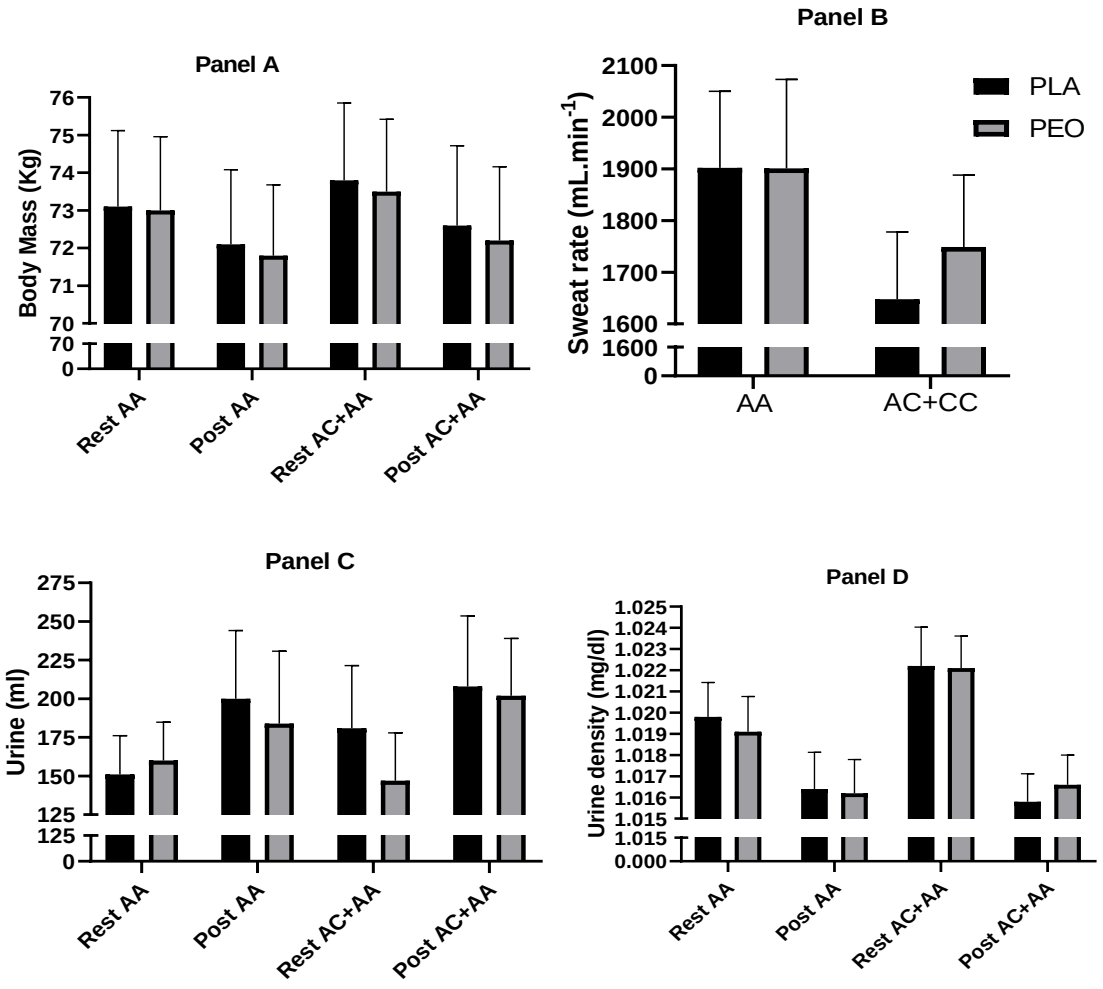

Fig 6 Panel A – Total Body Mass lost; Panel B – Sweat Rate; Panel C – Urine Volume; Panel D – Urine Density. Data are expressed as mean ± standard error. There was no statistical difference between the procedures for all moments and for intra and intergroup analyzes ($p>0.05$). Two-way ANOVA test for repeated measures with Bonferroni posthoc was adopted

DISCUSSION

This study demonstrated that a single dose of PEO supplementation improves the time to exhaustion of recreational runners who ran at 70% of VO_{2max}, but this ergogenic effect only occurred in carriers of the C allele (AC+CC genotype) of the CYP1A2 gene (rs762551). The physiological mechanisms evaluated did not explain this ergogenic response to PEO nor the absence of significant ergogenic effect in AA homozygotes.

This study reinforces a trend of the previous data that showed peppermint's ability to improve physical performance. While original studies demonstrated that PEO improved the time to exhaustion of recreational runners [9] and physically active students [8], there are

already reviews and meta-analyses indicating that menthol alone improves both the time to exhaustion and the race time (time trial protocols) of runners and cyclists [2-7]. Therefore, our data strengthens the concept that has been consolidated that menthol has an ergogenic effect both ingested alone and through its whole food, the PEO.

Despite these promising ergogenic findings from PEO, our data also corroborate previous observations that there is important individual variability in the magnitude and ergogenic responsiveness of this food. In data from Shepherd and Peart [10], the standard deviation corresponded to 33.5 % of the average time to exhaustion (587.04 ± 196.53 seconds) and in data from Parton et al. [11] it was 29.8% of the average (34.5 ± 10.3 minutes). Meanwhile, the standard deviation of the present study represented 31 % of the mean. Our study presented additional data to confirm this variability, which was the frequency of responders and non-responders to supplementation, that is, 32.5 % of runners did not improve their times with PEO intake (13 of the 40 athletes tested). Although this analysis has not been carried out in previous studies with the PEO, this variability in responsiveness frequency was also seen in a study with another food, grape juice, in which there was a significant improvement in the time to exhaustion, but 34.1 % did not respond to supplementation [13].

This individual variability in response to the same intervention has also been explained in the literature by the presence of genetic polymorphisms. The behavior of lipid peroxidation in response to exercise and time to exhaustion were shown to be genotype dependent for the genes MnSOD (pequi oil intake) [12] and SOD3 (grape juice intake) [13], respectively. Our data are the first to show genotypic influence of PEO for the CYP1A2 gene. They reinforce this new nutrigenomic trend by corroborating previous findings that genetic polymorphisms modify responsiveness to sports supplementation.

This nutrigenetic influence has also been demonstrated for the polymorphism in the CYP1A2 gene after caffeine intake, since a faster metabolism was demonstrated only in the presence of the AA genotype, accompanied by a lack of effect in the AC and CC genotypes [19]. Despite this, there are also studies that have shown the ergogenic advantage of caffeine with the presence of the C allele [22,41]. Therefore, despite this controversy about the genotypic advantage specifically for the effect of caffeine as a function of CYP1A2 activity, all these data together reinforce the need for this emerging line of research that investigates the ergogenic effect of foods as a function of nutrigenetics.

The effect size found for the genotypic influence of PEO on the time to exhaustion in the nutrigenetic analysis was much larger than the effect size in the analysis without considering the genotypic categorization carried out. This demonstrates the robustness of the genotypic

influence on the ergogenic effect of PEO. Being a very unprecedented finding, we still do not have an explanation for this phenomenon, however, it is known that CYP1A2's main function is to metabolize caffeine. In fact, previous data show that menthol inhibits CYP1A2 activity in an animal model [16]. It is also known that the plasma clearance of caffeine reflects the activity of CYP1A2 in vivo [20] and it has already been demonstrated that a single oral dose of menthol decreased caffeine clearance, generating higher plasma levels [17], which leads to a longer effect of caffeine on the body and consequently its ergogenic effects are observed for a longer time. Despite this, in our study, there was no intervention with caffeine, so that increase in time until exhaustion must have been directly mediated by menthol. The information we have for now is that people carrying the C allele of the CYP1A2 gene are slow metabolizers of caffeine, due to the lower activity of CYP1A2 [20], and it is precisely these carriers of the C allele who had the best increase time to exhaustion in the present study.

To confirm that the inhibition of CYP1A2 activity occurs more significantly in carriers of the C allele, data indicating a possible higher serum concentration of menthol in carriers of this allele would be necessary. As this was not done in the present study, we recommend this analysis in future experiments. Furthermore, the participation of CYP1A2 in menthol metabolism still needs to be further investigated, but evidence points to a possible involvement, as Sakuma et al. (1999) [45] demonstrated that phenobarbital activates the synthesis and activity of CYP1A2 in an animal model and the analysis of menthol metabolism showed that it was higher in animals treated with phenobarbital [46].

We analyzed some physiological variables to try to explain the ergogenic results found after ingesting PEO. The runners' hydration *status* data did not change statistically, even after division by genotypic group. The supplementation protocol was efficient in keeping the athletes well hydrated, and the fact that they had already started exercising with a good hydration *status* may have influenced the results. Thermal sensation and thermal comfort data also did not vary, even after the genotypic subdivision, despite the presence of menthol in PEO, which has been recognized as a cooling effect [5,7] and has been shown to influence the activity of CYP1A2 [16].

