UNIVERSIDADE FEDERAL DE MINAS GERAIS

Escola de Educação Física, Fisioterapia e Terapia Ocupacional

Programa de Pós-Graduação em Ciências do Esporte

Jefferson Fernando Coelho Rodrigues Junior

EFEITO DE UM PROTOCOLO CURTO DE ACLIMATAÇÃO AO CALOR NA RECUPERAÇÃO APÓS 10 KM DE CORRIDA EM INTENSIDADE AUTORREGULADA EM AMBIENTE QUENTE

Belo Horizonte

2025

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Tese apresentada ao Programa de Pós-Graduação em Ciências do Esporte da Escola de Educação Física, Fisioterapia e Terapia Ocupacional da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do título de Doutor em Ciências do Esporte.

Orientador: Prof. Dr. Samuel Penna Wanner
Coorientador: Prof. Dr. Thiago Teixeira Mendes

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UNIVERSIDADE FEDERAL DE MINAS GERAIS
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ATA DE DEFESA DE TESE

JEFFERSON FERNANDO COELHO RODRIGUES JÚNIOR

Às **13:00 horas** do dia **26 de junho de 2025**, a comissão examinadora, indicada pelo Colegiado do Programa de Pós-Graduação em Ciências do Esporte, reuniu-se por videoconferência, para julgar, em exame final, a tese intitulada "**Efeito de um protocolo curto de aclimatação ao calor na recuperação após 10 km de corrida em intensidade autorregulada em ambiente quente**". Abrindo a sessão, o presidente da comissão, Prof. Dr. Samuel Penna Wanner (UFMG), orientador, após dar a conhecer aos presentes o teor das Normas Regulamentares de Defesa do Trabalho Final, passou a palavra para o candidato, que realizou a apresentação da sua tese. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato. Logo após, a comissão se reuniu, sem a presença do candidato e do público, para julgamento e expedição do resultado.

Indicações dos membros da comissão examinadora:

Prof. Dr. Samuel Penna Wanner (UFMG - orientador) - Aprovado

Prof. Dr. Thiago Teixeira Mendes (UFBA - coorientador) - Aprovado

Prof. Dr. Cândido Celso Coimbra (UFMG) - Aprovado

Prof. Dr. Fabiano Trigueiro Amorim (University of New Mexico, EUA) - Aprovado

Prof. Dr. Mário Norberto Sevílio de Oliveira Júnior (UFMA) - Aprovado

Prof. Dr. Thales Nicolau Prímola-Gomes (UFV) - Aprovado

Após as indicações, o candidato Jefferson Fernando Coelho Rodrigues Júnior foi considerado: **APROVADO**.

Nada mais havendo a tratar, eu, Prof. Dr. Samuel Penna Wanner, presidente da comissão examinadora, dei por encerrada a reunião, da qual, para constar, lavrei a presente ata, que, lida e aprovada, vai por todos assinada eletronicamente.

Belo Horizonte, 26 de junho de 2025

Assinatura dos membros da banca examinadora:

Documento assinado eletronicamente por **Samuel Penna Wanner**, **Coordenador(a) de curso de pós-graduação**, em 26/06/2025, às 17:21, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Este trabalho foi realizado no Laboratório de Adaptações Cardiovasculares ao Exercício (LACORE) do Núcleo de Esportes da Universidade Federal do Maranhão em parceria com o Laboratório de Fisiologia do Exercício (LAFISE) da Escola de Educação Física, Fisioterapia e Terapia Ocupacional (EEFFTO) da Universidade Federal de Minas Gerais (UFMG), com os auxílios concedidos pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 407925/2023-6) e pela Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; APQ-01828-23).

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RESUMO

Protocolos de aclimatação ao calor, caracterizados por sessões de exercício físico sob estresse térmico ambiental, são estratégias efetivas para induzir adaptações fisiológicas e perceptivas e para melhorar o desempenho prolongado (aeróbico) em ambiente quente. O primeiro estudo desta tese investigou os efeitos do estresse térmico ambiental sobre variáveis fisiológicas e perceptivas durante corrida de longa duração em esteira rolante e sobre o desempenho neuromuscular de membros inferiores após a corrida. Treze corredores recreativos que participaram do estudo mantiveram menor velocidade média para completar os 10 km em intensidade autorregulada e apresentaram maiores valores de percepção de esforço durante a corrida a 35°C em comparação com 25°C. A altura do salto com contramovimento (SCM) também foi reduzida em 7% uma hora após o exercício, indicando fadiga neuromuscular, mas não houve diferença entre as duas temperaturas ambientais investigadas. O segundo estudo analisou respostas fisiológicas, perceptivas e de desempenho físico após uma corrida de 10 km em intensidade autorregulada a 35°C. 16 voluntários apresentaram aumento da fadiga, da dor muscular percebida e da gravidade específica da urina, além da redução do vigor, da recuperação percebida e da atividade parassimpática cardíaca após a corrida. Os dados também confirmaram a redução na altura do SCM 1 hora após a corrida. A maioria dessas alterações foi transitória e retornou aos valores basais após 24 horas, com exceção da recuperação percebida, que permaneceu reduzida. As alterações de desempenho neuromuscular não foram associadas às alterações perceptivas. O terceiro estudo avaliou os efeitos da aclimatação ao calor de curto prazo (5 dias) na recuperação após corrida em ambiente quente. Dezesseis participantes foram aleatoriamente alocados em dois grupos: controle ou aclimatação. A aclimatação ao calor resultou em melhor desempenho durante a corrida, sem alterar a recuperação, que foi semelhante entre os grupos. As exceções foram a menor reativação parassimpática cardíaca na primeira hora subsequente à corrida de 10 km e os efeitos benéficos mediados pela aclimatação ao calor nos valores de gravidade específica da urina e de fadiga percebida. Conclui-se que o ambiente quente prejudica o desempenho aeróbico e intensifica o estresse perceptivo em comparação com o ambiente temperado. Após a corrida em ambiente quente, observa-se alterações no neuromusculares, perceptivas, autonômicas e relacionadas ao equilíbrio hídrico-eletrolítico. Por fim, um protocolo de aclimatação ao calor de curto prazo melhora o desempenho e impacta minimamente a recuperação, ainda que os indivíduos aclimatados se exercitem a uma maior carga externa do que os controles.

Palavras chave: Aclimatação; Corrida; Desempenho Físico; Fadiga; Recuperação pós-exercício; Termorregulação.

ABSTRACT

Heat acclimation protocols, characterized by physical exercise sessions under environmental heat stress, are effective strategies for inducing physiological and perceptual adaptations and improving prolonged (aerobic) performance in hot environments. The first study of the thesis investigated the effects of environmental heat stress on physiological and perceptual variables during prolonged self-paced treadmill running and on lower limb neuromuscular performance after the run. The thirteen recreational runners who participated in the study maintained a lower average speed to complete the 10 km and showed higher ratings of perceived exertion during the run at 35°C compared to 25°C. Countermovement jump (CMJ) height was also reduced by 7% one hour after exercise, indicating neuromuscular fatigue; there was no difference in this reduction between the two environmental temperatures investigated. The second study analyzed physiological, perceptual, and physical performance responses after a 10 km self-paced run at 35°C. Data from the 16 volunteers indicated increased fatigue, muscle soreness, and urine specific gravity, as well as decreased vigor, perceived recovery, and cardiac parasympathetic activity after the 10 km run. The data also confirmed the reduction in CMJ height one hour after the run. Most of these changes were transient and returned to baseline after 24 hours, except for perceived recovery, which remained reduced. Neuromuscular performance changes were not associated with perceptual changes. The third study assessed the effects of short-term (5-day) heat acclimation on recovery after running in a hot environment. Sixteen participants were randomly allocated into one of the following two groups: control or acclimation. Heat acclimation resulted in better running performance without compromising recovery, which was similar between groups. The exceptions were the impaired cardiac parasympathetic reactivation during the first hour after the 10 km run and the beneficial effects mediated by heat acclimation on urine specific gravity and perceived fatigue values. In conclusion, a hot environment impairs aerobic performance and intensifies perceptual strain compared to a temperate environment. After running in the heat, changes are observed in lower limb neuromuscular performance, perceptual variables, and variables related to cardiac autonomic control and fluid-electrolyte balance. Finally, a short-term heat acclimation protocol improves performance and has minimal impact on recovery, even though acclimated individuals exercise at a higher external load than the controls.

Keywords: Acclimation; Running; Physical performance; Fatigue; Post-exercise recovery; Thermoregulation.

LISTA DOS ARTIGOS INCLUÍDOS NA TESE

ESTUDO 1. *Reduced running performance and greater perceived exertion, but similar post-exercise neuromuscular fatigue in tropical natives subjected to a 10 km self-paced run in a hot compared to a temperate environment*

*Artigo publicado no periódico *Plos One* no dia 17 de agosto de 2023 (CiteScore: 5,6; Qualis A1).

ESTUDO 2. *Time-course of physical performance, perceptual, and physiological changes following a 10 km self-paced run in the heat*

* Artigo submetido para o periódico Scandinavian Journal of Medicine and Science in Sports no dia 17 de julho de 2025 (CiteScore: 6,9; Qualis A1).

ESTUDO 3. ***Short-term heat acclimation has minimal impact on post-exercise recovery following self-paced 10 km run in the heat***

SUMÁRIO

1 INTRODUÇÃO

A intensificação das mudanças climáticas, diretamente ligada as atividades humana, resulta no aquecimento global e na maior frequência de condições climáticas extremas (Marto, 2005). Esses fenômenos impactam diretamente eventos esportivos realizados ao ar livre (outdoor), como as provas de corrida de rua, que vêm crescendo em popularidade mesmo em regiões com condições ambientais desafiadoras. A realização de provas em ambientes quentes e úmidos eleva o risco de desidratação e hipertermia graves, além uma redução no desempenho físico. A intensificação de eventos climáticos extremos aumenta a demanda por serviços de emergência, agravando condições de saúde crônicas (Bierlier; Scantamburlo, 2022).

A termorregulação é um conjunto de mecanismos que visa à manutenção da temperatura corporal interna em valores estáveis, em torno de 37ºC, através de respostas fisiológicas (autonômicas e somáticas) e comportamentais (Cheshire Junior, 2016; OSILLA; Marsidi; Sharma, 2018; Prímola-Gomes; Coimbra; Moreira; Wanner, 2024). Portanto, o corpo humano conta com diferentes mecanismos de produção, conservação e dissipação de calor que auxiliam na manutenção da temperatura interna, dentro de uma faixa estreita de oscilação, regulada pela região pré-optica hipotalâmica. A temperatura interna é controlada de maneira precisa, ainda que ocorram variações amplas na temperatura ambiente (Lim, 2020; Zhao; Ji; Deng; Wang *et al.*, 2024).

Diversos fatores endógenos e exógenos afetam o equilíbrio térmico corporal, modificando o gradiente de troca de calor entre a pele e o ambiente (Parkin; Carey; Zhao; Febbraio, 1999; standard, 2004; Zhao; Ji; Deng; Wang *et al.*, 2024). Um aumento de 13°C na temperatura ambiente pode, durante apenas 15 minutos de exercício em intensidade moderada, elevar a sobrecarga cardiovascular em 10% (Marins, 1996). Em provas de *endurance* (> 15 km), a partir de 15°C, a cada acréscimo em 1°C na temperatura ambiente, o tempo de prova aumenta em 1 min (Suping; Guanglin; Yanwen; Ji, 1992). Além da temperatura ambiente (T_A), a umidade relativa do ar (URA) e a radiação solar são outros fatores exógenos que podem intervir negativamente, principalmente em atividades físicas prolongadas (*endurance*), limitando a perda de calor e antecipando a fadiga (Maughan; Otani; Watson, 2012b). Conforme a URA aumenta, o tempo de exercício tolerado até a exaustão reduz (Che muhamed; Atkins; Stannard; Mündel *et al.*, 2016). Ademais, a radiação solar contribui para o desenvolvimento de doenças relacionadas ao calor (Nichols, 2014) e para a redução do desempenho aeróbico (Levels; De Koning; Broekhuijzen; Zwaan *et al.*, 2014; Otani; Kaya; Tamaki; Goto *et al.*, 2019).

A exposição ao ambiente quente pode reduzir significativamente o gradiente de temperatura entre a pele e o ambiente; este gradiente pode inclusive se inverter, principalmente quando a temperatura do ar é superior à temperatura da pele (Barros; Mendes; Pacheco; Garcia, 2013; Zhao; Ji; Deng; Wang *et al.*, 2024). Um menor gradiente reduz as trocas de calor por condução, convecção e radiação, limitando a capacidade de dissipação do calor corporal (Nielsen, 1938), enquanto a inversão do gradiente resulta em um maior ganho de calor a partir da exposição ao ambiente. Essas duas condições afetam o equilíbrio térmico do organismo (Niedermann; Wyss; Annaheim; Psikuta *et al.*, 2014).

Maiores valores de temperatura interna são observados durante o exercício em ambiente quente em comparação com ambientes frios ou temperados (Galloway; Maughan, 1997; Hunter; Gibson; Mbambo; Lambert *et al.*, 2002). Vale destacar que aumentos acentuados na temperatura interna podem afetar negativamente o desempenho aeróbico (Bongers; Daanen; Bogerd; Hopman *et al.*, 2018).

O aumento acentuado da temperatura interna modifica as propriedades contrateis do músculo, diminuindo sua capacidade de gerar força de forma voluntária (Todd; Butler; Taylor; Gandevia, 2005). Além disso, a realização de atividades físicas de longa duração no calor aumenta a utilização do glicogênio muscular, devido à elevação da temperatura corporal que estimula a glicogenólise. Esse consumo acelerado reduz os estoques de glicogênio de forma mais precoce, favorece o acúmulo de metabólitos, como os íons H+, e contribui para alterações no pH intramuscular. Esses fatores combinados comprometem a contração muscular e, consequentemente, antecipam a fadiga (Edwards; Harris; Hultman; Kaijser *et al.*, 1972; Júnior; Prado; Sena; Veneroso *et al.*, 2021).

Um estudo anterior relatou que, no ponto de fadiga voluntária, animais apresentavam temperaturas internas entre 40,1 °C e 40,2 °C (Fuller; Carter; Mitchell, 1998), ratos com temperatura corporal mantida acima de 40 °C correram por apenas metade do tempo em comparação àqueles cuja temperatura permaneceu abaixo de 38 °C (Caputa; Kamari, 1991).

Em homens treinados foram observadas temperaturas entre 40,1–40,3°C (González-Alonso; Teller; Andersen; Jensen *et al.*, 1999). No entanto, relatos sobre valores de temperatura interna superiores a 40°C são comuns em provas do atletismo, realizadas em ambiente *outdoor*. Um exemplo é um estudo com meio-maratonistas correndo em intensidade autorregulada em condições de calor e umidade elevada (27,9°C e 76,8%), no qual foram observadas temperaturas internas superiores a 41°C (Júnior; Mckenna; Amorim; sena *et al.*, 2020). Além de modificar o padrão de corrida (Christina; White; Mccrory, 1998; Dickinson; Cook; Leinhardt, 1985), a fadiga diminui o recrutamento muscular, resultando em uma diminuição da força e potência musculares após o esforço físico (Nicol; Komi; Marconnet, 1991).

Dentre as estratégias utilizadas atualmente para minimizar os efeitos prejudiciais do ambiente quente, a aclimatação ao calor é considerada a mais efetiva. A aclimatação vem sendo amplamente utilizada em diferentes populações e modalidades esportivas (Duffield, 2008; fry, 2020), sendo o principal método de proteção à saúde e de melhora do desempenho físico em atividades físicas realizadas em ambiente quente (Racinais; Alonso; Coutts; Flouris *et al.*, 2015). A aclimatação ao calor consiste na exposição repetida a ambientes quentes e possui diferentes metodologias de aplicação e controle, com sessões variando de 15 minutos até 4 horas, de acordo com a sessão de aclimatação (Flouris, 2014; Racinais; Alonso; Coutts; Flouris *et al.*, 2015). A duração da aclimatação pode ser curta (< 7 dias), intermediária (entre 8 - 14 dias) ou longa (≥ 15 dias) (Garrett; Rehrer; Patterson, 2011). Dias após o término das sessões de aclimatação, as adaptações são revertidas, levando à deterioração progressiva dos benefícios adquiridos (Armstrong; Maresh, 1991; Gibson; James; Mee; Willmott *et al.*, 2020).