In practical terms, we showed that a we showed that the administration of PEO in water just before and during running events is a low-cost and practical way to promote an increase in physical performance, considering the data from the present study and several other studies [5]. While previous studies were carried out with menthol alone, the present study and that of Meamarbashi and Rajabi [8] demonstrated that the ergogenic effect also occurs in the form of PEO, which is a more commercially available form, which facilitates the acquisition of the

product. However, although reviews and meta-analyses demonstrate this ergogenic effect, our study draws attention to the fact that the effect is robust (effect size = 0.80, considered large), but only occurs in carriers of the C allele.

As possible limitations of this study, we can mention the sample size. Larger and more homogeneous samples are recommended for the analysis of the three distinct genotypic groups (AA, AC and CC), so that the ergogenic responses to PEO for each genotype are accurately represented. We warn that the sample was composed only of recreational and not elite level runners, so these results could not be extrapolated to high-level athletes.

The ergogenic effect of menthol on performance in running and cycling events is well demonstrated, but the mechanisms involved are still poorly understood. This state of the art should guide future perspectives for this line of research, including studies with a larger sample size to verify the influence of body temperature as a mechanism of action and investigation of possible other mechanisms involved, since PEO is rich in other substances, such as antioxidants, which does not occur in isolated menthol. Regarding the participation of CYP1A2, we suggest future studies to verify the influence of this enzyme on the metabolism and serum levels of menthol. Furthermore, there are other genes and genetic polymorphisms involved in nutrigenetics that modulate the ergogenic effect of foods, such as the R577X polymorphism (rs1815739) in the ACTN3 gene, which is the polymorphism most associated with sports performance [42] and influenced the response to pequi oil [43] and the I/D polymorphism of the angiotensin-converting enzyme (ACE) (rs4646994), which is a polymorphism that has an influence on body temperature during exercise [44]. Finally, it is pertinent that future studies test the experimental protocol of this study also on high-level athletes.

In summary, we conclude that this study sheds new light on the following findings: First, it reinforced the results of recent studies, indicating PEO as a food with ergogenic capacity. Secondly, it reinforces a line of research that provides sufficient data for sports nutrition to move into the context of nutrigenetics, investigating the ergogenic effect depending on the various polymorphisms that can influence it. Within this context, the present study adds new results to the literature, as it showed that the ergogenic effect of PEO in improving physical performance in the running test to exhaustion in recreational runners is genotype dependent, identifying the genetic polymorphism of the CYP1A2 gene (rs762551) as at least one possible genetic variant that explains individual variability in response to PEO.

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DECLARATION OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

Manoel Miranda Neto, Darlene Camati Persuhn and Alexandre Sérgio Silva contributed to the study conception and design. Material preparation, data collection and analysis were performed by Manoel Miranda Neto, Ana Carolina Freitas Meireles, Zaira Batista de Queiroz Correia, Eriklys Cavalcante Barreto and Renata Lira de Assis. The first draft of the manuscript was written by Manoel Miranda Neto. The article was reviewed by Alexandre Sérgio Silva. All authors read and approved the final manuscript.

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APÊNDICE C - ARTIGO 3

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REVIEW — META-ANALYSIS

Influence of the administration form of menthol in physical performance in endurance exercise: A systematic review

Influence de la forme d'administration du menthol sur les performances physiques lors d'exercices d'endurance : revue systématique

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KEYWORDS
Cooling;
Ergogenic aid;
Mint;
Sports nutrition

Summary
Objectives. — Perform a systematic review of the literature and present the results of randomized clinical trials (RCTs) pointing out under which conditions and forms of administration menthol is really capable of improving performance in endurance exercise.
News. — Thirteen studies met the eligibility criteria. The sample size ranged from 6 to 12 athletes and the studies were evaluated between 5 and 9 on the PEDro scale. Six studies evaluated menthol mouth rinse, of which three demonstrated an increase in time to exhaustion between 6% and 9% and two demonstrated a decrease in time trial (2.7% and 3.5%). Five studies evaluated the topical application but without modification of physical performance variables. Two studies assessed the oral intake, of which in one of them there was a decrease in the time trial between 5.2% and 8.2%.

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Prospects and projects. — Current data point to a good perspective on the determination of menthol as an ergogenic resource, provided that further studies form a body of evidence on each of the forms of administration.
Conclusion. — The available evidence suggests that physical exercise practitioners who use menthol orally seem to be able to promoter sport advantage, but these results need to be weighed due to the evidence level is considered to be of low quality due to small sample size, heterogeneous supplementation and exercise protocols and low volume of studies.

MOTS CLÉS
Refroidissement ;
Aide ergogénique ;
Menthe ;
Nutrition sportive

Résumé
Objectifs. — Effectuer une revue systématique de la littérature et présenter les résultats d'essais cliniques randomisés (ECR) soulignant dans quelles conditions et formes d'administration le menthol est réellement capable d'améliorer les performances à l'effort d'endurance.
Actualités. — Treize études remplissaient les critères d'éligibilité. La taille de l'échantillon variait de 6 à 12 athlètes et les études ont été évaluées entre 5 et 9 sur l'échelle PEDro. Six études ont évalué les bains de bouche au menthol, dont trois ont démontré une augmentation du temps jusqu'à l'épuisement entre 6 % et 9 % et deux ont démontré une diminution du contre-la-montre (2,7 % et 3,5 %). Cinq études ont évalué l'application topique mais sans modification des variables de performance physique. Deux études ont évalué l'apport oral, dont dans l'une d'elles il y avait une diminution du contre-la-montre entre 5,2 % et 8,2 %.
Perspectives et projets. — Les données actuelles permettent d'avoir une bonne perspective sur la détermination du menthol comme ressource ergogénique, à condition que des études complémentaires constituent un corpus de preuves sur chacune des formes d'administration.
Conclusion. — Les preuves disponibles suggèrent que les praticiens de l'exercice physique qui utilisent le menthol par voie orale semblent être en mesure de promouvoir l'avantage du sport, mais ces résultats doivent être pondérés car le niveau de preuve est considéré comme de faible qualité en raison de la petite taille de l'échantillon et de la supplémentation hétérogène et protocoles d'exercice et faible volume d'études.

1. Introduction

Physical performance can be altered both by internal factors, such as level of training [1] and nutrition [2] as well as by external factors including room temperature and relative humidity air [3]. It is known that performance in endurance exercise, such as street and track races, cycling, triathlon, is reduced in places with a tropical climate with high temperatures and relative humidity because thermal stress increases the evapotranspiration process [4]. It is also known that hydration in these long-running sports is considered an important nutritional resource for improving physical performance, especially in hot environments, due to the maintenance of plasma volume, which leads to a control of the increase in body temperature [5]. Recent studies have tried to show that this improvement can also occur by minimizing the increase in body temperature during exercise [6]. Using adequate nutritional support, more specifically the adequate water intake, can help minimize the increase in body temperature leading to decreased performance loss as shown by Stevens et al. in a literature review [7].

Within this context, the use of menthol has emerged as a nutritional ergogenic alternative aiming to improve physical performance, and the scientific community has used some likely justifications to explain the improvement of this performance after menthol ingestion, such as the minimizing the rise in body temperature and perceptual cooling capacity [8] and ventilator [9], analgesic [10] and excitatory effects [11]. Therefore, as can be seen, several may explain the influence of menthol on physical performance, leaving doubts as to which would be the main one. Empirical studies suggest that the perceptual effects of menthol may be responsible for this improvement [8,12], but also physiological explanations have been proposed to explain the cooling. Rinsing the mouth with menthol has been used because there is a transfer of fresh sensations to the brain, through the stimulation of the trigeminal nerve [13,14] and in particular transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1). Menthol acts upon TRPM8 receptors by rapidly increasing intracellular calcium and mobilizing calcium flux through the channels to induce cold response signals at the application site, as Farco and Grundmann showed in a literature review [15]. The application of menthol to the skin causes a refreshing sensation with cutaneous vasoconstriction [16]. Intake of menthol-based beverages seems to influence body temperature and performance when drunk cold or associated with ice [6], with its action reduced when consumed in isolation by be quickly absorbed by the intestine and extracted by urine [17].

Studies with menthol in the form of drinks, gels, sprays have been proposed as shown in reviews [7,18] and in

systematic reviews and meta-analysis [19—21]. Recently it was also published a consensus statement on the safe and effective use of menthol in hot environments in preparation for the Tokyo 2021 Olympic Games [22]. As can be seen, the interest of the scientific community in studying the use of menthol as an ergogenic resource has grown and some evidence has been proposed to justify this interest. Despite this considerable number of reviews on the topic, the present study is justified due to some methodological differences in relation to the previous literature mentioned above. None of the previous studies included randomized clinical trials in the search strategies and some did not even include the inclusion criteria, a fact adopted in our methodology, offering greater quality in the evidence. The present study restricted interventions with menthol as the only cooling compound, a fact that is different from that adopted in two reviews that did not evaluate only menthol as a cooling compound, but instead associated with other compounds or simply other cooling compounds that were not just menthol. We focus on presenting the forms of administration of menthol and which one would be the most suitable for use.