Estudos anteriores observaram várias adaptações fisiológicas advindas da aclimatação, tais como redução da frequência cardíaca, expansão do volume plasmático (Guy; Deakin; Edwards; Miller *et al.*, 2015a) e diminuição em até 40% na concentração de eletrólitos no suor, resultando em um equilíbrio hidroeletrolítico mais estável (ARMSTRONG; Maresh, 1991; Chalmers; Esterman; ESton; Bowering *et al.*, 2014a; Chinevere; Kenefick; Cheuvront; Lukaski *et al.*, 2008; Racinais; Alonso; Coutts; Flouris *et al.*, 2015). Além desses ajustes, observa-se aumentos na taxa de sudorese e nas capacidades evaporativa e de resfriamento do corpo humano, os quais previnem o superaquecimento corporal (Klous; De Ruiter; Alkemade; Daanen *et al.*, 2020).

As primeiras adaptações a aclimatação podem ser observadas a partir do quarto dia de exposição (HORVATH (Chalmers; Esterman; Eston; Bowering *et al.*, 2014a; Horvath; Shelley, 1946), sendo que a magnitude e os benefícios deste fenômeno dependem do número de sessões de exposições (Houmard; Costill; Davis; Mitchell *et al.*, 1990). Após 8 e 22 dias de aclimatação, a temperatura interna em repouso reduziu em 0,20°C e 0,32°C, respectivamente (Patterson; Stocks; Taylor, 2004). Essa redução da temperatura interna em repouso favorece a diminuição da sobrecarga térmica durante o exercício de *endurance*, minimizando a desidratação (Racinais; Cocking; Périard, 2017), reduzindo o estresse induzido pelo calor e melhorando o desempenho físico (Douzi; Dugué; Vinches; Al sayed *et al.*, 2019).

Apesar de não existir um consenso sobre tempo mínimo, evidencias apontam que 300 min semanais (5 sessões de 60 min) são suficientes para induzir adaptações parciais. Protocolos de aclimatação iguais ou superiores a 450 min/sem (5 sessões de 90 min) reduzem o tempo para completar distâncias pré-determinadas em 6,6%, enquanto 240 min/sem (4 sessões de 60 min) induzem apenas diferenças perceptivas (Wardenaar; Ortega-Santos; Vento; Beaumont *et al.*, 2021).

Até este ponto, a introdução descreveu os efeitos do ambiente quente sobre as respostas fisiológicas e o desempenho físico de seres humanos. Além disso, foram apresentadas as adaptações induzidas pela aclimatação ao calor e a capacidade dessa intervenção em reduzir os efeitos deletérios causados pela realização de exercício sob estresse térmico ambiental. A partir daqui iremos discutir a recuperação pós-exercício, visto que o nosso estudo visa compreender a influência da aclimatação ao calor na recuperação após exercício realizado em ambiente quente.

A recuperação pós-exercício é um fenômeno multifatorial (fisiológico e psicológico) que tem por objetivo a retomada da homeostase, permitindo ao indivíduo estar apto a receber um novo estímulo (Kallus; Kellmann, 2016; Tomlin, D. L.; Wenger, H. A., 2001). Trata-se de um aspecto importante dentro de um programa de treinamento e entre competições para garantir que o atleta consiga manter um estado ótimo de desempenho. O tempo de recuperação varia de acordo com o tipo do exercício, o tempo entre as sessões de exercício, os objetivos do praticante e o seu nível de aptidão física (Calleja-González; Mielgo-ayuso; Ostojic; Jones *et al.*, 2019).

Após uma sessão de exercício vigoroso, o músculo esquelético perde, parcialmente, a sua capacidade de gerar força, reduzindo por consequência o desempenho físico. Isso ocorre devido a vários fatores, incluindo o desarranjo das fibras musculares causado por microlesões no tecido, a resposta inflamatória local aguda (Rose; Edwards; Siegler; Graham *et al.*, 2017) e dores musculares de início tardio (Dmit) (Cheung; Hume; Maxwell, 2003). Cleary, Sitler e Kendrick (2006) verificaram que participantes desidratados e hipertérmicos apresentaram menor capacidade de gerar força nos músculos flexores e extensores da perna, além de sintomas mais evidentes de DMIT.

Quando o tempo de recuperação é negligenciado, além da redução do desempenho decorrente das alterações estruturais nas fibras musculares e da não restauração dos substratos utilizados durante o exercício, aumentam-se as chances de lesões (Burke; Kiens; Ivy, 2004). De forma crônica, a sucessão inadequada do processo de estímulo-recuperação pode levar à síndrome de *overtraining* (Meeusen; DUclos; Foster; FRY *et al.*, 2013a), caracterizada pela redução no desempenho físico de maneira prolongada (Carrard; Rigort; Appenzeller-Herzog; Colledge *et al.*, 2022; Stellingwerff; Heikura; Meeusen; Bermon *et al.*, 2021). Os sintomas de overtraining afetam até 65% dos corredores de longa distância em algum momento de suas carreiras profissionais (Rogero; Mendes; Tirapegui, 2005) e os principais sintomas são cansaço excessivo, dores frequentes, aumento dos níveis de estresse e ansiedade (Ackel-d'elia; Vancini; Castelo; Nouailhetas *et al.*, 2010; Tomazini, 2014).

Devido à natureza multifatorial da recuperação, as alterações no desempenho devem ser analisadas de forma específica às características do exporte. Para isso são necessários testes fisiológicos e medidas de parâmetros perceptivos, bioquímicos e autonômicos para um melhor diagnostico das condições dos atletas e para que treinadores e preparadores físicos possam prescrever estratégias adequadas de recuperação (Kallus; Kellmann, 2016). As escalas psicométricas e a variabilidade da frequência cardíaca (VFC) são ferramentas que vem sendo utilizadas na quantificação do estado de recuperação (Borresen; Lambert, 2007). A VFC é utilizada no contexto esportivo por ser um método não invasivo, com baixo custo e boa aplicação (Plews; LAursen; Stanley; Kilding *et al.*, 2013). A VFC é uma variável importante para o controle do estado de recuperação obtida a partir dos intervalos R-R (Buchheit; Chivot;

Parouty; Mercier *et al.*, 2010; Reichel; Hacker; Palmowski; Boßlau *et al.*, 2022). É possível realizar uma análise detalhada da VFC (Abellán-aynés; López-plaza; Alacid; Naranjo-orellana *et al.*, 2019), utilizando índices como o RMSSD (raiz quadrada da média dos quadrados das diferenças sucessivas entre intervalos RR normais adjacentes), um indicador sensível da atividade parassimpática cardíaca, o pNN50 (porcentagem de intervalos NN sucessivos com diferença superior a 50 ms), outro marcador da modulação parassimpática, (Balocchi; Cantini; Varanini; Raimondi *et al.*, 2006), além do índice LF/HF (razão entre as potências de baixa e alta frequência), que reflete o balanço autonômico.

De modo a avaliar aspectos perceptivos da recuperação, vários questionários vêm sendo utilizados, como, por exemplo, a Escala de Recuperação de Qualidade Total (Wilke; Wanner; Penna; Maia-lima *et al.*, 2021), o perfil dos estados de humor (MCNAIR, 1992) e o questionário de estresse de recuperação (Kallus; Kellmann, 2016). Por exemplo, uma sessão de treinamento reduz a percepção de recuperação (WILKE; Fernandes; Martins; Lacerda *et al.*, 2019b) e modifica os estados de humor de atletas, aumentando a sensação de fadiga e reduzindo o vigor (Wilke; Wanner; Penna; MAIA-LIMA *et al.*, 2021), todas essas alterações perceptivas são transitórias e tendem, em apenas 24 h, a retornarem aos valores pré-sessão de treinamento. Após 10 semanas de pré-temporada, os atletas apresentaram menores alterações nos estados de humor induzidas por uma sessão de treinamento de intensidade elevada (Wilke; Wanner; Penna; Maia-Lima *et al.*, 2021), indicando que o treinamento esportivo pode modificar a recuperação perceptiva de atletas. Vale destacar que os efeitos da aclimatação ao calor em variáveis perceptivas, após um exercício físico extenuante, ainda são desconhecidos.

Além disso, estudos anteriores realizados com diferentes populações, encontraram uma forte correlação entre a redução da altura do salto com contramovimento (SCM) e o aumento nas concentrações de lactato e amônia no sangue (Jimenez-reyes; Pareja-blanco; Cuadrado-peñafiel; Morcillo *et al.*, 2016; Jiménez-reyes; Pareja-blanco; Cuadrado-peñafiel; Ortega-becerra *et al.*, 2019). Um estudo feito com 40 corredores verificou reduções de 17%, 22%, 7,1% e 7,4% na potência de membros inferiores, altura do SCM, força de preensão manual do braço direito e esquerdo, respectivamente, ao final da maratona realizada a uma temperatura de 27°C (del coso; fernández; abián-vicen; Salinero *et al.*, 2013). Neste contexto, vários estudos vem sugerindo a utilização da altura do SCM como ferramenta para monitorar o treinamento, quantificar a fadiga e avaliar a recuperação neuromuscular (García-pinillos;

Ramírez-campillo; Boullosa; Jiménez-reyes *et al.*, 2021; Thomas; Dos' Santos; Comfort; Jones, 2018).

A recuperação pós-exercício também sofre influência de fatores exógenos. Por exemplo, quando o exercício é realizado em ambiente quente, a recuperação pode ser mais demorada em comparação a ambientes temperados ou frios, devido ao alcance de maiores valores de temperatura interna, frequência cardíaca e percepção subjetiva do esforço (Carter iii; Cheuvront; Wray; Kolka *et al.*, 2005; Duffield, 2008). Ao comparar diferentes condições ambientais, foi observado um efeito moderado para a recuperação da contração voluntária isométrica máxima após esforço físico, sendo a recuperação 9% mais lenta no ambiente quente (Duffield; King; Skein, 2009). A contração voluntária máxima também recupera-se de forma mais lenta após exercício realizado em ambiente quente em comparação ao exercício realizado em ambiente temperado (Saboisky; Marino; Kay; Cannon, 2003; Todd; Butler; Taylor; Gandevia, 2005).

Dessa forma, investigar a recuperação após um protocolo de aclimatação ao calor é importante, pois permitirá otimizar a aplicação de estímulos de treinamento em ambientes quentes. Ou seja, o presente estudo permitirá compreender se a aclimatação ao calor pode ser utilizada como uma ferramenta para a melhora da recuperação, além do seu uso tradicional para a melhora do desempenho aeróbico.

Por outro lado, a aclimatação ao calor pode dificultar a recuperação pós-esforço físico. A aclimatação melhora o desempenho físico e, portanto, os indivíduos aclimatados irão percorrer ou pedalar uma determinada distância pré-establecida em menos tempo, ou seja, a uma maior velocidade. Isso significa que os indivíduos aclimatados irão realizar o exercício com uma maior carga mecânica (externa), o que pode acentuar a fadiga e prolongar a recuperação pós-exercício. Logo, frente à inexistência de informações na literatura, é difícil de avaliar se a aclimatação ao calor irá acelerar, retardar ou não interfere no curso temporal da recuperação.

Embora vários estudos tenham verificado os impactos de diferentes métodos e tempos de recuperação (Pastre; Bastos; Netto júnior; Vanderlei *et al.*, 2009), existe uma lacuna quanto ao efeito da aclimatação ao calor frente à recuperação após exercício de *endurance*

(Brocherie; Girard; Millet, 2015), tornando-se evidente a necessidade de se investigar alterações nas variáveis de recuperação mediante à aclimatação ao calor.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito de um protocolo curto de aclimatação ao calor na recuperação de corredores após 10 km em intensidade autorregulada e em ambiente quente.

Para isso, a tese foi dividida em três estudos, de acordo com os objetivos específicos descritos abaixo.

2.2 Objetivos específicos

Estudo 1

1) Comparar as respostas fisiológicas e perceptivas de nativos de regiões tropicais durante uma corrida de 10 km em esteira rolante, realizada em intensidade autorregulada, entre as condições ambientais temperada (25°C) e quente (35°C).

2) Comparar as alterações no desempenho do salto com contramovimento (CMJ) após as corridas entre as condições ambientais temperada (25°C) e quente (35°C).

Estudo 2

1) Descrever as alterações em variáveis fisiológicas, perceptivas e de desempenho físico induzidas por uma corrida de 10 km, em esteira rolante e em intensidade autorregulada, sob estresse térmico ambiental.

2) Investigar se as alterações em variáveis perceptivas e de desempenho induzidas por uma corrida de 10 km, sob estresse térmico ambiental, estão associadas.

3) Verificar se é possível agrupar os atletas em diferentes perfis de recuperação.

Estudo 3

3) Investigar os efeitos da aclimatação ao calor de curto prazo no curso temporal da recuperação de variáveis fisiológicas, perceptivas e de desempenho físico após uma corrida de 10 km realizada em intensidade autorregulada e em ambiente quente.

3 ESTUDO 1

Reduced running performance and greater perceived exertion, but similar post-exercise neuromuscular fatigue in tropical natives subjected to a 10 km self-paced run in a hot compared to a temperate environment

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Short title: Physiological and perceptual responses induced by exercise-heat stress

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Abstract

Environmental heat stress impairs endurance performance by enhancing exercise-induced physiological and perceptual responses. However, the time course of these responses during self-paced running, particularly when comparing hot and temperate conditions, still needs further clarification. Moreover, monitoring fatigue induced by exercise is paramount to prescribing training and recovery adequately, but investigations on the effects of a hot environment on post-exercise neuromuscular fatigue are scarce. This study compared the time course of physiological and perceptual responses during a 10 km self-paced treadmill run (as fast as possible) between temperate (25°C) and hot (35°C) conditions. We also investigated the changes in countermovement jump (CMJ) performance following exercise in these two ambient temperatures. Thirteen recreational long-distance runners (11 men and 2 women), inhabitants of a tropical region, completed the two experimental trials in a randomized order. Compared to 25°C, participants had transiently higher body core temperature (T_{CORE}) and consistently greater perceived exertion while running at 35°C ($p < 0.05$). These changes were associated with a slower pace, evidenced by an additional 14 ± 5 min (mean ± SD) to complete the 10 km at 35°C than at 25°C ($p < 0.05$). Before, immediately after, and 1 h after the self-paced run, the participants performed CMJs to evaluate lower limb neuromuscular fatigue. CMJ height was reduced by 7.0% (2.3 ± 2.4 cm) at 1 h after the race ($p < 0.05$) compared to pre-exercise values; environmental conditions did not influence this reduction. In conclusion, despite the reduced endurance performance, higher perceived exertion, and transiently augmented T_{CORE} caused by environmental heat stress, post-exercise neuromuscular fatigue is similar between temperate and hot conditions. This finding suggests that the higher external load (faster speed) at 25°C compensates for the effects of more significant perceptual responses at 35°C in inducing neuromuscular fatigue.

Keywords: countermovement jump; fatigue; heat; neuromuscular; performance; recovery; thermoregulation; treadmill running

Introduction

Robust evidence indicates that environmental conditions influence fatigue perception and performance during prolonged physical exercises [1–4]. For example, high ambient temperature (T_A) conditions impair endurance performance [5, 6], and this effect results from increased body core hyperthermia, leading to changes in brain electrical activity, augmented perceived exertion, and reduced neuromuscular drive [7, 8]. The accelerated fatigue during exercise-heat stress is also explained by enhanced cardiovascular strain [9] and altered thermal perception [10]. Even though the role of physiological and perceptual responses in impairing endurance performance under environmental heat stress is well acknowledged in the literature, the time course of these responses, particularly when comparing a self-paced treadmill run between warm/hot and cool/temperate conditions, was less studied.

While some studies only reported physiological and perceptual responses at the end of self-paced exercises at different T_As [11–13], Marino et al. [14, 15] described the time course of these responses in athletes subjected to 8 km treadmill runs in hot (35°C) and cool (15°C) conditions. However, in the experiments conducted by Marino and colleagues, the athletes ran at 70% of their peak speed for 30 min at 15°C or 35°C before the 8 km runs; therefore, athletes already started the self-paced exercise at 35°C with higher body core temperature (T_{CORE}), heart rate (HR), and rating of perceived exertion (RPE) than at 15°C. Also important, whether the changes caused by exercise-heat stress are evident in heat-acclimatized individuals still requires further investigation since inhabitants of tropical climates have adaptations that improve heat tolerance and reduce physiological and perceptual strain when exposed to hot conditions [16]. Thus, the current study fills these literature gaps by describing the time course of physiological and perceptual responses in recreational athletes, inhabitants of a tropical region, subjected to prolonged self-paced exercises in temperate and hot conditions.