There are many doubts about the ergogenic effect of menthol, there are several application methods used in the studies, as well as several mechanisms that are proposed to explain how menthol acts in the body, decreasing the temperature increase and improving performance. Another issue is that there are several exercise protocols and sample (athletes and non-athletes) used in the studies, just as of temperature and relative humidity. In view of all this information, it is still unclear the real capacity of menthol to improve the physical performance and what is the best exercise and supplementation protocol. Thus, the present systematic literature review presents, in a critical way, the results of randomized clinical trials (RCT) pointing out in what conditions and forms of administration menthol could be able to improve endurance exercise performance, which modalities and which populations would be effectively benefited by the use of menthol and the gaps that still need to be solved in future studies. Guidance for future research is also provided.

2. Evidence acquisition

2.1. Study design and eligibility criteria

This review has been written in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [23] and Cochrane Collaboration handbook [24]. Studies using the endurance exercise were selected as an independent experimental procedure and considering the following eligibility criteria: original; RCT; both sexes; age $\geq$ 18 years; practitioners of endurance exercises; supplemented with menthol or peppermint essential oil in a chronic or acute manner; usual nutritional habits during the intervention period; and a minimum score of 5 on the PEDro scale. Review studies, case studies, dissertations or theses and those that evaluated the ergogenic effect of menthol associated with other foods or active compounds were disregarded.

2.2. Data sources

The choice of articles was limited to RCT in the English language and published from 2010 to august 2021. The search was performed in the MEDLINE database (accessed by Pubmed), using a combination of the following terms or keywords: menthol, peppermint essential oil and exercise. The search strategy used was: (''peppermint essential oil'' [All Fields] OR (''menthol'' [MeSH Terms] OR ''menthol'' [All Fields])) AND (''exercise'' [MeSH Terms] OR ''exercise'' [All Fields]) AND (Clinical Trial [ptyp] AND ''2010/01/01'' [PDat]: ''2021/08/31'' [PDat] AND ''humans'' [MeSH Terms]).

2.3. Study selection and quality assessment

The search and selection of articles were carried out independently and blindly by M. M. N. and R. S. B. S., initially by titles and abstracts. After that, a complete reading of the manuscripts was carried out to reapply the eligibility criteria. Then, the authors compared the selected articles and, in cases of divergence, reapplied the inclusion criteria. When the disagreement still remained, a third reviewer (A. S. S.) was requested, which finally resulted in 13 articles. Qualifying manuscripts were assessed according to PEDro scale for appraising the quality of literature [25]. Fig. 1 shows the process of selecting studies and results of the number of articles found.

3. Results

According to what is shown in Fig. 1, the systematic search based on keywords and search strategy resulted in 23 articles found. Of these, five articles were excluded after analyzing the titles. After reading the abstracts of these remaining articles, the inclusion criteria led to the removal of five more article. Therefore, 13 articles were considered for analysis as to eligibility via reading the full text and all remained and were included in the review. Of these studies, six evaluated the mouth rinse with menthol (Table 1), five evaluated the topical use of menthol (Table 2) and two evaluated the oral intake of menthol or peppermint essential oil (Table 3). The characteristics of these thirteen studies are summarized in their respective tables.

The 13 selected studies include a total of 135 participants, so the study with the lowest mean age was 20 ± 1 years old [30] and the study with the highest mean age was 42 ± 13 years old [6], including healthy men and women with different levels of training, 12 studies that evaluated physically active individuals and only one study that evaluated elite athletes [34] and of the total of studies only two had its sample composed of both sexes [30,35], all the others only men. All studies were performed in a single dose, except one in which the supplementation was for 10 days [35] had cross-designs and evaluated endurance exercise practitioners. The exercise protocols involved time to exhaustion tests ($n = 5$) [26,28—30,35], time trial tests ($n = 6$) [6,8,9,27,32,33] and the remaining two studies used the perceived effort ($n = 2$) as the main performance evaluation variable [31,34]. While the ideal conditions for physical exercise are ambient temperature around 22 °C and relative humidity around 60% [36], all the studies evaluated were

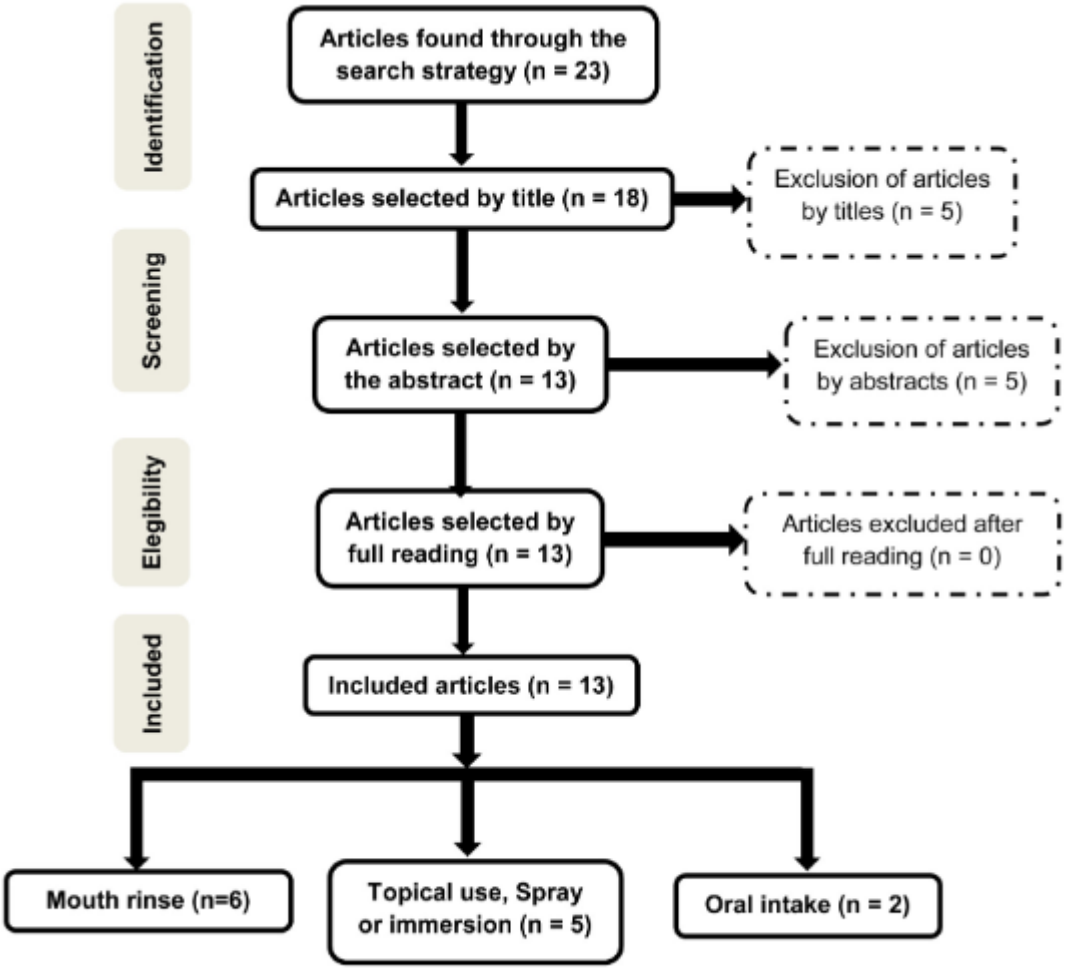

Figure 1 PRISMA search strategy.

carried out under unfavorable climatic conditions, in hot environments (between 29 and 35 °C) and relative humidity or low (between 27 and 50%) or high (between 70 and 78%). Only one study was carried out in ideal relative humidity (62%) [34], but in a hot environment (29.1 °C).

In the quality analysis using the PEDro Scale, an average of 7.15 ± 0.94 was observed, with the lowest value evaluated at 5 [31] and the highest value 9 [30]. Tables 1–3 shows the PEDro scale scores for each of the articles. The items that were least contemplated or that were not clearly stated in the studies were clarity regarding the distribution of the subjects blindly (only 4 studies made it clear) and the administration of supplementation blindly (only 3 studies made it clear). The authors stated that he was double blind, but in the majority, they did not make it clear who among the researchers was blinded. The products used as placebo or control were: one study used orange-flavored placebo solution [26], two studies used apple favored non-calorific artificial sweetener consisting of sucralose [28,30], four studies used ice slurry ingestion [6,9,27,29], four studies used 3% surfactants mixed in water [8,31–33], one study used cold water immersion [34] and one study used natural mineral water [35].

The most contemplated items were: the subjects were randomly assigned to groups (11 studies); in the beginning, the groups were similar in terms of the most important prognostic indicators (13 studies); participation of volunteers blindly in the study (10 studies); at least 85% of the sample participated in the measurement of at least one key result (13 studies); performing intergroup statistical comparisons for at least one key result (13 studies) and presenting precision and variability measures for at least one key result (13 studies). All 13 studies included in this review were published in indexed international journals with an impact factor (average of 3.945 ± 3.039, with the lowest impact factor being 1.637 and the highest 13.8).