Aside from changes occurring during exercise, another point that merits investigation is the effect of a self-paced run under hot environmental conditions on post-exercise neuromuscular performance and, consequently, neuromuscular fatigue. Monitoring muscle fatigue is paramount to adequately prescribing training loads and recovery [17, 18]. Fatigue is defined as an exercise-induced reduction in maximal voluntary muscle force or power production [19] and can be assessed by measuring physiological and perceptual variables and physical performance [20–22]. Due to its validity, reliability, and low cost, performance in the countermovement jump (CMJ) test has been widely used to monitor athletes' lower limb neuromuscular fatigue during and after training sessions [23–25]. For example, weekly averages of CMJ height significantly correlated with perceived exertion, distance covered, and training zone in elite middle- and long-distance runners [26]. Furthermore, from an acute fatigue perspective, CMJ height was reduced at 24 h and 48 h post-match compared to 24 h before the match in young Turkish football players [27].

Under hot conditions, a fixed-intensity endurance exercise to fatigue induces a greater reduction in neuromuscular performance compared to temperate conditions [8, 28]. However, exercising at a fixed intensity (*i.e.*, open-loop exercise) does not reproduce the demands of real-world sports competitions [29]. Therefore, we decided to investigate the influence of a hot environment on the level of neuromuscular fatigue induced by a self-paced (*i.e.*, closedloop) endurance exercise, in which the individuals adjust running speed to choose the best strategy to complete a predetermined distance as fast as possible [29–31]. In this regard, Périard et al. [32] reported that the loss of force production following self-paced cycling exercise in the heat was not exacerbated compared to the loss observed under temperate conditions. These authors investigated force production and voluntary activation of the knee extensors while subjects performed a maximal voluntary isometric contraction. In the present study, we propose measuring performance in a dynamic exercise, commonly used in training settings to monitor fatigue, to understand further whether the environmental heat stress interferes with the level of post-exercise neuromuscular fatigue.

Therefore, the current study compared the time course of physiological and perceptual responses in tropical natives during a 10 km self-paced treadmill run between temperate (25°C) and hot (35°C) conditions. In addition, this study investigated the changes in countermovement jump (CMJ) performance following 10 km runs in T_{A}s mentioned earlier. Considering the previous evidence (*e.g.*, [32]), we expected that running 10 km in the heat, compared to temperate conditions, would increase RPE and T_{CORE} at exercise completion (end-T_{CORE}) but would not exacerbate lower limb neuromuscular fatigue and the exercise-induced HR increase. Because our participants were heat acclimatized (*i.e.*, they lived in a tropical climate in Brazilian's northeast region), we also hypothesized that some effects induced by exercise-heat stress would be less evident in the current than in the previously-published studies.

Methods

Participants and ethical care

Thirteen recreational long-distance runners (11 men and 2 women) were recruited and completed the two experimental trials. All runners were born in Teresina (Brazil), a city located in a tropical region (latitude: 5°05'20" S, longitude: 42°48'07" W), with a monthly (calculated from 01/2013 to 12/2022; mean ± SD) average T_A and relative humidity of 28.0 ± 1.3°C and 72 ± 10%, respectively [33]. Of note, the maximum T_A surpasses 31°C in all months of the year and corresponds to a monthly average of 34.6 ± 2.2°C [33]. In addition, at the time of the experiments, the 13 participants lived in Teresina and were therefore considered heat-acclimatized individuals.

The inclusion criteria were: 1) to train regularly for at least two years; 2) to have a weekly training frequency of at least five days; 3) the absence of lower limb injuries in the six months before the experiments; 4) to not use any medication and nutritional supplements during the experiments; 5) to have completed a 10 km outdoor run in less than 45 min (men) or 55 min (women) in the six months before the experiments. This required performance could have been attained during training sessions or competitions. Of note, the two women participants used monophasic oral contraceptives and were tested during the active phase of the contraceptive pill, from the 2nd to the 21st day of use [34].

The study was approved by the Research Ethics Committee of the Universidade Federal do Maranhão (protocol number 1.548.709). The procedures conformed to the 1964 Helsinki Declaration and its later amendments. Moreover, the participants signed an informed consent form upon agreeing to participate in this study.

Sample size calculation

Sample size calculation was performed a priori using data from pilot experiments (n = 5) investigating the effect of a 10 km run under temperate conditions on the CMJ height. These experiments indicated that CMJ would possibly reduce at 1 h post-running, with an effect size (*i.e.*, Cohen's d) of 0.87. We then used the GPower software (version 3.1.9.7, Universität Düsseldorf, Germany) to calculate the required sample size according to the following additional parameters: the difference between two dependent means (matched pairs) when using a t-test, an alpha error = 0.05, and a power = 0.90. This calculation indicated that 13 participants were needed to identify significant changes in the CMJ height 1 h after finishing the 10 km.

Experimental design

The current experiments followed a crossover design, with each participant subjected to familiarization procedures and two experimental trials. Therefore, the participants visited the laboratory three times.

During the first visit, the experimental procedures were explained to the volunteers, who had the opportunity to ask questions about the protocol. Anthropometric measurements were performed, and then the participants were subjected, in the following order, to a familiarization session with CMJ, a maximal incremental cardiopulmonary exercise test, and a familiarization session with running on a motorized treadmill.

Experimental trials were carried out during the second and third visits (Fig 1). The volunteers were subjected to a 10 km self-paced run in a temperate (25°C) or hot environment (35°C), with relative humidity controlled between 40 and 50%. The order of the experimental trials was randomized. The interval between trials varied between 72 h and 96 h, and they were conducted at the same time of day (less than 1-hour difference when exercises were initiated), always in the afternoon (between 2:30 p.m. and 6:00 p.m.), to prevent circadian rhythm from interfering with the interpretation of our findings.

Fig 1. Timeline of the experimental trials. CMJ = countermovement jump.

The following variables were measured during exercise at 2 km intervals: HR, T_{CORE}, RPE, and thermal sensation. CMJ height, an indicator of lower limb neuromuscular fatigue, was measured before, immediately after, and 1 h after the 10 km run.

Procedures

Anthropometric measurements

Stature and body mass were determined, respectively, using a measuring tape and a bioimpedance scale (precision: 100 g; model HBF 514c, Omron Corporation, Kyoto, Japan). Skinfold thickness was measured in triplicates by the same investigator at seven sites – triceps, subscapular, pectoral, mid-axilla, abdominal, supra iliac, and mid-thigh – using a skinfold caliper (Lange, MI, USA). The body fat percentage was calculated according to Jackson and Pollock's equations [35]. Body surface area was also calculated according to the equation proposed by Du Bois & Du Bois [36].

Familiarization with CMJ

Initially, the individuals were subjected to a 10 min warm-up on a treadmill at a speed corresponding to 70% (*i.e.*, 8.4 ± 1.0 km·h^{-1}) of the average speed attained during their best 10 km performance. Previous evidence indicates that warming up muscles increases short-term high-intensity physical performance [37, 38]. This warm-up also preceded the CMJs performed before and 1 h after the 10 km self-paced exercise during the second and third visits.

After the standardized warm-up, the volunteers were familiarized with the jumping technique by performing 3 sets of 3 CMJ repetitions, with a 30-second interval between sets [39]. The participants initiated the jump from the upright position, with parallel feet on a force platform and hands on the hips to neutralize the influence of the upper limbs. At the examiner's signal, the individuals performed a continuous movement, with the concentric action preceded by a preparatory movement corresponding to an eccentric action until approximately 90° of knee flexion [39].

Cardiopulmonary exercise testing

After being familiarized with CMJs, the participants were subjected to an incremental cardiopulmonary exercise test to estimate peak oxygen consumption (VO_{2peak}) using a modified Åstrand protocol [40]. The test started at a speed of 11.3 $km \cdot h^{-1}$ and an incline of 0%. Speed was maintained at 11.3 $km \cdot h^{-1}$ throughout the protocol, with the incline increasing by 2.5% every 2 min; the speed and increment in incline were selected to exhaust most participants within 7 to 10 min of the test [41]. Resting VO_2 was summed to the VO_2 corresponding to both the horizontal and vertical components of the last stage completed to estimate VO_{2peak}. As proposed by the American College of Sports Medicine [42], the following criteria were used to interrupt the test: 1) the participant requested to interrupt the exercise or scored 10 on Borg's RPE scale [43]; 2) the presence of malaise signs, such as a pale appearance to the skin or cyanosis (signs of poor perfusion); 3) unusual shortness of breath; or 4) failure of the equipment. A chest-strapped monitor (Polar H10, Kempele, Finland) was used for the continuous HR measurement.

Familiarization with the treadmill

Forty min after the cardiopulmonary exercise test, participants were further familiarized with the motorized treadmill (model Evoque Jet 6, TGR Fitness, Blumenau – SC, Brazil) by running 5 km at the same speed as the warm-up described earlier (*i.e.*, 8.4 ± 1.0 $km \cdot h^{-1}$). The incremental test and the familiarization session were performed under temperate conditions (T_A = 25.5 ± 0.4°C and relative humidity = 51.9 ± 2.1%).

Experimental trials

On the day of the experiments, around 8:00 a.m., the volunteers ingested a telemetric capsule to allow the measurement of gastrointestinal temperature (*i.e.*, an index of T_{CORE}). At noon, the participants had lunch and arrived at the laboratory at 2:30 p.m., where they stayed in a temperate environment (~25°C) until the pre-race variables, body mass, and hydration status were measured. We ensured that the participants were not dehydrated when they started running, as indicated by a urine specific gravity equal to or less than 1.029 [44].

The participants were instructed to complete the predetermined distance as fast as possible and, while running, had only visual feedback of the distance covered. The environmental conditions selected for the experiments were based on a previous study from our laboratory, which showed that the time to complete a 30 km time trial on a cycle ergometer was 9% longer at 35°C than at 24°C [4]. T_A inside the experimental room was warmed up with the help of an electric heater (model AB1100N, Britânia, Brazil) or cooled down with a split air conditioning system (Springer Midea, Brazil). Whenever required, this split was also used in the dehumidification function. The dry and wet T_As were measured using the Thermal Stress Meter (TGD 400, Instrutherm, Brazil) and were used to calculate the relative humidity.

During the study period, the participants were instructed by a nutritionist to eat a standardized diet containing 6.5 g/kg of carbohydrates, 2.0 g/kg of fat, and 1.5 g/kg of protein, over five daily meals (breakfast, lunch, snack, dinner, and supper), according to the recommendations of the Academy of Nutrition and Dietetics, the Dietitians of Canada, and the American College of Sports Medicine [45]. The diets were prescribed individually, according to the participant's body mass. Food was weighed by the participants with an electronic kitchen scale (precision: 1.0 g) to ensure standardization.

Variables measured

Physical performance

The endurance performance corresponded to the time elapsed between the beginning and end of the 10 km self-paced run. The average speed at 2 km intervals was also recorded to understand how participants selected exercise intensity during the race. The time elapsed was measured with a stopwatch (precision of 0.01 s), and average speed was calculated as the distance traveled divided by a given time interval.

Lower limb neuromuscular power was determined by measuring the CMJ height. Athletes performed the CMJs on a custom-made force platform (Inovação em Tecnologia Esportiva, Belo Horizonte – MG, Brazil) with a 0.1 cm precision. The following equation accounted for the jump height: $h = g \times t^2 \times 8^{-1}$, where "h" is the height, "g" is the gravity acceleration, and "t" is the flight duration [46]. All jumps were performed at environmental conditions similar to those described for the familiarization procedures and cardiopulmonary exercise testing (~25°C). Each participant performed five trials and was instructed to jump as high as possible. The highest and lowest CMJ heights were excluded, and the arithmetic mean of the three remaining jumps was calculated [23].

Body core temperature (T_{CORE})

Due to the convenience of the technique and high correlation with rectal probe measurements [47], gastrointestinal temperature, an index of T_{CORE}, was monitored throughout the experiment using telemetric capsules (CorTemp® HQ Inc, model HT150002, Palmetto – FL, USA). Because of the temperature variations in the gastrointestinal tract, the participants were instructed to ingest the capsules between 7 h and 8 h before starting the 10 km run. Therefore, all procedures followed the recommendations of Byrne & Lim [48]. Our participants did not report any discomfort caused by ingesting the telemetric capsules.

Heart rate (HR)

HR was monitored every 2 km during the 10 km run using a Polar H-10 chest strap (Polar Electro Oy, Kempele, Finland), with a sampling frequency of 5 Hz. HR values were recorded by the Polar Beat application (version 2.5.1) and then transmitted to a smartphone, where they were analyzed by the Polar Flow application.

Perceptual variables

RPE and thermal sensation were recorded every 2 km during the race. RPE was determined using the 0 to 10-point Borg's Scale [43], whereas thermal sensation was determined by a 7-point scale: cold, cool, slightly cool, neutral, slightly warm, warm, and hot [49, 50]. This scale is suitable for describing a one-dimensional relationship between the physical parameters of indoor environments and subjective thermal sensation.

Calculated variable

Heat storage rate

Heat storage was calculated using the equation proposed by (NIELSEN, 1996): body mass (kg) × specific heat of body tissues (3480 J × $°C^{-1}$ × kg^{-1}) x change in T_{CORE} (°C). After that, the heat storage rate was calculated as follows: heat storage × $time^{-1}$ (s) × body surface $area^{-1}$ (m^2).

Statistical analysis

Homogeneity of variance was examined by Levene's test, whereas the data normality was investigated by the Shapiro-Wilk test; no significant effect was reported in either test. Unless otherwise stated, the data were reported as means ± standard deviation (SD). The time to complete the 10 km run and the change in the CMJ height were compared between trials (temperate vs. hot) using paired Student's t-tests. Two-way repeated-measures analyses of variance (ANOVAs) were performed to test differences in physiological and perceptual variables, pacing, and CMJ height between trials and distances (during the race) or between trials and time points (pre- vs. post-race). When significant differences were detected, Tukey's *post hoc* comparisons were performed. A p-value < 0.05 was considered to be statistically significant. Statistical analyses were carried out using SigmaPlot software (version 11.0, Systat Software Inc., San Jose - CA, USA).

We calculated Cohen's d effect size as a supplementary analysis to understand findings concerning performance better. This analysis assessed the magnitude of differences between data, and Cohen's d was calculated by subtracting the mean value of one trial from the mean value of the other trial to which it was being compared; the result was then divided by a combined standard deviation of the data. This calculation was further corrected by the correlation between measurements [52]. These analyses were performed using GPower version 3.1 (Universit¨at Düsseldorf, Germany). Effect size values were classified as trivial (d < 0.2), small (d = 0.2–0.6), medium (d = 0.6–1.2), or large (d ≥ 1.2) [53].

The intraclass correlation coefficient [ICC(3,k)] was calculated for the CMJ height, considering the values obtained before the two 10 km time trials; this calculation was performed in the IBM SPSS software (version 19.0, International Business Machines Corporation, Armonk – NY, USA). We also calculated the standard error of the measurement (SEM) using the following equation [54]: $SEM = SD \times \sqrt{(1 - ICC)}$.

Results

The participants' characteristics were as follows: age 31.5 ± 6.6 years, body mass 64.7 ± 9.4 kg, height 1.67 ± 0.08 m, body fat 12.3 ± 1.8%, body surface area 1.7 ± 0.1 m^2, estimated VO_{2peak} 54.2 ± 2.6 $mL \cdot kg^{-1} \cdot min^{-1}$.

The endurance performance was impaired in the heat, as evidenced by the additional 14.1 ± 4.8 min to complete the 10 km time trial at 35°C than at 25°C (72.7 ± 10.3 min vs. 58.6 ± 7.6 min; $p < 0.001$; d = 2.93). Indeed, impaired endurance at 35°C was observed in all 13 participants (Fig 2A) and corresponded to a large effect size. Regarding the average speed to complete 2 km intervals, a significant trial × distance interaction was observed ($F = 7.09$, $p < 0.001$; Fig 2B). The participants maintained a constant average speed throughout the 10 km at 25°C but presented a decreased speed from the 4th-6th km interval onwards at 35°C. As expected, the average speed was always slower at 35°C than at 25°C.

Fig 2. Endurance performance. The time to complete the 10 km self-paced run was measured under temperate (25°C) and hot (35°C) conditions (panel A). Data are expressed as means ± SD and individual data (*i.e.*, lines); all participants had decreased performance in the heat. Panel B shows the average speed at different distance intervals during the self-paced run in the two environmental conditions. Data are expressed as means ± SD. * indicates a significant difference compared to the control trial at 25°C, $p < 0.05$.

Significant main effects of distance were observed for HR (F = 862.02, $p < 0.001$; Fig 3A), T_{CORE} (F = 167.78, $p < 0.001$; Fig 3B), and heat storage rate (F = 23.83, $p < 0.001$; Fig 3C). For example, HR and T_{CORE} were markedly increased during both running exercises, attaining average values above 180 bpm and 39.2°C, respectively, at the end of the 10 km runs. Neither a significant main effect of trial (F = 0.80, $p = 0.387$) nor a significant trial × distance interaction (F = 1.11, $p = 0.364$) was observed for HR. In contrast, significant interactions were observed for T_{CORE} (F = 2.55, $p = 0.037$) and heat storage rate (F = 4.22, $p = 0.005$). More specifically, despite no differences at the end of running, T_{CORE} was transiently higher at 35°C than at 25°C in the 6th and 8th km. The heat storage rate was higher at 35°C between the 4th and 6th km but lower in the first and final 2 km compared to 25°C. One hour following the 10 km run, T_{CORE} was not different between experimental conditions (t = -2.046; $p = 0.063$) and corresponded to 37.49 ± 0.23°C and 37.59 ± 0.26°C at 25°C and 35°C, respectively.

Fig 3. Physiological responses during the 10 km self-paced run. The following physiological variables were measured under temperate (25°C) and hot (35°C) conditions:

heart rate (panel A), core temperature (panel B), and heat storage rate (panel C). Data are expressed as means ± SD. * indicates a significant difference compared to the control trial at 25°C, $p < 0.05$.

Regarding the perceptual variables, significant main effects of distance were observed for RPE (F = 105.45, $p < 0.001$; Fig 4A) and thermal sensation (F = 39.40, $p < 0.001$; Fig 4B), which increased markedly during both running exercises. For example, in the hot environment, RPE and thermal sensation corresponded to 9 ± 1 and 7 ± 0, respectively, when participants completed the predetermined distance. For the two variables, significant main effects of trial (RPE: F = 17.64, $p = 0.001$; sensation: F = 148.03, $p < 0.001$), but no significant trials × distance interactions (both $p > 0.430$), were observed.

Fig 4. Perceptual responses during the 10 km self-paced run. The following perceptual variables were measured under temperate (25°C) and hot (35°C) conditions: rating of perceived exertion (RPE; panel A) and thermal sensation (panel B). Data are expressed as means ± SD. # indicates a significant difference compared to the control trial at 25°C (main effect of ambient temperature), $p < 0.05$.

Before exercise, the CMJ height was not different between the two experimental trials (Fig 5A). CMJ height under baseline conditions corresponded to an ICC(3,k) of 0.984 and an SEM of 0.6 cm. A significant main effect of time point was observed for CMJ height (F = 9.07, $p =$ 0.001), with the values being lower at 1 h after (31.2 ± 4.0 cm, d = 1.00, *grouped data from the two trials*) but not immediately after running (32.6 ± 5.1 cm, d = 0.31), compared to before running (33.6 ± 4.4 cm). The reduction observed 1 h after the self-paced exercise corresponded to a medium effect size. Neither a significant main effect of trial ($F = 0.33$, $p = 0.576$) nor a significant trial × time point interaction ($F = 1.36$, $p = 0.276$) was observed for the CMJ height. We then compared the reduction in the CMJ height between the two trials immediately after ($p = 0.989$, d = 0.00; Fig 5B) and 1 h after the run ($p = 0.162$, d = 0.40; Fig 5C) and observed no differences; the effect sizes for comparisons between trials were classified as trivial (immediately after) and small (1 h after).

Because one participant had exaggerated reductions in the CMJ height following the run at 35°C (approximately 11 cm), we repeated the analyses excluding this participant. Again, no inter-trial differences were observed in the reduction of the CMJ height immediately after ($p = 0.115$, d = 0.47) and 1 h after the 10 km run ($p = 0.283$, d = 0.29; Fig 5C); these two comparisons were classified as small effect sizes. Therefore, including this participant did not influence the findings regarding CMJ height.

Fig 5. Lower limb neuromuscular performance before and after running. The countermovement jump (CMJ) height was measured before, immediately after, and 1 h after the 10 km self-paced run under temperate (25°C) and hot (35°C) conditions (panel A). Data are expressed as means ± SD. + indicates a significant difference compared to before running (main effect of time point), $p < 0.05$. Panel B shows the reduction in the CMJ height calculated by subtracting the immediately after from before running, whereas panel C shows the reduction calculated by subtracting the 1 h after from before running. In these two panels, data are expressed as means ± SD and individual data (*i.e.*, lines). The black and green lines indicate, respectively, greater and lower performance reductions after running at 35°C than at 25°C.

Discussion

The objectives of the current study were twofold. First, we assessed the influence of T_A on the time course of physiological and perceptual responses in tropical natives during a 10 km self-paced run. Second, we investigated the changes in lower limb neuromuscular performance, as determined by CMJ height, following exercise in these two environmental conditions. Our main findings were that, compared to 25°C, endurance performance was reduced, the perceptual strain was increased, while HR and end-T_{CORE} were unchanged at 35°C. In addition, CMJ height reduced by 7.0% (2.3 cm on average) 1 h after the run compared to pre-exercise values, and this reduction was not different between the environmental conditions studied. These findings confirm our first hypothesis, except for the end-T_{CORE} that was unaffected by T_A in the current investigation.

As expected, the participants spent additional time (*i.e.*, 14.1 ± 4.8 min) to finish the 10 km run at 35°C than at 25°C. This finding corroborates with previous studies on the detrimental effects of hot environments on endurance during self-paced exercises [4, 55, 56], including when individuals ran on motorized treadmills [11, 12, 14, 15]. For example, Marino et al. [14] reported that well-trained athletes struggled to run 8 km as fast as possible at 35°C. While all participants completed this task at 15°C, 4 of the 9 participants did not succeed at 35°C, and even those athletes that could run the 8 km in the heat selected a slower pace.

Our findings contradict the second hypothesis and were somehow surprising because the participants lived in a tropical climate: the average maximum T_A in Teresina was equal to or above 35°C (*i.e.*, the hot condition in the current study) in 40% of the months in the period comprised between 2013 and 2022 [33]. Therefore, we expected that heat-acclimatized tropical natives would be less sensitive to the performance-impairing effects of environmental heat stress, which was not the case. Our participants reported higher scores of thermal sensation (*i.e.*, greater body sensation of heat) and perceived exertion at 35°C than at 25°C throughout the treadmill run. Notably, they usually train early in the morning when T_A and solar radiation are not at their highest levels and are exposed to environments with controlled T_A through air-conditioning in several buildings. Thus, some habits of these tropical natives may limit the full development of a heat acclimatization state. Moreover, previous evidence indicates that adaptive pressure on the thermoregulatory function is more intense during artificial acclimation (*e.g.*, exercise-heat stress) than the pressure associated with residing in a hot climate [57]. In this regard, individuals living in tropical climates also benefit from engaging in a heat acclimation protocol [58], and some adaptations induced by acclimation (*e.g.*, the reduction in heart rate during fixed-intensity walking in a hot environment) did not differ when the protocol was carried out at the end of summer or winter [59].

At 25°C, the participants maintained a constant pace during the 10 km; *i.e.*, the speed to cover the 2 km intervals ranged from 10.2 to 10.7 $km \cdot h^{-1}$. The selected speed was slower throughout the 10 km at 35°C than at 25°C, reproducing previous data obtained in marathons [60] and cycling time trials [61]. At 35°C, the participants considerably decreased their pace from the 4th-6th km interval until the end of the race. At this interval, the heat storage rate, perceived exertion, and thermal sensation were significantly augmented at 35°C compared to 25°C, suggesting that altered physiological and perceptual responses induced by the environmental heat stress contributed to reducing the athletes' pace. Trubee et al. [60] pointed out that non-elite men and women runners, as our participants, may consider implementing a slightly slower initial speed to maintain or increase speed in the latter stages of a race in hotter temperatures to enhance performance. Nevertheless, we did not record a faster speed in the last 2 km, agreeing with the recent findings showing that non-elite runners could not sprint at the end of a 10 km run at 33°C [62].

T_{CORE} was transiently higher at 35°C than at 25°C due to a reduced ability to dissipate the metabolic heat produced while running under hot conditions. Interestingly, when individuals decreased their running speed by ~2.0-2.5 $km \cdot h^{-1}$ at 35°C, the heat storage rate decreased, and T_{CORE} became similar between the two environments. This finding disagrees with the observations by Périard et al. [32], who observed a 0.8°C higher rectal temperature at the end of a 40 km time trial at 35°C compared to 20°C. The contradictory results between studies may be explained by the different exercise protocols used (running vs. cycling), T_A of control conditions (25°C vs. 20°C), and the more evident reduction in performance we observed compared to Périard's study (24% vs. 8%). The accentuated performance impairment in the current investigation contradicts our expectations since the inhabitants of Teresina lived much closer to the Equator line than the Australian participants of the 2011 study.

At the end of the run, our participants exhibited average T_{CORE} values close to 39.5°C in both environments. This level of hyperthermia produces significant physiological changes; for example, it is associated with augmented intestinal permeability [63]. A peak T_{CORE} value of 40.3°C was recorded in an individual, even though the present study consisted of a race simulation, where athletes could select their preferred pace to achieve the best performance while maintaining their physiological responses within safe limits [61]. Still, this peak T_{CORE} is lower than values reported in previous studies investigating thermoregulatory responses during half marathons in hot environments: 40.7°C [64] and 41.5°C [65].

When completing the 10 km, the participants were running at 95.5 ± 6.5% (mean ± SD; at 25°C) and 94.4 ± 4.5% (at 35°C) of their maximum HR obtained in the incremental test conducted at temperate conditions (T_A = 24°C and relative humidity = 50%). These data indicate that the individuals exercised near their maximum capacity when they finished the race. Importantly, these similar HR values were attained even with the participants being, on average, 2.5 km·h^{-1} slower during the last 2 km at 35°C than at 25°C. Similar observations (*i.e.*, slower speed or lower power output in the heat but similar HR values) were reported by Marino et al. [14], Périard et al. [32], and Maia-Lima et al. [4]. These findings suggest that individuals selected an exercise intensity according to the cardiac strain experienced in two different environmental conditions.

CMJ height decreased 1 h after the 10 km run compared to pre-exercise height. The reduction corresponded, on average, to 2.3 cm, which exceeds by more than three times the SEM, indicating that decreased performance does not result from measurement error. Hyperthermia caused by exercise in the heat reduces voluntary muscle activation. As reviewed by Nybo et al. [66], whole-body hyperthermia is associated with central fatigue as evidenced by direct measures of voluntary activation during sustained isometric contractions or repeated isokinetic contractions, thus leading to lower force production. A previous study reported a lower force development during sustained handgrip contractions in hyperthermic individuals after a cycling exercise in the heat [7]. The authors concluded that the attenuated ability to activate the skeletal muscles in the hand did not depend on whether the muscle group was active during the preceding lower limb cycling exercise [7]. Thus, central fatigue induced by exercise-induced hyperthermia may represent a mechanistic link between prolonged exercise and the reduced CMJ height, even though treadmill running and jumps are substantially different: *e.g.*, metabolic pathways involved in energy supply (aerobic metabolism vs. high-energy phosphates breakdown).

In the current study, the similar T_{CORE} attained at the end and 1 h after the 10 km may help explain the similar reduction in lower limb power in the two T_{A}s investigated. Nevertheless, CMJ height only decreased 1 h after the 10 km, when average T_{CORE} was 37.5°C, approximately 0.2°C higher than pre-exercise values. Périard et al. [32] reported that the additional increase in T_{CORE} (using rectal probes) did not exacerbate the loss of force production of the knee extensor following self-paced cycling exercise in the heat. Therefore, the changes in T_{CORE} and the resulting central fatigue (*e.g.*, impaired neural drive; [7]) are not the sole explanation for the reduction in lower limb power, which can also result from peripheral fatigue (*e.g.*, impaired excitation-contraction coupling). A decline in Ca^{2+} sensitivity, metabolic acidosis, and impaired action potential may occur in contracting muscles during strenuous aerobic exercises, promoting peripheral fatigue and reducing CMJ height [67, 68].

Interestingly, CMJ height was not significantly reduced immediately post-exercise compared to pre-exercise in our experiments. Similarly, CMJ performance was not impaired immediately after simulated tennis matches in cool (21.8°C) and hot environments (36.8°C) [69]. These findings are supported by two recent meta-analyses that indicated no changes in muscle power within 15 min after an endurance exercise session [70] and a delayed reduction in CMJ height following female soccer matches [71]. In the latter meta-analysis, the athletes jumped a lower height at 24 and 48 h following the matches but not immediately after [71]. However, the current data do not agree with an investigation that reported reduced CMJ height immediately, 3 h, and 24 h after a half marathon compared to the pre-race values [72]. Therefore, we cannot rule out that the magnitude and time course of post-exercise changes in CMJ performance depend on the intensity and duration of the previous endurance exercise.

Our data indicate that running 10 km in a hot environment induced similar HR values and neuromuscular fatigue than running the same distance under temperate conditions, although the average speed was considerably lower in the heat. This finding suggests that the higher external load (*i.e.*, the faster speed) at 25°C compensates for the effects of a transiently higher T_{CORE} and more significant perceptual responses at 35°C in inducing neuromuscular fatigue. From an applied perspective, this observation suggests that, at specific training microcycles, exercising under environmental heat stress may be an adequate strategy to maintain the physiological stimulus when a reduction in external load is desired. Notably, heat acclimation was recently proposed to increase training intensity without a subsequent increase in force production in well-trained rowers, thus limiting lumbar spine compressive forces and stress and reducing lesion incidence [73].

This study is not free of limitations. First, lower limb neuromuscular fatigue was assessed only at two points following the 10 km run. Therefore, we must recognize that differences between experimental conditions could exist after the last time point investigated (1 h after the run). Second, among our 13 participants, only two were women. Although we intended to study a similar number of male and female athletes, recruiting women who fulfilled the inclusion criteria was challenging. Finally, our post-exercise neuromuscular fatigue analysis was restricted to CMJ height. Future investigations should also investigate the changes in the force-time profile of these jumps [74] or use a different test protocol (*i.e.*, maximum voluntary contraction or muscular contraction evoked during electrical stimulation) that allows identifying the origin (central or peripheral) of neuromuscular fatigue [7, 75, 76].

In conclusion, hot conditions impair endurance performance, enhance perceptual responses, and transiently changes physiological strain during a self-paced run compared to temperate conditions. Moreover, lower limb neuromuscular fatigue, determined by the reduction in CMJ height, is evident 1 h after the 10 km run. Despite the marked endurance performance impairment induced by exercise-heat stress, post-exercise neuromuscular fatigue is similar between temperate and hot conditions.