3.1. Mouth rinse with menthol

The practice of rinsing the mouth with a liquid solution of menthol and then spitting it out, either before or during the experimental exercise, was adopted in six studies, in physically active men and women with average age between 20 and 33 years old and 22 years old, respectively. The same volume and concentration of the menthol solution were used (25 mL at 0.01%) (Table 1). The time to exhaustion was assessed in four of these studies [26,28–30] with significant improvement found in three of them (6%, 7.7% and 9% increase) [26,28,29] and one study showed no significant improvement [30]. The other two studies evaluated the time trials as variable of physical performance [9,27], and both showed that there was a significant decrease in this time (2.7% and 3.8% of improvement). Another variable used to assess physical performance was perceived exertion, used in

Table 1 Studies that examined the effects of mouth rinse with menthol on physical performance and body temperature of endurance practitioners.

Authors/(PEDro)	Subjects	Exercise protocol	Supplementation protocol	Variables — Results
Mundel and Jones (2010) [26] (7)	9 physically active men 25 ± 7 years 54 ± 5 mL/kg/min (VO2máx)	Pedaled to exhaustion on an exercise bike at 65% of VO2máx Laboratory Conditions: 34 ± 1 °C RAH: 27 ± 4%	Cross over EXP condition: 25 mL menthol (0.01%) CON condition: orange solution dissolved in water in the same volume and concentration Instructed to gargle the solutions around the mouth for 10 seconds and then spit without swallowing (every 10 min during exercise)	Time to exhaustion - ↑ (9%) Exhaled air volume - ↑ (12.5% at 20 minutes and 10.6% at 40 minutes) Perceived effort - ↓ (15%) Rectal temperature - → Skin temperature - → Heart rate - → Oxygen uptake or carbon dioxide production - → Blood glucose or lactate - → Sweat rate or water ingested - →
Stevens et al. (2016) [9] (7)	11 men, recreational runners 29 ± 9 years Running time 18—22 min in 5 km running	10-Minutes warm-up followed by a 5 km time trial run on a treadmill Laboratory Conditions: 32.6 ± 0.2 °C RAH: 45.8 ± 5.7%	Cross over EXP condition: Rinse in the mouth for 5 seconds of 25 ml of L-menthol (0.01%) every 1 km during exercise and then spit without swallowing Positive CON condition: pre-cooling with ingestion of 7.5 g/kg ice paste (1 °C) before heating Negative CON condition: no intervention	Time trial- ↓ (2.7%) when compared to negative CON condition and ↓ (3.8%) when compared to positive CON condition Thermal sensation - ↓ ≅ (20%) Exhaled air volume - ↑ ≅ (6.5%) Prolactin concentration in blood compared to CON negative condition only - ↑ ≅ (63%) Sweat rate - → Rectal Temperature - → Skin temperature - → Blood lactate - → Perceived effort - → Gastrointestinal discomfort - →
Stevens et al. (2017) [27] (7)	11 men recreational runners 30 ± 9 years 61 ± 6 mL/kg/min (VO2máx)	20 min warm-up with 70% VO2max run followed by 3 km running on a treadmill Laboratory Conditions: 32.5 ± 0.18 °C RAH: 46.8 ± 7.9%	Cross over EXP condition: Oral rinse of 25 mL of menthol (0.01%) + water spray on the face [procedure done before training (every 5 minutes) and during training (every 1 km)] EXP condition 2: immersion in cold water at 23 °C for 30 minutes + ingestion of ice paste (7.5 g/kg) pre-workout EXP condition 3: combination of the two previous conditions CON condition: no cooling procedure	Time trial- ↓ (3.5%) in both EXP and EXP 3 vs. COM conditions. In the condition EXP 2 ↓ (2.1%), but without statistical difference vs. CON Respiratory exchange ratio ↑ (2.6% and 3.3% respectively) in EXP and EXP 3 vs. CON Thermal sensation - ↓ (6%, 6% and 8.1%) in EXP, EXP 2 and EXP 3 vs. COM Sweat rate - ↓ (26.6% and 35.7%) in EXP 2 and EXP 3 vs. CON Forehead Temperature - ↓ ≅ (1.1%) EXP 1 vs. CON Skin Temperature - ↓ ≅ (1.3%) EXP 3 vs. EXP 1 and ≅ (2.5%) EXP 3 vs. CON Rectal Temperature - ↓ ≅ (20%) in EXP 2 and EXP 3 vs. EXP 1 and CON Blood Prolactin Concentration - ↓ ≅ (45%) at all times vs. CON Exhaled air volume - ↑ ≅ (11%) at all times vs. CON Heart Rate - ↓ ≅ (6%) in EXP 2 Blood Lactate - → Perceived effort - → Gastrointestinal discomfort - →

Table 1 (*Continued*)

Authors/(PEDro)	Subjects	Exercise protocol	Supplementation protocol	Variables — Results
Flood et al. (2017) [28] (8)	8 physically active men 26 ± 5 years 55.4 ± 6.0 mL/kg/min (VO2máx)	Maximum sprint 5 s on stationary bike (5 min rest) followed by a test until exhaustion (keeping 16 on the Borg scale) and another maximum Sprint 5 s Laboratory Conditions: 35.0 ± 0.8 °C RAH: 47.8 ± 2.3%	Cross over EXP condition: 25 mL oral rinse of 0.01% L-menthol solution before and every 10 minutes during exercise CON condition: Oral rinse with placebo, using 25 mL of a non-calorie artificial apple sweetener in the same concentration and at the same times.	Time to exhaustion - ↑ (7.7%) Power - ↑ (3.5%) Peak isokinetic power of the declined sprint - ↓ (9.0%) pre-post in the EXP condition but without statistical difference not in the CON condition ↓ (3.4%) Thermal sensation - ↓ ≅ (5.4%) Perceived effort - ↓ ≅ (2.5%) Thermal comfort - → Rectal temperature - → Skin Temperature - → Cardiorespiratory variables Oxygen consumption (VO2max) - → Respiratory rate - → Tidal volume - → Ventilation - → Heart rate - →
Jeffries et al. (2018) [29] (8)	10 physically active men 33 ± 9 years 52.4 ± 5.3 mL/kg/min (VO2máx)	Time to exhaustion on a bicycle at 70% Wmax Baseline (85% of the time until baseline exhaustion) Laboratory Conditions: 35 ± 0.2 °C RAH: 40 ± 0.5%	Cross over EXP condition: mouth rinse with 25 mL of L-menthol (0.01%) Positive CON condition: ice paste intake (1.25 g/kg^{-1}) Negative CON condition: mouth rinse with 25 mL of a raspberry flavored placebo (0.01%)	Exhaustion in relation to baseline performance ($P = 0.036$): L-menthol - ↑ (6%) Ice paste - ↑ (7%) Placebo - → (1%) L-menthol vs. Ice paste - → Rectal temperature - → Skin temperature - → Heart rate - → Thermal sensation - → Thermal comfort - → Perceived effort - →
Parton et al. (2021) [30] (9)	11 physically active men 20 ± 1 years 53.9 ± 6.9 mL/kg/min (VO2máx) 11 physically active women 22 ± 2 years 43.5 ± 2.9 mL/kg/min (VO2máx)	Time to exhaustion on a bicycle such that a rating of perceived exertion 16 on Borg scale was maintained The trial was stopped when power output fell below 70% of this initial value for 30-s Laboratory Conditions: 34.9 ± 0.5 °C RAH: 40.6 ± 2.2%	Cross over EXP condition: mouth rinse with 25 mL of L-menthol (0.01%) CON condition: apple flavoured non-calorific artificial sweetener, consisting of sucralose dissolved in 25 mL of deionised water (0.01%) Solution to rinse 30 seconds prior to the main fixed RPE trial and at regular 10-min intervals	Time to exhaustion - → Thermal comfort - → Thermal sensation - ↓ ≅ (45%) Power output - ↑ (6.5%) in men Tympanic temperature - → Heart rate - → Whole-body sweat loss - →

RAH: relative air humidity; EXP: experimental; CON: control.

all six studies, showing a significant decrease in two of them (2.5% and 15%) and without variation in the other four.

Rectal temperature was measured in five studies, but only one showed significant variation with a 20% decrease [27]. The forehead temperature was measured in only one study with a significant decrease of 1.1% [27]. The skin temperature was measured in five studies, but only one had a significant decrease 1.3% and 2.5% depending on the application protocol [27]. This temperature was measured at four specific points, depending on the study, the points varied [forehead, back of the hand, lumbar and calf (three studies) or pectoralis major, triceps brachii, rectus femoris

Table 2 Studies that examined the effects of applying menthol through spraying or immersion in a solution containing menthol on the physical performance and body temperature of endurance practitioners.