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Figure 1

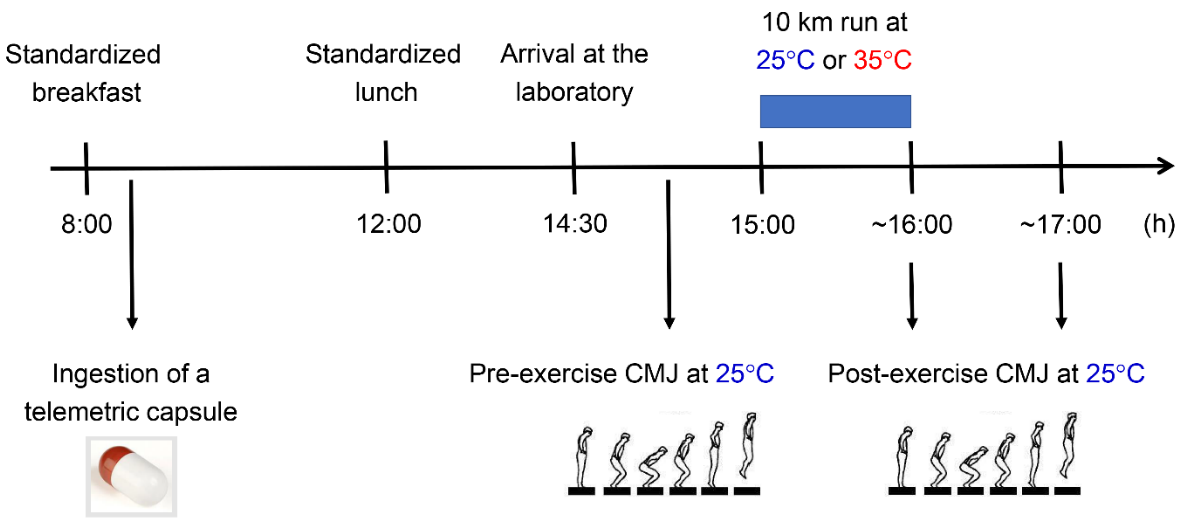

Standardized breakfast
Standardized lunch
Arrival at the laboratory
10 km run at 25°C or 35°C
8:00
12:00
14:30
15:00
~16:00
~17:00
(h)
Ingestion of a telemetric capsule
Pre-exercise CMJ at 25°C
Post-exercise CMJ at 25°C

Figure 2

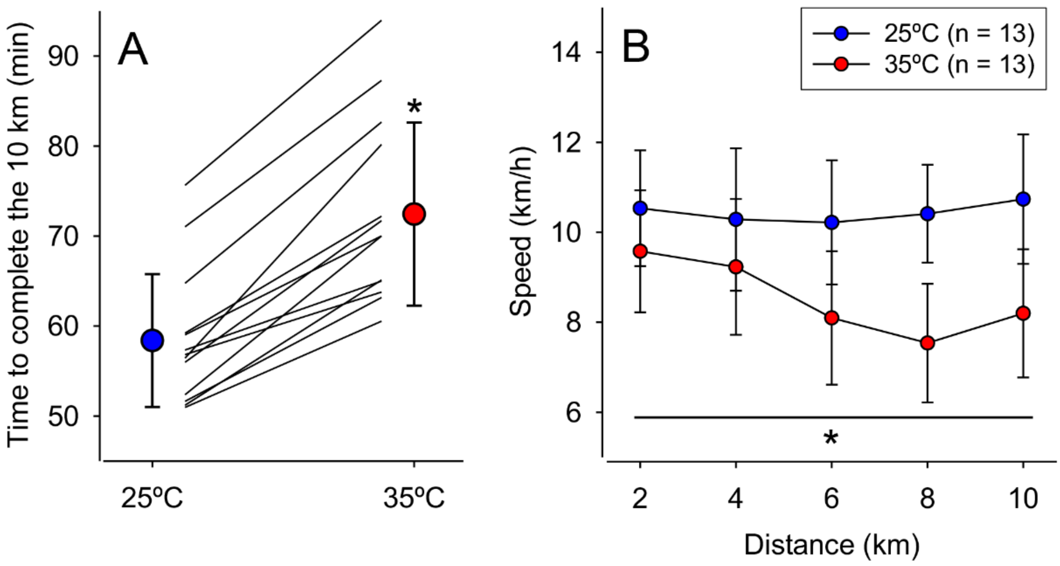

A
Time to complete the 10 km (min)
90
80
70
60
50
25ºC
35ºC
*
B
25ºC (n = 13)
35ºC (n = 13)
Speed (km/h)
14
12
10
8
6
*
2
4
6
8
10
Distance (km)

Figure 3

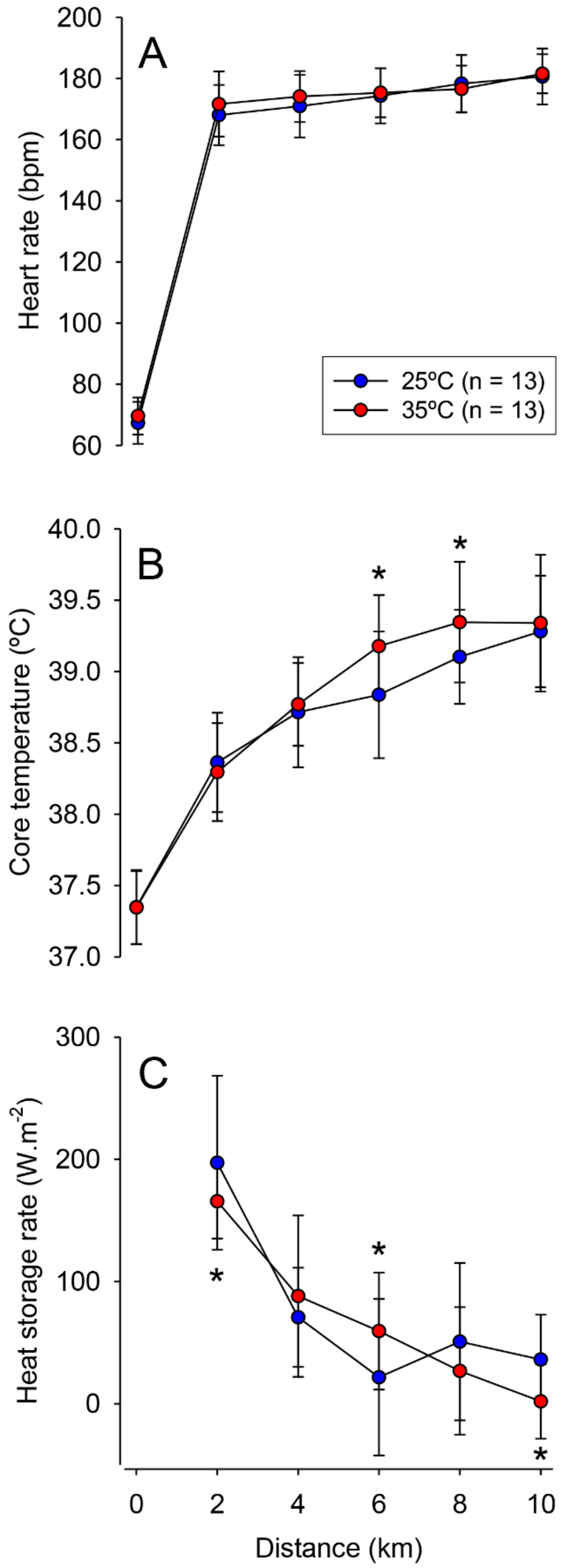

A
Heart rate (bpm)
200
180
160
140
120
100
80
60
25ºC (n = 13)
35ºC (n = 13)
B
Core temperature (ºC)
40.0
39.5
39.0
38.5
38.0
37.5
37.0
*
*
C
Heat storage rate (W.m-2)
300
200
100
0
*
*
*
0
2
4
6
8
10
Distance (km)

Figure 4

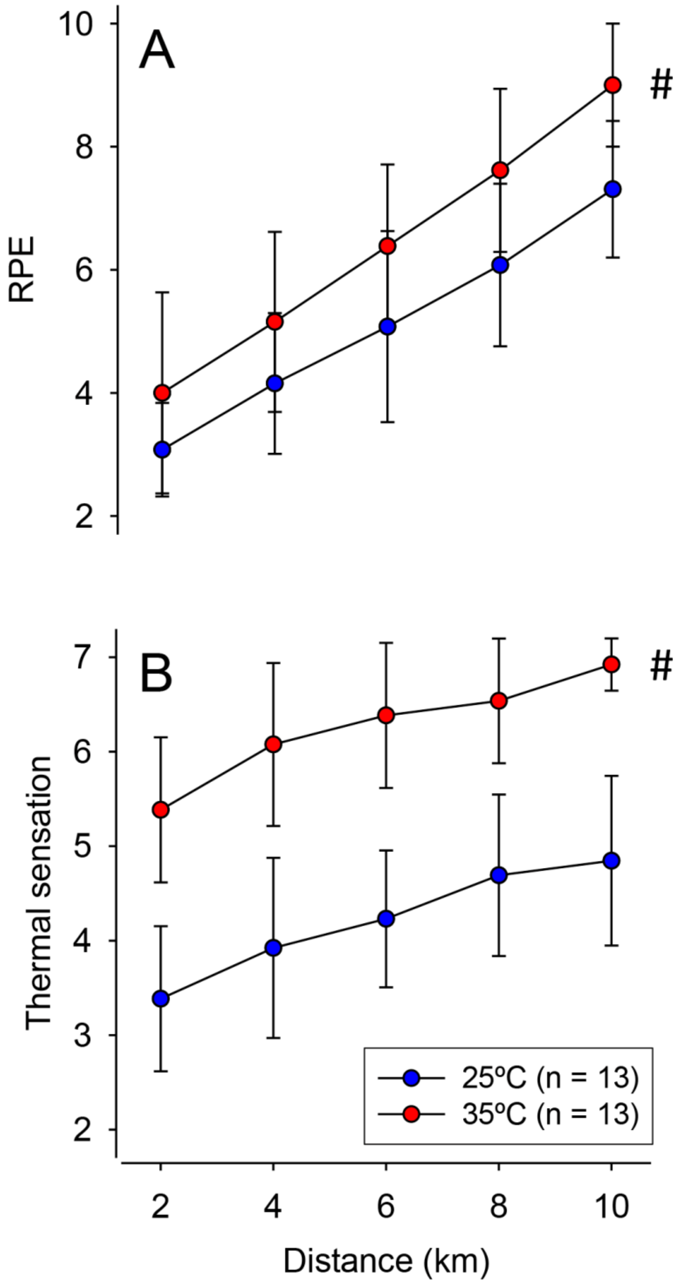
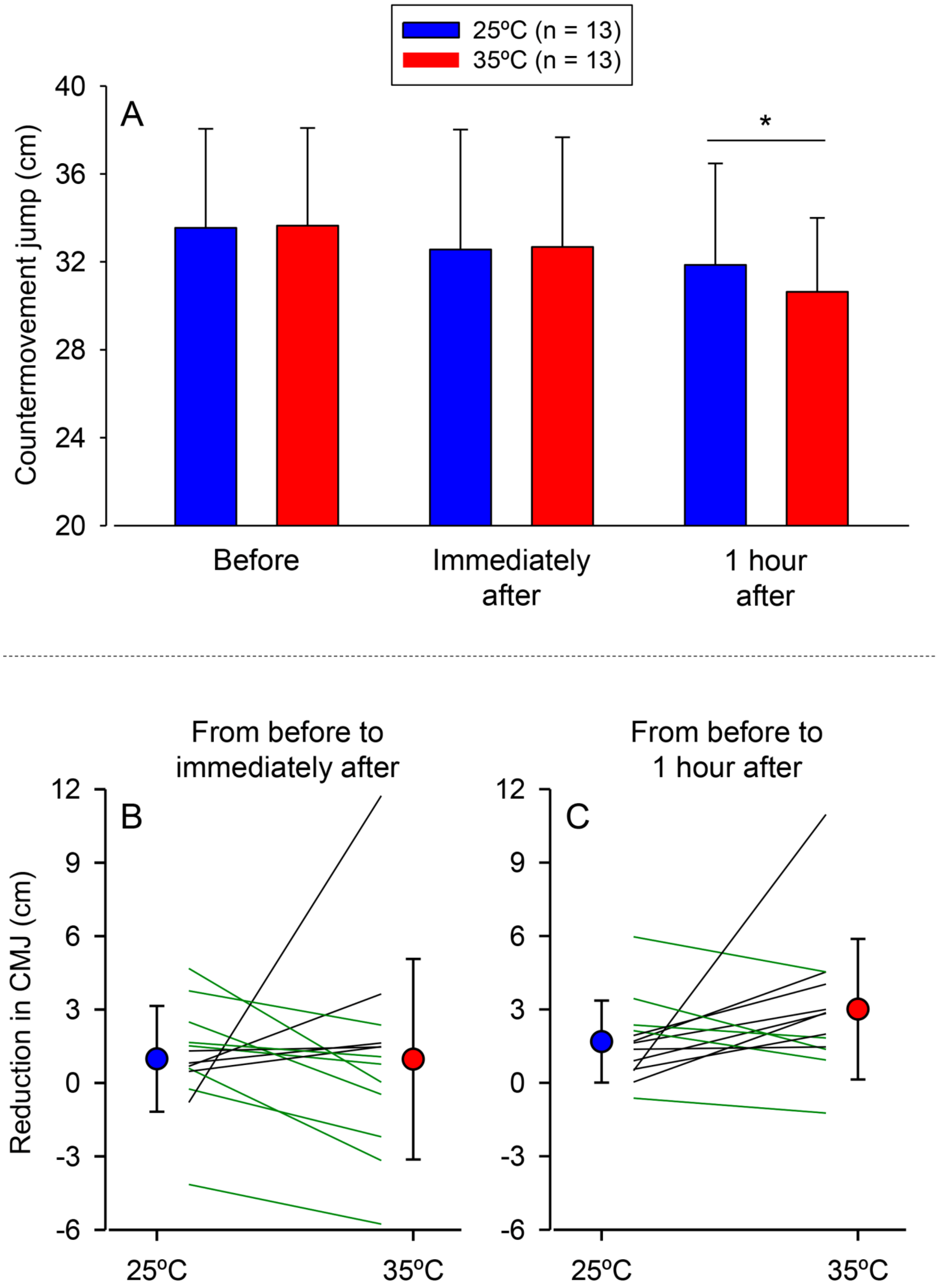

A
B
RPE
Thermal sensation
Distance (km)
#
25ºC (n = 13)
35ºC (n = 13)
10
8
6
4
2
7
5
3

Figure 5


25ºC (n = 13)
35ºC (n = 13)
A
*
Countermovement jump (cm)
40
36
32
28
24
20
Before
Immediately after
1 hour after
From before to immediately after
From before to 1 hour after
B
C
Reduction in CMJ (cm)
12
9
6
3
0
-3
-6
25ºC
35ºC

4 ESTUDO 2

Time-course of physical performance, perceptual, and physiological changes following a 10 km self-paced run in the heat

ABSTRACT

This study investigated the time course of changes in physical performance [countermovement jump (CMJ) height and handgrip force), perceptual (fatigue, vigor, perceived recovery and muscle soreness), and physiological (heart rate variability and urine markers of dehydration)] variables following a self-paced run in the heat. Sixteen long-distance recreational runners ran 10 km as fast as possible on a treadmill at an ambient temperature of 35°C. Data were analyzed using normality tests (Shapiro-Wilk) and homogeneity tests (Levene), repeated-measures ANOVA with Tukey's post hoc tests, Pearson's correlations, and a hierarchical cluster analysis based on the area under the curve of recovery variables over the 24-hour period. Exercise induced physiological and perceptual strain, characterized by increased body temperatures, heart rate, rating of perceived exertion, and thermal perception. Compared to pre-exercise values, CMJ height was reduced 1 hour after the exercise, whereas handgrip strength was unaltered. Post-exercise perceptual changes were observed, including increased fatigue and muscle soreness, alongside decreased vigor and perceived recovery. Urine markers of dehydration increased, whereas markers of parasympathetic cardiac modulation decreased immediately following exercise-heat stress. Almost all variables returned to baseline levels within 24 hours. Next, we assessed whether the reduced CMJ height was associated with altered perception 1 hour after the exercise; no significant correlations were observed. One cluster showed no reduction in handgrip strength and attenuated fatigue, while a second cluster was characterized by increased muscle soreness. In conclusion, the current data provide a unique overview of recovery following a prolonged treadmill run in the heat and help optimize the training and recovery prescription of long-distance runners who train and compete under thermally stressful conditions.

Keywords: athletic performance, autonomic nervous system, cluster analysis, dehydration, fatigue, hot temperature, muscle strength, post-exercise recovery

INTRODUCTION

Long-distance running events (> 3 km) are popular worldwide for elite and recreational athletes (Vitti; Nikolaidis; Villiger; Onywera *et al.*, 2020; Witthöft; Marcin; Thuany; Scheer *et al.*, 2025). Preparation for these events includes high training volumes, as indicated by elevated distances covered during a week of regular training. For example, well-trained male long-distance runners covered, on average, approximately 72 km per week during their regular training (Luden; Hayes; Galpin; Minchev *et al.*, 2010), while athletes who finished a marathon reported running 52 ± 38 km (mean ± SD) per week (Yeung; Yeung; Wong, 2001). Even greater weekly volumes can be found in the literature, such as the regular training volume of 129 ± 53 km self-reported by senior British athletes who had competed at a national, international, or Olympic level (Spilsbury; Fudge; Ingham; Faulkner *et al.*, 2015). Given these training characteristics, it is paramount to characterize the level of fatigue experienced by long-distance (endurance) runners and understand how they recover between training sessions and after competitions.

Among different available definitions, fatigue can be defined as a disabling symptom in which physical and cognitive functions are limited by interactions between performance fatigability and perceived fatigability(Enoka; Duchateau, 2016). Performance fatigability is the decline in an objective measure of performance over a discrete period caused by impairments in muscle contraction function and activation (Enoka; Duchateau, 2016). The reduced CMJ height following a 10 km self-paced run(Rodrigues; Mendes; Gomes; Silami-garcia *et al.*, 2023) and the lower force produced during 2-minute isometric maximum voluntary contractions after a submaximal exercise to fatigue(Moraes; Paulinelli-júnior; Teixeira-coelho; Cançado *et al.*, 2019) are examples of performance fatigability. On the other hand, perceived fatigability corresponds to the changes in sensations that regulate the integrity of athletes; it is derived from changes in modulating factors (*e.g.*, core temperature, hydration, and motivation) and is used to regulate the pace of the performance and thereby control the development of fatigue (Enoka; Duchateau, 2016).

Recovery is a multifaceted (*e.g.*, physiological and psychological) restorative process relative to time (Kellmann; Bertollo; Bosquet; Brink *et al.*, 2018) aimed, for example, at restoring homeostasis of the body's physiological systems (Peake, 2019) allowing the athlete to receive a new training stimulus. Due to the multifactorial nature of recovery, its assessment should include performance measures (*i.e.*, most specific to the sports discipline as possible) alongside physiological and psychological measures (Kellmann; Bertollo; Bosquet; Brink *et al.*, 2018). Such measures subsidize athletes and coaches to monitor and control the training load better, avoiding fatigue accumulation. Acutely, insufficient recovery may impair readiness for the next training session; chronically, it may lead to overtraining syndrome (Meeusen; Duclos; Foster; Fry *et al.*, 2013b).

Despite the well-known negative impacts of environmental heat stress on performance in endurance events (El Helou; Tafflet; Berthelot; Tolaini *et al.*, 2012; Ely; Cheuvront; Roberts; Montain, 2007; Rodrigues; Mendes; Gomes; Silami-garcia *et al.*, 2023), research on how athletes recover following exercise under these adverse conditions is still lacking. Most studies on the topic have compared recovery following exercises performed in hot versus control environments and provided contradictory findings: some studies showed more significant impairments in performance measures following exercise in the heat (Nybo; Nielsen, 2001), whereas other studies did not (Moraes; Paulinelli-júnior; Teixeira-coelho; Cançado *et al.*, 2019; Rodrigues; Mendes; Gomes; Silami-garcia *et al.*, 2023). However, the time-course in performance, perceptual, and physiological changes following a prolonged run in the heat, including multiple post-exercise measurements, have not been investigated in an integrated manner, according to the proposed multifaceted concept of recovery. Such an investigation is essential considering ongoing global warming, which is not expected to be reversed in the following decades (Bitencourt; Muniz Alves; Shibuya; DE Angelo Da Cunha *et al.*, 2021) and will force runners to train and compete more frequently under undesirable heat stress conditions (Souza-júnior; Carmo; Wanner, 2024).

Another relevant point is that high inter-individual variability of the recovery time-course exists, often influenced by a variety of external (*i.e.*, training/match loads, sleep, and nutrition) and internal factors (physical capacities) (Johnston; Gabbett; Jenkins; Hulin, 2015; Wanner; Wilke; Duffield, 2016), creating further challenges to interpret recovery. Previously, we clustered futsal players into different recovery profiles that were labeled faster recovery, slower physiological recovery, and slower perceptual recovery profiles (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a). Whether athletes from an individual sport (*i.e.*, middle-distance runners) can be clustered into different recovery profiles is still to be determined. The ability to identify different recovery profiles may aid the prescription of training loads and recovery strategies, particularly when the coaching staff is responsible for several athletes that train simultaneously with diverse requirements alongside restricted facilities and staff availability (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a).

Therefore, the present study aimed to describe the changes in performance, perceptual, and physiological variables following a self-paced 10 km treadmill run under environmental heat stress. In addition, this study investigated whether the post-exercise changes in performance and perceptual variables were associated and then tried to cluster the athletes into different recovery profiles. We hypothesized that (a) exercise-heat stress would induce significant changes in the variables measured following the 10 km run; (b) these changes would not be associated due to different recovery timelines for performance and perceptual variables, as previously described (Gagge; Stolwijk; Hardy, 1967; Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a); (c) the athletes could be clustered into different recovery profiles.

METHODS

Ethical care

The current study received approval from the Research Ethics Committee of the Universidade Federal de Minas Gerais (protocol number: CAAE 67163323.7.0000.5149) and followed the principles outlined in the 1964 Declaration of Helsinki and its subsequent amendments, ensuring that all experimental procedures were conducted with the highest ethical standards. All participants were informed about the study's objectives, procedures, and any potential risks and benefits associated with participation. Subsequently, they signed an informed consent form confirming their voluntary participation in the study.

Participants

The participants were long-distance recreational runners (14 men and 2 women), tropical natives living in São Luís, a city located in the northeast region of Brazil (latitude: 2° 31′ 51″ South, longitude: 44° 18′ 24″ West). The monthly average ambient temperature (T_A) and relative humidity (RH) in São Luís were 26.6 ± 2.2°C and 82.3 ± 11.5%, respectively, based on data from the period between 2020 and 2024 (INMET, 2025).

Participants met the following inclusion criteria: 1) they responded “no” to all questions of the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas; Reading; Shephard, 1992), 2) trained regularly for at least two years; 3) maintained a weekly training frequency of at least five days; 4) were free of lower limb injuries within the six months before the experiments; and 5) completed a 10 km run outdoor in less than 50 min (men) or 55 min (women) within the six months prior to the experiments. This performance could have been achieved during training sessions or competitions. The female participants were using monophasic oral contraceptives and were tested during the active pill phase, specifically from the 2nd to the 21st day of use (Andrade; Nunes-Leite; Bruzzi; Souza *et al.*, 2023; Sims; Heather, 2018). Moreover, the participants abstained from using medications and nutritional supplements during the experiments.

Experimental design

The present study is part of a broader investigation into how short-term heat acclimation affects athletes’ recovery following a treadmill run in the heat. The experiments were carried out between August and December, the hottest months of the year in São Luís: average monthly T_A = 27.7 ± 2.0°C and RH: 74.5 ± 9.8%, with the highest T_A exceeding 30°C in all four months (INMET, 2025).

Each participant visited the laboratory three times. During the first visit, they signed the informed consent form, and their body mass and stature were measured using a digital scale coupled to a stadiometer (Welmy, model W200), with resolutions of 50 g and 0.5 cm, respectively. These measurements were used to calculate body mass index and surface area according to the equations proposed by Keys et al. and Du Bois and Dubois(Bois, 1989; Keys; Fidanza; Karvonen; Kimura *et al.*, 2014). Body fat percentage was estimated using a bioimpedance device (OMRON, model HBF– 514C). The participants were then familiarized with using the perceptual scales and the dynamometer and with performing countermovement jumps (CMJ). Next, they performed a 1-mile (1.6-kilometer) test to estimate their maximum rate of oxygen consumption (VO_{2MAX}) under temperate conditions: T_A = 24.4 ± 0.4°C and RH = 55 ± 4%. Upon finishing this test, they ran an additional 8.4 km at 70% of the average speed of their best self-reported 10 km performance (*i.e.*, 9.7 ± 1.3 km.h^{-1}); this extra distance was covered at the same T_A as the 1-mile test and aimed to familiarize the athletes with running for a prolonged period on a treadmill.

The second visit took place 24 h after the first one. The participants ran 10 km at a self-paced intensity under temperate conditions: T_A = 35.4 ± 0.4°C, RH = 60 ± 8%. Physiological (*i.e.*, heart rate, aural canal temperature, and mean skin temperature) and perceptual variables (RPE, thermal comfort, and thermal sensation) were measured every km throughout the 10 km run. Moreover, several variables were collected before, immediately after, 1 h after, and 24 h after the treadmill run to assess post-exercise recovery. These variables included CMJ height, handgrip force, heart rate variability, urine specific gravity, urine color, mood status, perceived recovery, and perceived muscle soreness (Figure 1). The measurements taken 24 h after the treadmill run corresponded to the participants' third visit to the laboratory. During this last visit, they also completed a 1-mile test.

Experimental procedures

1-mile test

This test was performed to estimate the participants' VO_{2MAX} (on the first visit) and analyze their recovery (on the third visit). The athletes were instructed to run the one mile as fast as possible on a treadmill (Matrix model T1X, Johnson Health Tech) and had no feedback regarding their performance while exercising; they could only visualize the distance covered. Following the recommendations by the American College of Sports Medicine for maximal testing (Mahler; Froelicher; Miller; York, 1995) the 1-mile (1,600-kilometer) test would be interrupted in the event of equipment failure, at a participant's request, or if a participant showed an unusual lack of breathing or signs of malaise, such as pale appearance of the skin or cyanosis (signs of poor perfusion). None of these conditions were observed in the current study.

VO_{2MAX} was estimated using the following equation developed specifically for physically active young Brazilians (Almeida; Campbell; Pardono; Sotero *et al.*, 2010): $VO_{2MAX} = 8.101 + 0.177 \times S_{AVG}$, where S_{AVG} is the average speed in $m.min^{-1}$ to complete one mile.

Familiarization with the handgrip test and CMJs

Initially, the participants ran 10 min at 70% of the mean speed obtained during their best 10 km performance to increase lower limb muscle temperature. Elevated muscle temperature improves performance in high-intensity short-duration exercises (Carmo; Goulart; Cabido; Martins *et al.*, 2023).

After the standardized warm-up, the athletes were familiarized with CMJs by completing 3 sets of 3 repetitions, with a 30-second interval between sets, as previously described (Claudino; Mezêncio; Soncin; Ferreira *et al.*, 2012; Rodrigues; Mendes; Gomes; Silami-Garcia *et al.*, 2023). Participants initiated the jump from a standing position, with their feet positioned in parallel on a contact mat (model SH5001, Saehan Corporation, South Korea) and both hands akimbo to avoid the influence of upper limbs on jump height. At the experimenter's signal, the individuals performed a continuous movement consisting of quickly flexing the knees to 90° (eccentric action) and then jumping (concentric action) as high as possible (Claudino; Mezêncio; Soncin; Ferreira *et al.*, 2012).

The participants were then familiarized with the procedures to assess right handgrip strength using a hand dynamometer (model SH5001, Saehan Corporation, South Korea). The handgrip test was conducted according to the recommendations of the American Society of Hand Therapists, with the volunteer sitting on a chair with the shoulder adducted, the elbow flexed at 90°, the forearm in a neutral position, and the wrist position varying from 0 to 30° of extension (Bellace; Healy; Besser; Byron *et al.*, 2000). The participants repeated the test until three consecutive consistent measurements of handgrip strength were recorded.

Measurements before the 10 km run

The experimental procedures were always carried out between 2:30 and 6:00 p.m. The 10-kilometer runs were initiated with less than 1 h difference between participants to standardize the circadian rhythm effects on the variables measured.

Upon arriving at the laboratory, the participants rested 10 minutes in a room at temperate conditions (T_A = ~25°C) to establish baseline measurements. Their hydration status was checked using a refractometer (model RTP-20 ATC, Instrutherm®), and a urine specific gravity of 1.020 or less indicated a euhydrated status (Armstrong; Maresh; Castellani; Bergeron *et al.*, 1994). If a participant was found dehydrated, he/she was provided with 500 mL of water and his/her urine specific gravity was tested again 30 to 60 min later.

Heart rate variability (HRV), an indirect indicator of autonomic cardiac modulation, was assessed through the analysis of R-R intervals recorded at rest. Data collection was performed using an H10 chest strap, with signals transmitted to a smartphone running the Kubios HRV software (Elite HRV, Asheville, NC, USA). The following time-domain HRV parameters were analyzed: the mean R-R interval (hereafter referred to as R-R interval), the root mean square of successive differences between adjacent R-R intervals (RMSSD), and the standard deviation of normal R-R intervals (SDNN). Both RMSSD and SDNN are commonly used to reflect parasympathetic activity, particularly in short-term recordings. Mood state was assessed through the Brunel Mood Scale (BRUMS), translated and validated in Portuguese (Rohlfs; Rotta; Luft; Andrade *et al.*, 2008). The BRUMS has six sub-scales (i.e., anger, confusion, depression, fatigue, tension, and vigor), each composed of four items. Each item is preceded by the question “How do you feel right now?” and is answered on a 5-point scale (from 0 to 4). We only assessed the fatigue and vigor sub-scales, usually affected by a single exercise session (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a).

Perceived muscle soreness was determined using a visual analog scale (VAS) (Miller; Duncan; Brown; Sparks *et al.*, 2003). A 10-centimeter line was drawn; 0 corresponded to no muscle soreness, while 10 corresponded to the maximum muscle soreness imaginable. The experimenter palpated the thigh and calf regions, and then the athlete marked a point in the straight line, indicating their current perceived muscle soreness. Perceived muscle soreness was determined separately for the tight and calf, and these scores were summated to indicate lower limb muscle soreness. Perceived recovery was determined using the 15-point total quality of recovery (TQR) scale (Kenttä; Hassmén, 1998) ranging from 6 (worse than very, very poor recovery) to 20 (better than very, very good recovery).

The athletes then performed a warm-up as described earlier, followed by three CMJs and three repetitions of the handgrip test. The arithmetic mean performance of the three jumps and handgrip repetition were computed. Next, the athletes were weighed and directed to the heated room, where the 10 km run was conducted.

10 km run

The exercise took place in a small room (3.0 m in length x 2.5 m in width x 2.2 m in height), and the ambient temperature was increased with the help of three electrical heaters (Britânia, model AB1100N). The athletes were instructed to run the 10 km as fast as possible and again had no feedback about their performance. They could drink refrigerated water *ad libitum*, and no electrical fans were used to generate artificial during the treadmill run.

Aerobic performance corresponded to the time to complete the 10 km run. The time spent covering each km was also recorded and then converted into average speed; this information allowed us to analyze the participants' running strategy. In addition, the following variables were collected at 1 km intervals: heart rate (HR), auricular canal temperature, skin temperatures, perceived exertion (RPE), thermal comfort, and thermal sensation.

Auditory canal temperature was measured using an infrared thermometer (model AOJ-20A, AOJ Medical, China) with a display resolution of 0.1°C. The thermometer was inserted into the auditory canal while the participants were running. Previous data from our laboratory indicate that auditory canal temperature agrees with rectal temperature during exercise in the heat (Moraes et al., unpublished observations). An infrared digital thermometer (Fluke, model 62 MAX+) was used to measure skin temperature (T_{SKIN}) at three skin sites on the body's right side, using the following anatomical references: chest – 3 cm beside the nipple, close to the midline; arm – lateral portion at half the distance between the shoulder and elbow; thigh - anterior surface at half the distance between the hip and knee. The infrared thermometer was placed 20 cm from the skin surface, and the sites of interest were marked with a whiteboard pen. Mean weighed T_{SKIN} was calculated using the equation proposed by Roberts et al. (Roberts; Wenger; Stolwijk; Nadel, 1977): mean $T_{SKIN} = (T_{CHEST} \times 0.43) + (T_{ARM} \times 0.25) + (T_{TIGHT} \times 0.32)$.

HR was monitored using a Polar H-10 chest strap (Polar Electro Oy, Kempele, Finland) with a sampling frequency of 5 Hz. RPE was determined using the 15-point Borg scale (Borg, 1982), ranging from 6 to 20. Thermal sensation was determined by a 7-point scale (Gagge; stolwijk; Hardy, 1967), ranging from 1 (very cold) to 7 (very hot); in this scale, the score of 4 corresponds to a neutral sensation. Thermal comfort was assessed by a four-point scale (Gagge; Stolwijk; Hardy, 1967) ranging from 1 (comfortable) to 4 (very uncomfortable).

Measurements after the 10 km

Body mass was measured immediately after the exercise. Thereafter, measurements were made as previously described and in the following order: HRV, CMJ height, handgrip strength, BRUMS, TQR, and perceived soreness. The athletes could not provide a urine sample at this time point due to dehydration caused by exercise-heat stress.

One hour after the exercise, procedures for determining HRV were carried out, and then the participants were subjected to another warm-up session. Additional measurements were made in the following order: CMJs, handgrip strength, BRUMS, TQR, and perceived soreness. Similar procedures were made 24 h after exercise, except a 1-mile test that was conducted as the final assessment before the participants were cleared to leave the laboratory.