Authors/(PEDro)	Subjects	Exercise protocol	Supplementation protocol	Variables — Results
Gillis et al. (2010) [31] (5)	12 physically active men 22 ± 2.9 years 47.4 ± 6.2 mL/kg/min (VO2máx)	15 min rest and 45 min exercise at 45% peak power on a cycle ergometer Followed by three counterbalanced exercises Laboratory Conditions: 30 °C RAH: 70%	Cross over EXP condition 1: Spray with 100 mL with 0.05% l-menthol EXP condition 2: Spray with 100 mL with 0.2% l-menthol CON condition: spraying of control solution without l-menthol	Rectal temperature - ↑ ≅ (0.2%) EXP 1 and EXP 2 vs. CON Thermal sensation - ↓ ≅ (30%) in EXP 1 and EXP 2 vs. CON Body temperature - → Sweat hate - → Blood flow from the skin - → Heart rate - → Thermal comfort - → Perceived effort - → Both doses induced skin irritation
Barwood et al. (2012) [32] (7)	11 physically active men 30 ± 8.1 years 40 km cycle < 70 min	Cycling time trial for 40 km Laboratory Conditions: 32 °C RAH: 50%	Cross over EXP condition: Menthol sprayed on the cycling jersey (106 mL at 0.05% between warm-up and time trial) Positive CON condition: Control solution sprayed on the cycling jacket (same volume, moments and concentration) Negative CON condition: no spraying	Time trial- → Thermal Sensation - ↓ ≅ (25%) EXP vs. negative and positive CON Thermal comfort - ↑ ≅ (21%) EXP vs. negative and positive CON Power - ↑ ≅ (5%) in the last 5 km of the time trial in the condition EXP vs. CON negative and positive Skin temperature - → Subjective effort perception - →
Barwood et al. (2014) [33] (7)	6 physically active men 21 ± 1 years 1.80 ± 0.07 meters in height 78.9 ± 6.9 kg body weight	15 min pre-exercise with fixed intensity and then 5 km time trial Laboratory Conditions: 34 °C RAH: 50%	Cross over EXP condition: Menthol sprayed on the cycling jersey (106 mL at 0.05% between warm-up and time trial) Positive CON condition: Spray control on the cycling jersey (same volume, moments and concentration) Negative CON condition: no spraying	Time trial- → Thermal Sensation - ↓ ≅ (45%) EXP vs. negative CON and ≅ (35%) vs. positive CON at the start of the time trial Thermal comfort - ↑ ≅ (45%) EXP vs. negative CON and positive CON at km 1 of the time trial Heart Rate - → Perceived effort - → Skin temperature - →
Barwood et al. (2015) [8] (7)	8 physically active men 21 ± 2 years 1.81 ± 0.07 meters in height 83.1 ± 11.1 kg body weight	Cycling time trial for 16.1 km Laboratory Conditions: 34 °C RAH: 33%	Cross over EXP condition: Menthol sprayed on the cycling jersey (106 mL at 0.05% between warm-up and time trial) CON condition: Control solution sprayed on the cycling jacket (same volume, moments and concentration)	Time trial- → Thermal sensation - ↓ Thermal comfort - ↑ Perceived effort - ↓ Skin temperature - ↓ (2%) Body temperature - → Rectal temperature - → Power - →
Rinaldi et al. (2018) [34] (6)	8 male elite cyclists 24.1 ± 4.4 years 321 ± 41 Watts (maximum aerobic strength)	Two 20 minute cycling trials interspersed with a 10-minute immersion in cold water or menthol in cold water Laboratory Conditions: 29.1 °C RAH: 62%	Cross over EXP condition: immersion in water at 10 °C and 0.01% menthol CON condition: immersion in water at 10 °C and 0.01% placebo	Thermal sensation - ↓ ≅ (8.5%) Subjective perception of effort - ↓ ≅ (28%) Power - ↑ ≅ (15.6%) Rectal temperature - ↓ ≅ (17%) Skin temperature - ↓ ≅ (24%) Thermal Comfort - →

RAH: relative air humidity; EXP: experimental; CON: control.

Table 3 Studies that examined the effect of oral intake of menthol or peppermint essential oil on physical performance and body temperature of endurance practitioners.

Authors/(PEDro)	Subjects	Exercise protocol	Supplementation protocol	Variables — Results
Riera et al. (2014) [6] (7)	12 physically active men (cyclists and triathletes) 42 ± 13 years 59.9 ± 10.4 mL/kg/min (VO2máx)	15 min of free warm-up followed by 20 km of pedaling against the clock at full speed Laboratory conditions: 30.7 ± 0.8 °C RAH: 78 ± 0.03%	Cross over EXP condition: Drink 190 mL of menthol (0.05%) before and after warming up, before exercise and every 5 km during exercise CON condition: Intake of 190 mL of control drink without menthol (0.05%) at the same times The drinks were at three different temperatures: 23 °C (neutral) 3 °C (cold) −1 °C (ice paste)	Intra groups time trial With menthol vs. without menthol: 23 °C - → With menthol 3 °C - ↓ ≅ (5.2%) With menthol −1 °C - ↓ ≅ (5.4%) Inter group time trial: Menthol cold - ↓ ≅ (8.2%) and menthol ice cream - ↓ ≅ (6.9%) as opposed to neutral without menthol, neutral with menthol and cold without menthol Central Temperature - → Heart Rate - → Perceived effort - → Thermal Comfort - → Thermal Sensation - →
Shepherd and Peart (2017) [35] (8)	4 physically active men and 3 women 24.57 ± 3.95 years 3.54 ± 1.52 L/min (VO2peak)	Bicycle exhaustion test, which started at 0 W and increased by 30 W every 60 seconds (1 W every 2 seconds) until exhaustion Cardiopulmonary testing Laboratory temperature and RAH conditions not reported	Cross over EXP condition: oral intake for 10 days of 0.05 mL of peppermint essential oil diluted in 500 mL of water CON condition: oral intake for 10 days of 500 mL of water	In the Bicycle test: Time to exhaustion - → Peak force - → Total work - → In the cardiopulmonary test: Peak VO2 - → Spirometric parameters - → Heart rate - → Systolic and diastolic blood pressure - →

RAH: relative air humidity; EXP: experimental; CON: control.

and gastrocnemius (two studies)]. Average temperature of these points it was used to determine the skin temperature. Tympanic temperature was performed in only one study, with no significant variation [30].

Still in Table 1, the other variables used as possible explanations for these good results found in physical performance were a greater volume of exhaled air during exercise in the three studies [9,26,27] in which it was evaluated (increase between 6.5% and 12.5%). The thermal sensation was measured in five studies [9,27–30], all showing a decrease between 5.4% and 20%. Thermal comfort was evaluated in only three studies [28–30] and showed no variation. The concentration of prolactin in the blood has been shown to vary widely in the two studies [9,27] in which it was evaluated (increase of 63% and decrease of 45%). Different behavior was observed for blood lactate, which showed no variation in the two studies [26,27] in which it was measured. Heart rate did not vary in four studies [26,28–30] and only one showed a decrease of approximately 6% [27]. Four studies evaluated the sweat rate [9,26,27,30] and only one showed a decrease between 26.6% and 35.7% [27].

both did not change. Only one of the studies, body temperature and skin temperature (2% decrease) were evaluated.

Still in Table 2, the other variables used were the measurement of the thermal sensation, being evaluated in all five studies, and all of them showed a decrease ranging between ≅ 8.5% and 45%. Thermal comfort was also assessed in all studies, with an increase in three of them (between ≅ 21% and 45%). Heart rate did not vary in the two studies in which it was measured [31,33]. Sweat rate and blood flow in the skin were evaluated in only one study, but without changes [31].

3.3. Oral menthol intake

One study evaluated the oral intake of menthol in physically active men with an average age of 42 years [6] and another study evaluated the oral intake of peppermint essential oil in men and physically active women with an average age of 24 years [35] (Table 3). In the study with menthol intake, there was a decrease in the time trial between 5.2% and 8.2%, depending on the temperature of the drink. Other variables analyzed were perceived exertion, central temperature, heart rate, thermal sensation and thermal

3.2. Use of menthol by spraying or immersion in menthol solution (topical use)

Another practice that has been used is the use of menthol through spraying or immersing the body in a solution containing menthol. Five studies evaluated these application methods, four studies with spraying [8,31–33] and one study with immersion [34], in physically active men or elite athletes aged between 21 and 30 years who used 106 mL to 0.05% menthol [8,32,33], 100 mL at 0.05% or 0.2% menthol [31] and immersion in water at 10 °C and 0.01% menthol [34] (Table 2). The time to exhaustion (for a standardized exercise protocol) has not been evaluated by any study using this application method. The time trial was evaluated in three studies [8,32,33], but also without modification. Another variable used to assess physical performance was perceived exertion, assessed in all five studies, showing a decrease in two of them (≅ 28% in one and in the other we were unable to determine clearly) [8,34] and without variation in the other three [31–33].

Rectal temperature was measured in three studies, with different behaviors in each of them, increasing 0.2% in one study [31], decreasing ≅ 17% in another study [34] and without varying in the third study [8]. The skin temperature was measured in four studies [8,32–34] but in only two of them there was a decrease of 2% [8] and ≅ 24% [34]. This temperature was measured using different methods depending on the study, where two studies evaluated eight sites (left pectoral, right scapula, left biceps, left dorsal hand, vastus medialis right, left hamstrings, right anterior tibial, right dorsal foot) one study evaluated six sites (biceps, chest, subscapularis, forearm, thigh and calf) and one study assessed four sites (chest, arm, leg and forehead), averaging the temperature of these sites to determine skin temperature. Two studies measured what they called body temperature, that is, the temperature of the entire body, being measured at the same locations as the skin temperature (eight and six locations) but using different formulas for averaging and

studies that evaluated the time to exhaustion, one evaluated the intensity of 65% of VO2 max. [26], another one of 70% of Wmax. [29], two evaluated the intensity through the ability to maintain 16 on the Borg scale [28,30] and one study followed the intensity by increasing by 30 W every 60 seconds [35], all five studies on stationary bicycle. Meanwhile, from six studies that evaluated the race time (time trial), one was 3 km [27], two 5 km [9,33], one 16.1 km [8], one 20 km [6] and the other 40 km [32]. We intended to do a meta-analysis to determine the degree of evidence, with physical performance as the main outcome. However, with such different exercise protocols, this action was not prudent for this moment. So, clearly, more studies are needed and with a larger sample size in each one of them so that a meta-analysis between them can be possible. After the final inclusion of the 13 articles that are part of this systematic review, we note that another 10 articles could have been part of this work, but were not included in the systemic research because one did not is in Pubmed and the other nine that are, are not classified as clinical trial, as for example, we can mention the studies by Tran Trong et al. [37] and the most recent article published on the topic written by Gavel et al. [38].

The main strengths considered by us were that the studies were all carried out in challenging environments for thermoregulation, always in hot environments and with either low or high relative humidity, with just one exception. The products used as placebo or control in the 13 studies of this

comfort, but none of them showed variation. In the study that evaluated the oral intake of peppermint essential oil, there was no improvement in the variables of physical performance evaluated (time to exhaustion, peak strength and total work) as well as none of the other cardiorespiratory variables (peak VO2, heart rate, blood pressure and spirometric parameters).

4. Discussion

Of the eight studies that evaluated the ergogenic effect of oral ingestion or mouth rise of menthol, six demonstrated an improvement in the physical performance of recreational athletes who practice aerobic exercise. On the other hand, topical application or immersion in liquid does not result in benefit. However, this evidence must be weighed according to the quality and some important methodological aspects of the selected studies.

Considering the quality criteria adopted, an issue that deserves consideration is the sample size of the studies, which was initially considered by us as an important methodological limitation. The study by Barwood et al. [33] was carried out with only six individuals and eleven studies have a sample between seven and twelve individuals, with the exception of the study by Parton et al. ($n = 22$) [30]. Despite this finding, after performing the sample calculation *a posteriori*, we realized that in the five studies in which there was no improvement in physical performance, the estimated effect size was between 0.01 and 0.15, so that the result would not be positive even with a larger sample size, thus ruling out the chance of a false negative. On the other hand, in all studies in which performance improvement was observed, the sample size was smaller than that calculated *a posteriori*. In summary, all studies were performed with insufficient sample size.

The selected studies are very difficult to compare because, despite well-defined supplementation protocols, the physical test protocols are very diverse. Of the five

solutions used was followed, depending on the method of application. Only one study did not use menthol in its isolated form, using the oral ingestion of peppermint essential oil, which has most of its composition composed of menthol (70%) [39], so the amounts of menthol provided were similar and justifies the maintenance of this study in the review. We can also mention the accomplishment of experimental protocols in laboratory environment with control of the ambient temperature and relative humidity of the air done in 12 of the 13 studies. Body temperature was also measured in 12 of the 13 studies, with the measurement method varying according to the measurement location (forehead, rectal, skin, body and tympanic). The time between procedures is an important methodological parameter, as it minimizes the effects of physiological adaptation and interference from one session to the other. Based on this, we observed that 12 of the 13 studies included in this review reported having performed a washout between 24 h and 10 days.

Some authors hypothesized that, since the effect of menthol is probably explained by a cooling action, application to the skin (topical and immersed in liquid) would also have an ergogenic effect. However, this was not confirmed in any of the five studies that used this means of application, at least to improve physical performance (a reduction in thermal sensation was observed in some studies, accompanied by greater thermal comfort and lower body temperature). Therefore, these data indicate the continuation of this line of research only with oral application of menthol. This evi-

review were diverse, depending on the method of ingestion or application, the product varied and in some of them it represented a problem regarding blinding, especially when the product was ingested or mouth rinse. Mundel and Jones [26] stated that to mask the experimental hypothesis and blind the subjects as much as possible with regards to the purpose of the study, they were informed that the aim of the study was to compare the flavor of two commercially available sports drinks. Parton et al. [30] and Flood et al. [28] stated that blinding participants to the cooling sensation induced by l-menthol is difficult and so chose to use a bitter tasting placebo solution to equally stimulate the oral cavity due to the difficulty replicating the taste of menthol without eliciting a cooling effect. Shepherd and Peart [35] recognized that mineral water may not be as appropriate as a placebo with a matching flavor, but stated that this could not be achieved, as the odor of peppermint may be as ergogenic as ingestion. We understand that the liquid mint essence (absence of menthol), not used by the authors, could be a more interesting and reliable alternative as a placebo. Data from our laboratory (not yet published) indicated that approximately 90% of more than 30 people who were tested for the organoleptic properties (taste, aroma and color) of peppermint essential oil and liquid mint essence (placebo) failed to notice the difference between the two drinks. Reinforcing that this placebo would have similar characteristics to the experimental product, with the exception of the cooling capacity, characteristic of menthol.

Was noted that similar supplementation methodologies within each application method, with three well-defined forms of administration, which increases the power of evidence of the results found. Of the thirteen studies evaluated, twelve used menthol in its isolated form, and a standard of quantity and concentration of the menthol improvement in physical performance, but we still cannot rule out other objective variables that may also influence physical performance.

Physiological mechanisms involved have been poorly investigated. Of the thirteen studies in this review, none investigated the possible explanatory mechanisms for improving performance after using menthol; only half dealt with the matter in a speculative manner, suggesting mechanisms that could participate in this process. Mundel and Jones [26] speculated that menthol would promote increased ventilation promoted by cooling and activate the brain area associated with taste and reward, decreasing the stimuli associated with the feeling of fatigue, as seen by Guest et al. [41]. Similar reasoning was speculated by Shepherd and Peart [35] who hypothesized a possible mechanism based on a previous study, such as the relaxation of bronchial smooth muscle tone [42].

Riera et al. [6] speculated that the thermoreceptors in the hypothalamus do not detect increases in temperature due to the cooling effects of menthol and, therefore, no inhibitory signals would be sent to the motor control center. This could also prevent redistribution of blood from the nucleus to the periphery, with adequate cardiac output being maintained. Flood et al. [28] speculated a similar situation, that the role of menthol in modulating arousal or motivational levels can be considered part of the mechanistic process. The author explained his theory based on a literature review conducted by Zheng and Hasegawa [43] that showed that central dopaminergic neurotransmission plays an important role in thermoregulation and performance during long duration endurance exercise and that dence must be weighed, however, by the fact that only one study evaluated elite athletes (topical application) [34] and two studies evaluated women [30,35]. Thus, we cannot affirm that menthol would be an ergogenic resource indicated for high-performance athletes and women until further studies consider these populations. On the other hand, recreational sport has evolved a lot, so that street running has become one of the most popular sports practices in recent years, be it competitive or recreational. Participation in street racing worldwide has increased by more than 50% over the past ten years, gaining millions of fans [40] and this population can benefit from the use of menthol.

The mechanisms of action that can explain this improvement in physical performance are still little known and controversial. Body temperature, perception of body temperature and sweat rate were variables considered in the studies. In relation to body temperature, all studies evaluated this variable, but only in three studies there was a significant reduction, so it is still a controversial explainer. The sweat rate was evaluated in only four studies and in a single study this rate was reduced, so it does not seem to influence the improvement of physical performance, but this result must be ponder by the low number of studies that evaluated this variable. Thermal comfort was evaluated in nine studies but in only three studies there was an improvement in this comfort to the athlete, so it seems that this variable is also controversial in explaining the improvement in physical performance. Regarding thermal sensation, eleven studies evaluated this variable and in nine of them there was a decrease in thermal sensation, so it seems to be consensual that this variable can be one of the explanations of the improvement in physical performance. In view of this, these results suggest that subjective measures (thermal sensation) may be the action mechanisms that best explain the include women and high-level athletes, as they are scarce in the literature. They should also explore the mechanisms by which oral administration of menthol (ingestion or rinsing) improves physical performance. Another point is that, although the result of the a posteriori calculation revealed the non-interference of the small sample size in the results, we believe it is necessary to carry out studies with larger samples. The studies available until this systematic review of the literature do not allow us to determine whether menthol has an ergogenic effect also in high-performance athletes, in women, and they have not investigated the mechanisms by which menthol can improve physical performance.