Calculated variables

Heart rate variability (HRV), an indirect marker of autonomic regulation of cardiac function, was assessed through the recording of R-R intervals during resting conditions. Data were collected using an H10 chest strap and transmitted to a smartphone running the Kubios HRV software (Elite HRV, Asheville, NC, USA). The following variables in the time domain of HRV were assessed: the mean duration of R-R intervals (R-R interval, for simplicity), the root mean square of successive differences in R-R intervals (RMSSD), and the standard deviation of normal R-R intervals (SDNN). Changes in RMSSD and SDNN estimate the parasympathetic cardiac activity in short-term recordings (SHAFFER; GINSBERG, 2017).

The volume of water ingested was calculated by determining the difference between the amount of water available before and after the exercise. Whole-body sweat loss was calculated as the difference between the pre-exercise (BM_{PRE}) and post-exercise body mass (BM_{POST}), corrected by the volume of water ingested, according to the following equation: sweat loss = $BM_{PRE} – BM_{POST}$ + water ingested. This equation generated results in mg that were converted to mL. Of note, no participant asked to urinate between the two body mass measurements. Sweat rate ($L \cdot h^{-1}$) was calculated by dividing whole-body sweat loss by the time to complete the 10 km. The percentage change in body mass (%ΔBM) was determined using the following equation: $\%\Delta BM = [(BM_{PRE} – BM_{POST}) / BM_{PRE}] \times 100$.

Statistical analysis

The homogeneity of variance was assessed using Levene's test, and data normality was checked with the Shapiro-Wilk test, with no significant effects observed in either test. Data were presented as means ± standard deviation (SD). Repeated-measures one-way analyses of variance (ANOVAs) were applied to test differences in the variables assessed between distances (during the 10 km run) and between time points (pre vs. post-treadmill run). When significant differences were detected, post hoc comparisons using the Tukey's test were performed.

The association between changes in performance (*i.e.*, reduction in CMJ height) and perceptual variables (fatigue, vigor, muscle soreness, and perceived recovery) was assessed using the Pearson's correlation coefficient. A p-value of < 0.05 was considered statistically significant. All statistical analyses were conducted using IBM SPSS (version 22), while graphs were prepared in SigmaPlot software (version 11.0, Systat Software Inc., San Jose, CA, USA). Effect sizes were reported based on partial eta squared (ηp^2) for ANOVAs. Benchmarks to interpret ηp^2 values have been defined by Cohen (1988): small ($\eta p^2 = 0.01$), medium ($\eta p^2 = 0.06$), and large ($\eta p^2 = 0.14$) effects.

As a final analysis, we investigated whether the participants could be clustered into different recovery profiles, following the procedures described by Wilke et al. (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a). First, the percentage difference between the pre-run and each post-run time point was determined. These values were then transformed to a z-score and used to calculate the area under the curve (AUC) of the entire 24-hour post-run timeline for each variable via the trapezoidal method as a representation of the post-exercise recovery time course.

Then, using the AUC of the seven recovery parameters (i.e., CMJ height, handgrip strength, BRUMS fatigue, BRUMS vigor, TQR, perceived soreness, and RMSSD), an agglomerative hierarchical cluster analysis based on Euclidean distance and average linkage criteria was performed (IBM SPSS version 22). SDNN data were very similar to RMSSD data, and therefore, we decide not to include SDNN in analyses. Moreover, urine specific gravity and urine color were not measured 1 hour after the exercise, thus justifying their absence in the cluster analysis. Each subject's data for each measure was plotted in a multidimensional plan, and the Euclidean distance between subjects was calculated. The lower the distance between two subjects, the more similarities they share (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a). The threshold difference of 10 was used to optimize clustering based initially on dendrogram differentiation and then on the theoretical and meaningfulness of the resulting clustering.

RESULTS

Participants

Sixteen long-distance recreational runners (14 men and 2 women) completed all experimental procedures. Their characteristics are presented in Table 1. Of note, their mean training experience and mean estimated VO_{2MAX} were, on average, 4.4 years and 46.6 mL·kg^{-1}·min^{-1}, respectively.

Table 1. Participants' characteristics.

Variables	Mean	SD	CV (%)	Min.	Max.
Age (years)	33.4	10.3	30.8	18.0	49.0
Body mass (kg)	67.7	8.3	12.3	54.9	83.0
Height (cm)	169.5	8.5	5.0	150.0	186.0
BMI (kg·m^{-2})	23.6	2.3	10.0	19.0	28.0
Body fat mass (%)	21.2	7.1	33.7	10.8	34.8
Training experience (years)	4.4	2.2	51.4	2.0	10.0
VO_{2MAX} (mL·kg^{-1}·min^{-1})	51.9	5.8	11.1	41.7	62.2

Legend: BMI = body mass index; CV = coefficient of variation; Max. = maximum; Min. = minimum; SD = standard deviation; VO_{2MAX} = maximum rate of oxygen consumption.

10 km self-paced run

The average speed of the participants during the 10 km run was 10.9 ± 1.4 km/h, meaning that they lasted 55.9 ± 7.2 min to complete the predetermined distance in the heat (Figure 2). This performance was, on average, 10.1 ± 3.9 min ($p < 0.001$) slower compared to the best self-reported time in official 10 km races or training sessions. When analyzing the average speed values for each km interval (Figure 1), a significant increase was observed from the second to the fifth km compared to the first km ($F = 5.907$, $p < 0.001$, $\eta p^2 = 0.283$). The average speed increased from 11.1 ± 1.7 km/h in the first km to 12.3 ± 1.7 km/h in the fourth km. Thereafter, variability around mean speed values was increased, and no significant differences were observed compared to the first km.

Figure 1. Average speed for each kilometer during the 10 km self-paced run in the heat. Data are expressed as mean ± SD. * $p < 0.05$ vs. the first km.

During the 10 km race, the expected increases for the heart rate ($F = 170.600$, $p < 0.001$, $\eta p^2 = 0.919$), auricular temperature ($F = 93.828$, $p < 0.001$, $\eta p^2 = 0.862$), and mean skin temperature ($F = 24.271$, $p < 0.001$, $\eta p^2 = 0.618$; Figure 2) were observed. Similarly, the exercise-heat stress caused perceptual strain, as indicated by the increased scores of RPE ($F = 46.030$, $p < 0.001$, $\eta p^2 = 0.754$), thermal comfort ($F = 44.790$, $p < 0.001$, $\eta p^2 = 0.749$), and thermal sensation ($F = 25.829$, $p < 0.001$, $\eta p^2 = 0.633$). At the end of the 10 km run, the participants had a heart rate of 175 ± 14 bpm and an auricular temperature of 39.12 ± 0.46°C. Moreover, they indicated the following scores for perceptual variables upon completing the predetermined distance: RPE = 18 ± 1, thermal comfort = 4 ± 1, and thermal sensation = 6 ± 1.

Figure 2. Physiological and perceptual variables during the 10 km self-paced run in the heat: heart rate (panel A), auricular temperature (B), mean skin temperature (C), rating of perceived exertion (D), thermal comfort (E), and thermal sensation. Data are expressed as mean ± SD. * $p < 0.05$ vs. the first km.

The exercise-heat stress induced a whole-body sweat loss of 848.2 ± 333.8 mL, leading to a sweating rate of 15.7 ± 7.5 mL/min. The average fluid intake was 225.7 ± 230 mL, which corresponded to 26.6% of the sweat losses. The volunteers exhibited absolute and percentage reductions in body mass of 1.3 ± 0.7 kg and 1.9 ± 1.0% compared to pre-exercise body mass.

Table 2. Water balance during the 10 km run self-paced run in the heat.

Variables	Mean	SD	CV (%)	Min.	Max.
Change in body mass (kg)	1.3	0.7	56.6	0.4	3.4
Change in body mass (%)	1.9	1.0	52.8	0.6	4.6

Water intake (mL)	225.7	230.0	101.9	0	650.0
Sweat loss (mL)	848.2	333.8	39.4	407.8	1783.4
Sweat rate ($mL \cdot min^{-1}$)	15.7	7.5	47.6	7.5	38.7

Legend: CV = coefficient of variation; Max. = maximum; Min. = minimum; SD = standard deviation.

Post-exercise recovery

Physical performance tests assessed the CMJ height, handgrip strength, and time to complete 1 mile (Figure 3). CMJ height decreased following exercise-heat stress ($F = 4.794$, $p = 0.006$, $\eta p^2 = 0.242$). More specifically, CMJ height was lower 1 h after the exercise than before and immediately after. In contrast, no significant changes were observed for handgrip strength ($F = 0.158$, $p = 0.924$, $\eta p^2 = 0.010$). Finally, the participants took 0.38 ± 0.55 min less to complete the 1-mile test conducted 24 h after the 10 km run compared to the test conducted during the first laboratory visit ($t = 2.760$, $p = 0.015$).

Figure 3. Time course of changes in physical performance tests following the 10 km run in the heat: CMJ height (panel A), handgrip strength (panel B), and time to complete the 1-mile test (panel C). Data were obtained before, immediately after, 1 h after, and 24 h after the exercise, except for the 1-mile test data, which were obtained before (i.e., in the first visit) and 24 h after the exercise. Data are expressed as mean ± SD. * $p < 0.05$ vs. pre-exercise values; # $p < 0.05$ vs. immediately after the exercise.

Concerning the perceptual variables (Figure 4), fatigue ($F = 14.848$, $p < 0.001$, $\eta p^2 = 0.497$) and vigor ($F = 14.499$, $p < 0.001$, $\eta p^2 = 0.492$), both measured using the BRUMS questionary, were altered following the 10 km run in the heat. Fatigue was increased immediately and 1 h after the run compared to the pre-exercise values and 24 h after; the changes in vigor were similar except for the facts that scores were reduced immediately and 1 h after instead of being increased and that no significant difference was noted between 1 h and 24 h after. Perceived recovery also reduced following the treadmill run ($F = 19.756$, $p < 0.001$, $\eta p^2 = 0.568$), with lower values reported in all three post-exercise time points than at pre-exercise. The perceived muscle soreness also changed following the 10 km run in the heat

($F = 5.895$, $p = 0.013$, $\eta p^2 = 0.282$). Indeed, muscle soreness was higher immediately and 1 h after compared to 24 h after the exercise and almost reached statistical significance for higher values immediately after compared to pre-exercise ($p = 0.072$).

Figure 4. Time course of changes in perceptual variables following the 10 km run in the heat: fatigue (panel A), vigor (panel B), perceived recovery (panel C), and perceived muscle soreness (panel D). Data were obtained before, immediately after, 1 h after, and 24 h after the exercise. Data are expressed as mean ± SD. * $p < 0.05$ vs. pre-exercise values; @ $p < 0.005$ vs. 24 h after the exercise.

The recovery time course of physiological variables related to cardiac autonomic control (*i.e.*, RMSSD and SDNN) and water-electrolyte control (USG and urine color) was also assessed (Figure 5). RMSSD ($F = 16.710$, $p < 0.001$, $\eta p^2 = 0.527$) and SDNN ($F = 11.986$, $p < 0.001$, $\eta p^2 = 0.444$) were reduced immediately after the exercise compared to the other three time points analyzed. Finally, concerning hydric-electrolytic control, USG ($F = 18.829$, $p < 0.001$, $\eta p^2 = 0.557$) and urine color ($F = 27.562$, $p < 0.001$, $\eta p^2 = 0.648$) were increased immediately after the run in the heat compared to pre-exercise and 24 h after.

Figure 5. Time course of changes in physiological variables following the 10 km run in the heat: root mean square of successive differences in R-R intervals (RMSSD, panel A), standard deviation of normal R-R intervals (SDNN, panel B), urine specific gravity (USG, panel C), and urine color (panel D). Data were obtained before, immediately after, 1 h after, and 24 h after the exercise. Data are expressed as mean ± SD. * $p < 0.05$ vs. pre-exercise values; + $p < 0.05$ vs. 1 h after the exercise; @ $p < 0.005$ vs. 24 h after the exercise.

Next, we verified whether the changes in CMJ height 1 hour after the 10 run were associated with changes in perceptual variables at the same time. When using the absolute changes, the reduction in CMJ height was not significantly associated with the increases in fatigue ($r = 0.115$, $p = 0.671$) and perceived muscle soreness ($r = -0.226$, $p = 0.400$) or with the reductions in vigor ($r = -0.114$, $p = 0.674$) and perceived recovery ($r = -0.029$, $p = 0.916$). Similarly, when analyzing the percentage changes, the reduction in CMJ height was not significantly associated with the increases in fatigue ($r = -0.502$, $p = 0.251$) and perceived muscle soreness (r = -0.162, p = 0.580) or the reductions in vigor (r = -0.230, p = 0.391) and perceived recovery (r = -0.013, p =0.962). The correlations between the percentage reduction in CMJ and the percentage increases in fatigue and perceived muscle soreness were conducted with 7 and 14 observations, respectively (*i.e.*, we did not calculate percentage increases when the scores of fatigue and perceived muscle soreness corresponded to zero); all the other correlations considered 16 observations.

Next, we investigated whether the 16 participants could be clustered into different groups according to the recovery timelines of seven variables: CMJ height, handgrip strength, fatigue, vigor, perceived recovery, perceived muscle soreness, and RMSSD. Our analysis identified three clusters (two with 5 individuals and one with 4 individuals). Two individuals could not be clustered into any of these three groups (Figure 6).

Figure 6. Dendrogram from the cluster analysis.

The average data regarding the area under curves for z-scores were compared between the three groups (Table 3). No significant differences were observed in CMJ height ($F = 1.034$, $p = 0.388$), vigor ($F = 0.136$, $p = 0.874$), perceived recovery ($F= 1.981$, $p = 0.184$), and RMSSD ($F = 0.480$, $p = 0.631$). However, the area under curves for handgrip force and fatigue were, respectively, higher ($F =22.026$, $p < 0.001$) and lower ($F = 8.739$, $p = 0.005$) in Cluster 1 than in Clusters 2 and 3. Moreover, perceived muscle soreness was higher in Cluster 2 than in Clusters 1 and 3 ($F = 10.866$, $p = 0.002$).

Table 3. Comparison of the areas under curves for z-scores between the three groups (clusters) identified.

Variable	Cluster 1	Cluster 2	Cluster 3
CMJ	7.03 ± 9.31	10.25 ± 12.33	-0.55 ± 12.47
Handgrip	19.73 ± 10.20	-3.57 ± 9.68 [a]	-18.69 ± 4.45 [a]
Fatigue	-17.23 ± 11.34	15.50 ± 8.55 [a]	6.46 ± 18.01 [a]
Vigor	2.09 ± 8.88	2.44 ± 15.61	-2.47 ± 20.93
Perceived recovery	8.32 ± 20.62	5.12 ± 13.55	-14.04 ± 18.75
Muscle soreness	-6.85 ± 8.86	21.03 ± 19.17 [a b]	-16.96 ± 3.47
RMSSD	4.82 ± 12.83	3.47 ± 17.79	-3.75 ± 7.61

Legend: CMJ = counter movement jump, RMSSD = root mean square of successive differences in R-R intervals. Data are expressed as means ± SD. [a] $p < 0.05$ vs. Cluster 1; [b] $p < 0.05$ vs. Cluster 3.

To confirm the differences reported in Table 3, we compared the recovery time course of handgrip force, fatigue, and perceived muscle soreness between the three identified clusters using mixed-model two-way ANOVAs (Figure 7). Concerning the handgrip force, no significant main effect of time ($F = 0.096$, $p = 0.856$, $\eta p^2 = 0.009$) and no significant time x cluster interaction ($F = 2.358$, $p = 0.108$, $\eta p^2 = 0.300$) were observed. However, a significant main effect of the cluster was observed ($F = 7.702$, $p = 0.008$, $\eta p^2 = 0.583$), with the values in Cluster 1 being significantly higher than in Cluster 3.

No significant time x cluster interaction ($F = 2.536$, $p = 0.075$, $\eta p^2 = 0.316$) was observed for fatigue. However, significant main effects of time ($F = 17.774$, $p < 0.001$, $\eta p^2 = 0.618$) and cluster ($F = 4.188$, $p = 0.044$, $\eta p^2 = 0.432$) were reported, with the values in Cluster 1 being significantly lower than in Cluster 2. Regarding perceived muscle soreness, significant main effects of time ($F = 12.307$, $p = 0.001$, $\eta p^2 = 0.528$) and cluster ($F = 6.354$, $p = 0.003$, $\eta p^2 = 0.536$) and a significant time x cluster interaction ($F = 10.564$, $p = 0.003$, $\eta p^2 = 0.658$) were reported. More specifically, muscle soreness in Cluster 2 was higher than in Clusters 1 and 3.