The results of this review are easily applicable in practice, as there are data in favor of (oral) menthol, as it can be applied in a single dose, be easily ingested or rinsed off before or during endurance competition or training, and have good acceptance. Due to the pleasant sensation of pleasure and cooling, its use does not imply any extra activity for the athlete, as hydration is already part of the routine before, during and after training or competition, so the only change would be the addition of menthol to the liquid to be ingested. This administrative feasibility, associated with the pleasant sensation of refreshment, especially during endurance exercise, is potentially stimulating for the adoption of this ergogenic resource. But despite this, the results of the present study must be considered and cannot yet be translated into clinical evidence for nutritionists, coaches and athletes, until further methodologically improved studies, with larger samples and similar protocols are carried out.

annulled inhibitory signals from the central nervous system result in cessation. Exercise due to hyperthermia, therefore the removal of a ''thermoregulatory brake''.

Rinaldi et al. [34] and Jeffries et al. [29] raised the hypothesis that an anticipatory mechanism was involved to limit heat storage related to metabolic production and environmental stress, through the TRPM8 receptor, a peripheral cold receptor, which can also be found in the oral cavity and considered the main environmental cold detector, so that its activation induces a reduction in the thermal sensation, despite not having been observed by Jeffries et al. [29]. Meanwhile, it has also been proposed by these authors that the application of menthol could also inhibit the TRPA1 receptor, a pain-sensitive receptor, thereby mediating responses to pain and reducing a possible ergolytic influence of pain sensations. These receptors have an analgesic effect through peripheral and central mechanisms [44], which increases performance and probably limits the anticipatory mechanism. Despite these findings, studies with topical application have not had a positive result.

Although studies are limited in quantity and with some methodological issues that need improvement, orally administered menthol (mouth rinse and ingestion) has been shown to improve physical performance (time to exhaustion, race time, and perceived exertion) of recreational athletes aerobic exercise practitioners. Despite this, these results need to be considered and are suggestive in the sense that more work needs to be done so that we have more robust evidence of the ergogenic effect of oral menthol. These studies need to better standardize supplementation and exercise protocols that will test the use of menthol and should also

5. Conclusions

Data from the studies indicate that the administration of menthol orally (ingestion and mouth rinse) improves the sports performance of recreational athletes practicing aerobic exercises and that the topical application does not generate influence. However, these data cannot yet be considered as concrete scientific evidence intended by a systematic review of the literature that can support clinical practice because of methodological issues of the studies, such as the small sample size, experimental protocols of supplementation and exercise different. This translates to a PEDro scale mean of only 7.15 ± 0.94. Therefore, it is prudent to wait for new methodologically improved studies, with larger sample sizes, similar supplementation and exercise protocols, to be carried out until the use of menthol in clinical practice can be indicated. Even with the lack of effect of topical application, we cannot rule out the possible ergogenic capacity of this form of use, because the small sample size is unable to give consistency to the null hypothesis that was adopted in these studies. We recommend that all future studies be carried out using an a priori estimation of the sample size.

Disclosure of interest

The authors declare that they have no competing interest.

Author contributions

Manoel Miranda Neto and Alexandre Sérgio Silva contributed substantially to the conception and design of the study and the acquisition, analysis and interpretation of data; wrote the article and made critically revise it for important intellectual content; and approved publication of the final version. Raquel Suelen Brito da Silva contributed to write the article, analysis and interpretation of data; approved publication of the final version.

Data availability statement

The data of this study are available from the corresponding author Alexandre Sérgio Silva upon reasonable request.

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APÊNDICE D – RESULTADOS COMPLEMENTARES

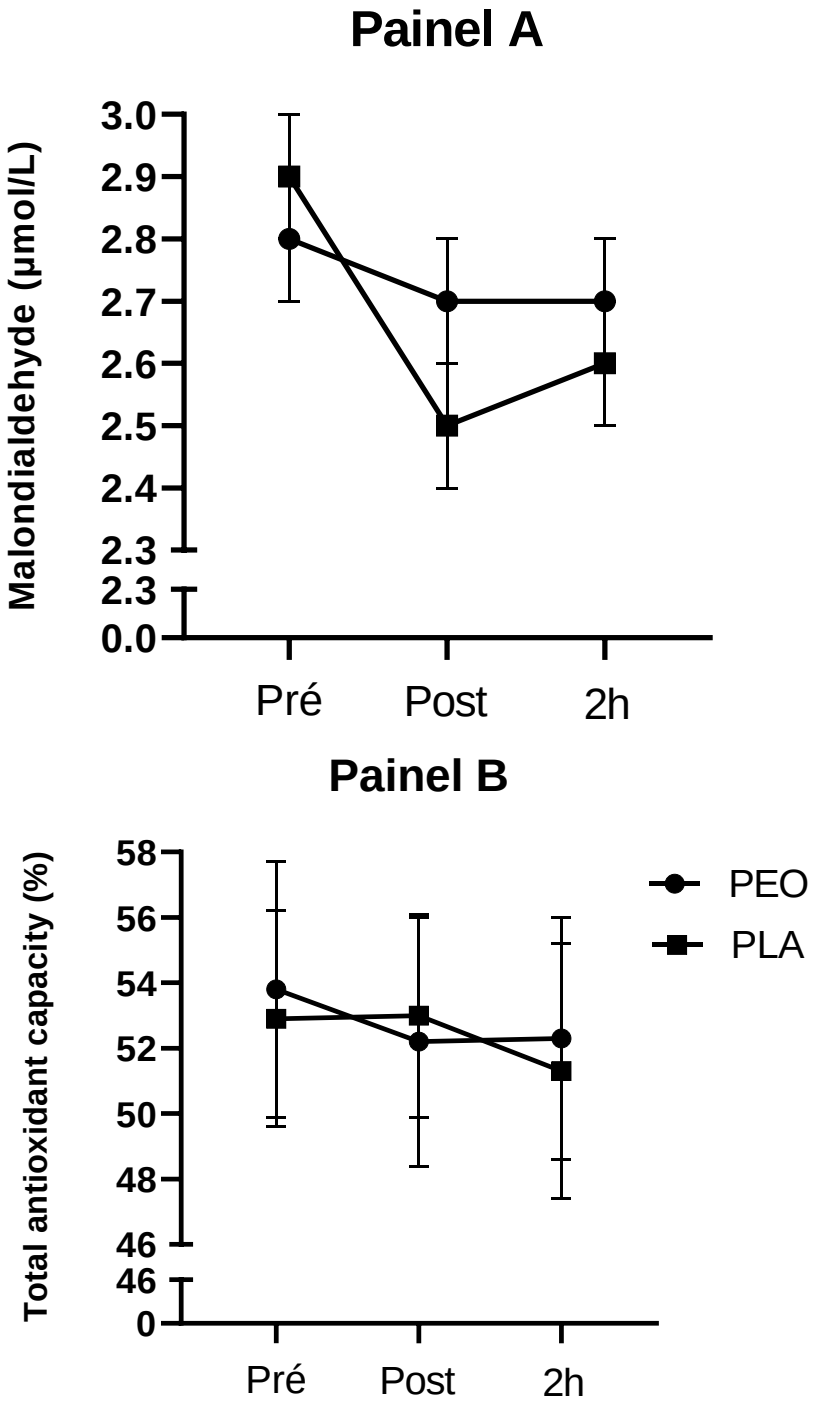


Figura 7. Balanço redox. Painel A: Atividade Pró oxidante (Malondialdeído); Painel B: Capacidade antioxidante total. Dados são expressos como média ± erro padrão. Não foram observadas diferenças estatísticas entre os procedimentos em nenhum dos momentos avaliados (p>0,05).

APÊNDICE E - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

_____

Esta pesquisa é intitulada **INFLUÊNCIA DO POLIMORFISMO DO GENE DA ECA SOBRE OS EFEITOS DA SUPLEMENTAÇÃO COM O ÓLEO ESSENCIAL DE HORTELÃ-PIMENTA (*Mentha piperita* L.) NO DESEMPENHO FÍSICO DE CORREDORES** e está sendo desenvolvida por MANOEL MIRANDA NETO, aluno do Curso de Pós-Graduação em Ciências da Nutrição da UFPB, sob a orientação do Prof. Dr. ALEXANDRE SÉRGIO SILVA.

A finalidade deste trabalho é investigar se uma bebida enriquecida com óleo essencial de hortelã-pimenta melhora o seu desempenho durante uma corrida e se esta melhora do desempenho é porque o óleo essencial de hortelã-pimenta faz com que seu corpo produza menos calor durante o exercício. Tem ainda por finalidade investigar se ocorre menor estresse oxidativo, desgaste da sua musculatura e se sua característica genética influencia esses resultados.

Para sabermos se a bebida que você vai ingerir realmente melhora seu desempenho, solicitamos sua colaboração para realizar o seguinte protocolo experimental: você vai vir ao laboratório uma primeira vez e deverá beber 500 ml de uma dose da bebida experimental ou controle, e, 30 minutos depois será realizada uma sessão de corrida até a fadiga, devendo permanecer no laboratório em um repouso de 2h após o exercício. A bebida experimental será um suco adicionado do óleo essencial de hortelã-pimenta, aquele mesmo hortelã natural que talvez você já tenha usado em sucos detox. A bebida controle será uma bebida isotônica (aquela que você costuma tomar em treinos e competições, e que compra sob o nome de Gatorade ou pawerade ou similares). Uma semana depois você deverá retornar ao laboratório para ingerir a bebida que não havia ingerido na semana anterior e repetir o mesmo exercício até a fadiga e o mesmo tempo de repouso.

Terminada esta fase do estudo, iniciaremos outra fase que agora vai durar 30 dias. Neste caso, você não precisará ir ao laboratório durante estes 30 dias. Deverá tomar 500ml da bebida por dia, sempre no turno da tarde. Neste período você poderá treinar normalmente. Vinte e quatro horas depois da ingestão do trigésimo dia, você deverá voltar ao laboratório para repetir o teste até a fadiga e o mesmo tempo de repouso.

Antes e durante os três exercícios de corrida até a fadiga, faremos as seguintes avaliações com você: coleta do material genético através da sua saliva, medidas de temperatura do corpo serão feitas por meio de um termômetro que é colocado no seu ouvido, um aparelho feito para isso que não gera dor nem desconforto. Além disso, você deverá responder em uma escala numérica o quanto o exercício está cansativo. Também será medida a sua Frequência Cardíaca (FC). Coletas sanguíneas serão realizadas antes, imediatamente após os três exercícios de corrida até a fadiga e duas horas após o exercício para dosagem de marcadores bioquímicos de dano muscular (CK e LDH), estresse oxidativo (MDA e CAT). As coletas sanguíneas serão feitas por enfermeira devidamente experiente. Coletas da urina para obtenção do sumário de urina.

Uma semana antes de tudo isto, nós te encaminharemos para uma clínica cardiológica da cidade para realizar um teste ergoespirométrico para determinação do consumo máximo de

oxigênio e limiar anaeróbio. Esse teste ergoespirométrico é uma corrida em uma esteira com velocidade que inclinação da esteira que vai aumentando gradualmente até a sua fadiga. Trata-se daquele conhecido teste de esforço que é realizado pelos médicos cardiologistas, sendo que neste caso é um teste voltado para atletas. Este teste é feito com você utilizando uma máscara para acompanhar sua respiração. Junto com esse teste teremos todo um aparato médico de reabilitação, caso lhe ocorra algo.

Tanto este teste ergoespirométrico quanto as corridas até a fadiga vão lhe trazer os mesmos desconfortos de uma corrida, uma vez que você deverá se esforçar ao máximo, até atingir a sua fadiga. Exercícios físicos sempre trazem riscos. Por isso, no teste ergoespirométrico feito uma semana antes do inicio da pesquisa, o cardiologista vai fazer um eletrocardiograma junto com a sua corrida para atestar a sua segurança cardiovascular para esforços máximos. Se for verificado algum problema em seu coração, lhe avisaremos e você não mais realizará o estudo.

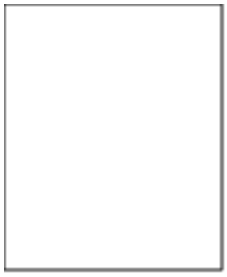
Mesmo avaliando sua segurança cardíaca, informamos que os procedimentos desta pesquisa podem gerar desconfortos como: sensação de mal-estar, tonturas, ânsia de vomito, desequilíbrio nas passadas na esteira devido ao cansaço, como também um possível roxeado no braço no momento da coleta sanguínea. Entretanto, os riscos previsíveis serão diminuídos pelo acompanhamento de profissionais experientes da área e treinados. Caso ocorram problemas maiores, serão cessados com a imediata interrupção da sessão de exercício ou da coleta sanguínea e será realizado os primeiros socorros de forma imediata. Além disso, o responsável pela pesquisa estará de prontidão com infraestrutura e equipamentos adequados para qualquer eventualidade caso venha ocorrer e coloquem em riscos os participantes. Nos casos em que os voluntários apresentem desconfortos ou sejam detectadas alterações que necessitem de assistência médica encaminharemos imediatamente para o hospital mais próximo (Hospital Universitário Lauro Wanderley), que fica a poucos metros do local das coletas.

Solicitamos a sua colaboração e autorização para coletar os dados necessários para esse estudo, assim como, apresentar os resultados obtidos em eventos da área de saúde e publicar em revistas científicas garantindo que seus dados pessoais serão mantidos em absoluto sigilo. Importante informar que a participação no estudo é voluntária, de modo que você não é obrigado a fornecer as informações e/ou colaborar com as atividades solicitadas pelo pesquisador. Caso decida não participar do estudo ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano, nem haverá modificação na assistência que vem recebendo na Instituição. Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido (a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

_____

Assinatura do Participante da Pesquisa
ou Responsável Legal



OBERVAÇÃO: (em caso de analfabeto - acrescentar)

Espaço para Impressão dactiloscópica

_____
Assinatura da Testemunha

Caso necessite de maiores informações sobre o presente estudo, favor entrar em contato com o pesquisador responsável**:**

Manoel Miranda Neto
Endereço: Rua Valdemar Chianca, 352, Ed. Maranata, apt 401 A, Jardim Oceania. CEP: 58037-255, João Pessoa-PB
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OU
Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba Campus I - Cidade Universitária - 1º Andar – CEP 58051-900 – João Pessoa/PB
☎ (83) 3216-7791 – E-mail: eticaccsufpb@hotmail.com

Atenciosamente,

_____
Assinatura do Pesquisador Responsável

_____
Assinatura do Pesquisador Participante

Obs.: O sujeito da pesquisa ou seu representante e o pesquisador responsável deverão rubricar todas as folhas do TCLE apondo suas assinaturas na última página do referido Termo.

APÊNDICE F - RECORDATÓRIO ALIMENTAR DE 24 HORAS

RECORDATÓRIO ALIMENTAR DE 24 HORAS

1°( ) 2°( ) 3°( )

Nome:_____ _____ Idade:_____

Data: _____ /_____ /_____

Refeição/horário	Preparação e/ou alimentos	Medida caseira	Quantidades (gramas/ml)	Observações
Desjejum				
Lanche				
Almoço				

Lanche	
Jantar	
Colação	
Faz uso de algum suplemento alimentar (proteínas, carboidratos, vitaminas, minerais, nutricosméticos, emagrecedores, etc)? ( )Sim ( )Não	
Se sim, qual (is) tipo, marca, quantidade e horário de ingestão do (os) suplemento (os)?	

ANEXOS

ANEXO A – ESCALA DE PERCEPÇÃO SUBJETIVA DE ESFORÇO DE BORG

Figura 8. Escala de Percepção Subjetiva de Esforço de Borg.

Fonte: https://www.google.com.br/imagens/escaladeborg

ANEXO B – ESCALA DA SENSAÇÃO TÉRMICA

Figura 9. Escala de Sensação Térmica.

Fonte: Escala subjetiva de sete pontos baseada em Hodder e Parsons (2007)

ANEXO C – ESCALA DO CONFORTO TÉRMICO

Figura 10. Escala de Conforto Térmico.

Fonte: Escala subjetiva de quatro pontos baseada em Hodder e Parsons (2007)

ANEXO D – PARECER CONSUBSTANCIADO DO CEP

UFPB - CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INFLUÊNCIA DO POLIMORFISMO DO GENE DA ECA SOBRE OS EFEITOS DA SUPLEMENTAÇÃO COM O ÓLEO ESSENCIAL DE HORTELÃ-PIMENTA (Mentha piperita L.) NO DESEMPENHO FÍSICO DE CORREDORES

Pesquisador: Manoel Miranda Neto

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 17130619.2.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1381887.pdf	07/08/2019 16:24:32		Aceito
Outros	Certidao_AD_REFERENDUM_PPGCN.jpg	07/08/2019 16:22:11	Manoel Miranda Neto	Aceito
Declaração de Instituição e Infraestrutura	Carta_de_anuencia_LETFADS.jpeg	01/08/2019 15:00:34	Manoel Miranda Neto	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Doutorado.pdf	31/07/2019 18:41:57	Manoel Miranda Neto	Aceito
Declaração de Instituição e Infraestrutura	Carta_de_anuencia_LEMAC.pdf	09/07/2019 10:59:57	Manoel Miranda Neto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	09/07/2019 10:59:16	Manoel Miranda Neto	Aceito
Folha de Rosto	Folha_de_rosto_comite_de_etica.pdf	09/07/2019 10:45:42	Manoel Miranda Neto	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 26 de Agosto de 2019