Figure 7. Comparisons of changes in handgrip strength, fatigue, and perceived muscle soreness following exercise-heat stress between the three clusters. Data are expressed as mean ± SD. [a] $p < 0.05$ vs. Cluster 1; [c] $p < 0.05$ vs. Cluster 3.

DISCUSSION

Running 10 km in the heat at a self-selected pace induced the expected changes in physiological and perceptual variables, such as the increases in body temperatures, heart rate, RPE, and scores of thermal perception. After exercise-heat stress, CMJ height was reduced, handgrip strength remained unaltered, and the time to complete the 1-mile test was improved. In addition, mood states, perceived recovery, and perceived muscle soreness were altered post-exercise, as well as autonomic cardiac activity and measurements related to the water-electrolyte balance. Interestingly, these changes were transient, and almost no residual effects on performance, perceptual, or physiological variables were observed 24 h after the treadmill run in the heat; only perceived recovery was still reduced on the next day. These exercise-induced changes in perception/mood states were not correlated with changes in performance. Moreover, cluster analysis indicated that individuals could be grouped into three clusters characterized by different physical performance and perceptual responses following exercise. Altogether, these findings confirmed the three hypotheses of the present study.

The time to complete the 10 km at 35°C was 22.6 ± 9.8% longer than the best performance reported by the participants. This finding should be interpreted cautiously because the conditions (environmental conditions, time of day, date, official race or training, indoor vs. outdoor run) associated with the best performance were not registered. Importantly, the fact that participants ran significantly worse is suggestive that the environmental conditions of current experiments were challenging.

The 10 km run in the heat produced significant physiological and perceptual strain, as evidenced by the increases in the heart rate, auricular temperature, and mean skin temperature, as well as the increases in RPE, thermal comfort, and thermal sensation scores. These findings reproduce previous observations in individuals running under environmental heat stress (Andrade; Nunes-leite; BruzzI; Souza *et al.*, 2023; Andrade; Wanner; Santos; Mendes *et al.*, 2024; Júnior; Mckenna; Amorim; Sena *et al.*, 2020; Rodrigues; Mendes;

Gomes; Silami-Garcia *et al.*, 2023). A significant and apparent increase in mean skin temperature was reported; this finding suggests that high relative humidity (i.e., 64 ± 10% in the last kilometer) has impaired the evaporative heat loss, thereby limiting the cooling effect caused by sweat evaporation on the participants' skin (Bright; Clark; Jay; Périard, 2025; Maughan; Otani; Watson, 2012a).

Exercise-heat stress impaired lower-limb neuromuscular function, as evidenced by a 5.6% reduction in CMJ height 1 hour after the treadmill run, agreeing with a previous report (Rodrigues; Mendes; Gomes; Silami-Garcia *et al.*, 2023). Reduced lower-limb neuromuscular performance following aerobic exercises can be explained by central and peripheral mechanisms. Marked increases in core temperature impairs the neural drive to contracting muscles, resulting in central fatigue (Moraes; Paulinelli-Júnior; Teixeira-Coelho; Cançado *et al.*, 2019; Nybo; Nielsen, 2001). Alternatively, a decline in Ca^{2+} sensitivity, metabolic acidosis, and impaired action potential may occur in contracting muscles during strenuous aerobic exercises, impairing excitation-contraction coupling (Fitts, 2016; Place; Yamada; Bruton; Westerblad, 2010) and reducing CMJ height.

Handgrip force did not change during the 24 h after exercise-heat stress. The test used in the current experiments assesses force production in muscles not exercised during the treadmill run and, therefore, was conducted to determine the level of central fatigue due to running-induced hyperthermia. Previous observations by Nybo and Nielsen (Nybo; Nielsen, 2001) indicated that maximal voluntary contraction measured with a handgrip apparatus was not different after cycling to fatigue at 40°C and 18°C, although force production during isometric contractions lasting 2 min was reduced at 40°C. This finding suggests that analyzing force production during sustained handgrip contractions is possibly a more sensitive test to assess central fatigue and describe the time course of performance recovery after a prolonged run in the heat.

The 1-mile test was conducted to analyze recovery using a test that required participants to perform the same type of activity with similar muscle recruitment to that of a 10 km run. However, the distance covered by the participants was reduced to avoid repeating 10 km runs in two consecutive days. This test was not sensible in detecting some level of residual fatigue because the participants completed the 1 mile faster at 24 h after the run compared to baseline. Although the improved post-exercise performance suggests that 24 hours was enough time for the individuals to recover fully, it is more likely that a learning effect mediated their faster running speed. Indeed, our participants had large weekly training volumes and were used to participating in races farther than 1,600 m. The anaerobic system contributes approximately 16-20% of the total energy supply during 1,500 m races (Hill, 1999; Spencer; Gastin, 2001). Longer distances are associated with greater aerobic contribution. For example, (Duffield; Dawson; Goodman, 2005) reported that the aerobic system contributed 86%-94% of the energy supply during a 3,000 m race. Therefore, the contribution of the energetic systems greatly varied between the 1-mile and 10-kilometer races. Further studies should test alternative measures to assess recovery using specific activities, such as measuring VO_2 and then determining running economy.

Perception was significantly altered following exercise-heat stress. Fatigue, vigor, perceived recovery, and perceived muscle soreness were all modified immediately and 1 h after the run, but returned to pre-exercise levels at 24 h after. The only exception was perceived recovery, which was still reduced 24 h after the exercise. The recovery time course of perceptual variables greatly depends on the sport analyzed. For example, after futsal high-intensity training sessions, fatigue and perceived recovery returned to baseline levels at 24 h post-training, whereas vigor only increased toward baseline levels at 48 h post-training (Wilke; Wanner; Penna; Maia-Lima *et al.*, 2021). In football, a sport that markedly augments markers of muscle damage in circulation, post-matches changes in perceived muscle soreness are longer lasting: male players indicated increased levels of perceived muscle soreness 72 h after matches (Silva; Rumpf; Hertzog; Castagna *et al.*, 2018), whereas female players showed increased perception 24 h but not 72 h after matches (Goulart; Coimbra; Campos; Drummond *et al.*, 2022).

RMSSD and SDNN, indices of parasympathetic cardiac activation, were reduced immediately after the treadmill run, indicating a predominant autonomic cardiac regulation via sympathetic outflow during and after exercise cessation. This finding corroborates previous investigations demonstrating suppressed parasympathetic activity during vigorous and prolonged physical exertions (Brenner; Thomas; Shephard, 1998; Kenny; Mcginn, 2017). Indeed, as reviewed by Junglee et al. (White; Raven, 2014), autonomic cardiac control is characterized by predominant sympathetic activity in exercise intensities leading to heart rate values higher than 140 bpm, a level of tachycardia that was surpassed in the current study. The changes in cardiac autonomic activity in our participants were transient, and 1 h was enough time for parasympathetic reactivation following exercise.

The values of urine markers of dehydration increased after running at 35°C, confirming previous reports in which the participants exercised under environmental heat stress (Junglee; Di Felice; Dolci; Fortes *et al.*, 2013; Périard; Eijsvogels; DAANEN, 2021). These changes agree with the 1.9% reduction in body mass and the ingestion of a low amount of fluid during the 10 km run. Therefore, the participants only replaced approximately 27% of their significant water losses through sweating. The participants finished the exercise with augmented values of urine specific gravity and urine color compared to pre-exercise, but these values are not high enough to classify them as dehydrated (Armstrong; Maresh; Castellani; Bergeron *et al.*, 1994).

Almost all variables investigated returned to pre-exercise levels 24 h after the 10 km at 35°C. This rapid recovery was observed despite the marked physiological and perceptual strain imposed by the 10 km run in the heat, indicating that the participants could effectively restore their functional capacities within a short time, being ready for a subsequent training stimulus. This rapid recovery timeline is coherent with the high training volume of long-distance runners (Luden; Hayes; Galpin; Minchev *et al.*, 2010). Further studies should investigate whether these athletes will still recover within 24 h when training in the heat is conducted across multiple days of a microcycle.

Next, we tested whether the changes induced by exercise-heat stress in physical performance and perceptual variables were associated. No significant correlations were noticed between the reduced CMJ height and the altered perceptual variables (i.e., fatigue, vigor, perceived recovery, or muscle soreness) 1 h after the run. These findings suggest that altered mood states or increased levels of perceived fatigue or muscle soreness do not explain the reduced participants' jumping ability; noteworthy, it is always important to indicate that correlations do not test causal relationships. Moreover, the lack of associations supports the notion that post-exercise changes in physical performance and perception are governed by different mechanisms, as evidenced by several reports showing different timelines of performance and perception recovery (Skorski; Mujika; Bosquet; Meeusen *et al.*, 2019) and by a study that identified a cluster of team sport players with slow perceptual recovery despite a "normal" performance recovery (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a).

Finally, cluster analyses indicated the existence of three subgroups within our study sample. Cluster 1 had no reductions in handgrip strength and attenuated increases in fatigue following exercise-heat stress compared to the other two clusters. In contrast, the athletes in cluster 2 reported augmented muscle soreness. These findings suggest that individuals in clusters 1 and 2 would be ready for a new training stimulus, respectively, earlier and later than the others. Cluster analysis has been used to classify individuals in training (Bautista; Chirosa; Robinson; Van Der Tillaar *et al.*, 2016; Gagge; Stolwijk; Hardy, 1967; Xie; Gou; Bai; Yang *et al.*, 2023) and medical research (Mclachlan, 1992). Although classifying individuals into different recovery profiles is a promising strategy for better individualization of training and recovery prescription, we recommend caution in interpreting our findings due to the limited number of athletes in the three clusters and because literature has not investigated the reliability of the current analytic procedures to determine different recovery profiles.

The current study provides practical information to help manage post-exercise recovery in long-distance runners. In addition, heat acclimation training has been recently advocated as a practical strategy with the potential to improve performance in endurance athletes, including cyclists preparing for prolonged stage races (Nybo; Rønnestad; Lundby, 2024). Despite these strengths, our study also has limitations. First, recovery timelines were only followed up to 24 h after the exercise; although most variables returned to baseline within this time interval, more time was needed for the participants' perceived recovery to attain pre-exercise levels. Second, the tests used to assess performance were all practical to be applied in training settings, but only the CMJ was sensible to detect changes in performance due to fatigue associated with exercise-heat stress. Third, the participants were classified into three clusters based on their recovery time courses; however, the sample size in each cluster was small (4-5 individuals), limiting us from characterizing the different recovery profiles better.

In conclusion, recreational athletes showed significant physiological and perceptual strain while running 10 km at a self-paced intensity in the heat. After the exercise-heat stress, changes were noted in performance, physiological, and perceptual variables; almost all changes were reversed the following day, indicating that long-distance runners recovered fast from their training sessions. Moreover, the recovery timeline of performance and perceptual variables were not associated, and the participants were classified into three clusters with different recovery profiles. These data are paramount for optimizing training and recovery prescription in long-distance runners.

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Figure 1

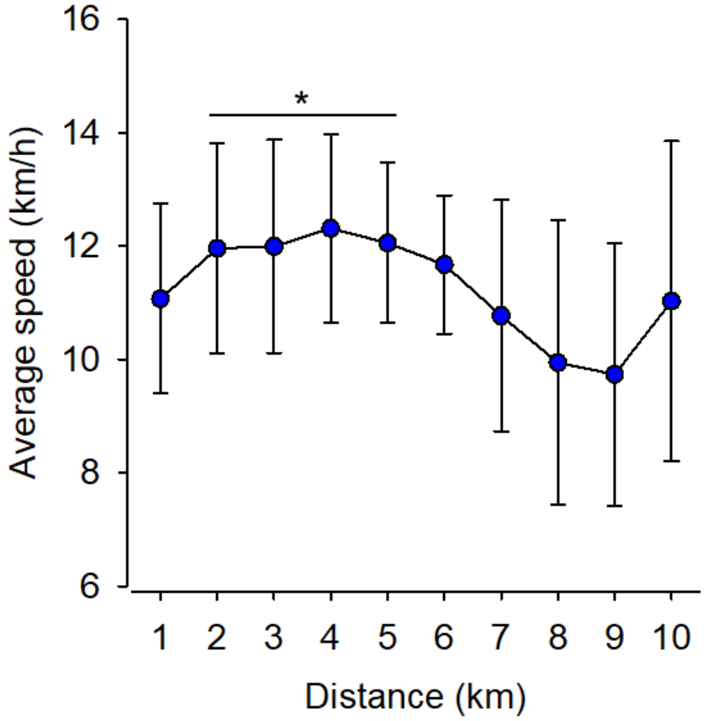


Figure 2

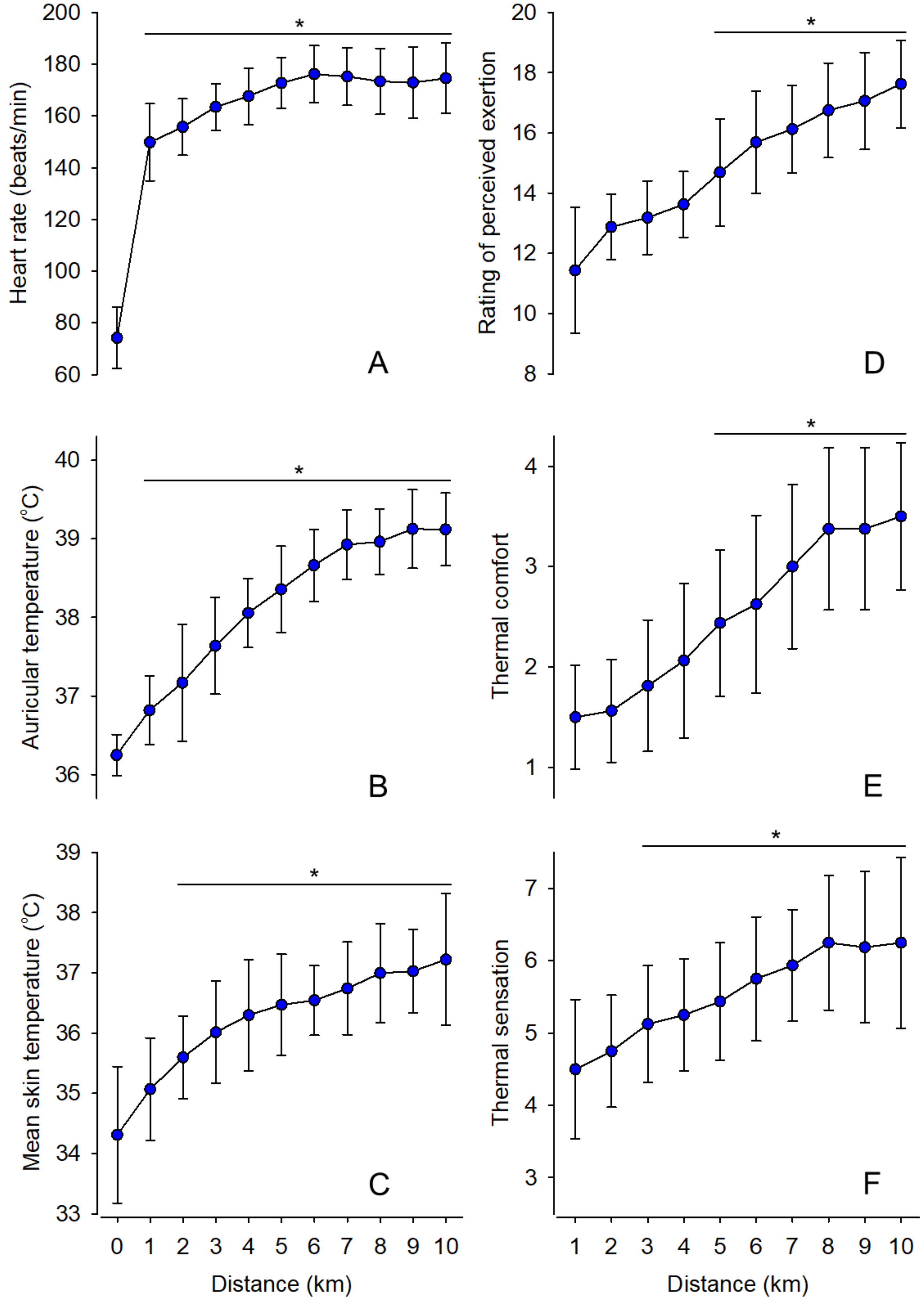

Heart rate (beats/min)
A
Rating of perceived exertion
D
Auricular temperature (°C)
B
Thermal comfort
E
Mean skin temperature (°C)
C
Thermal sensation
F
Distance (km)
Distance (km)
*

Figure 3

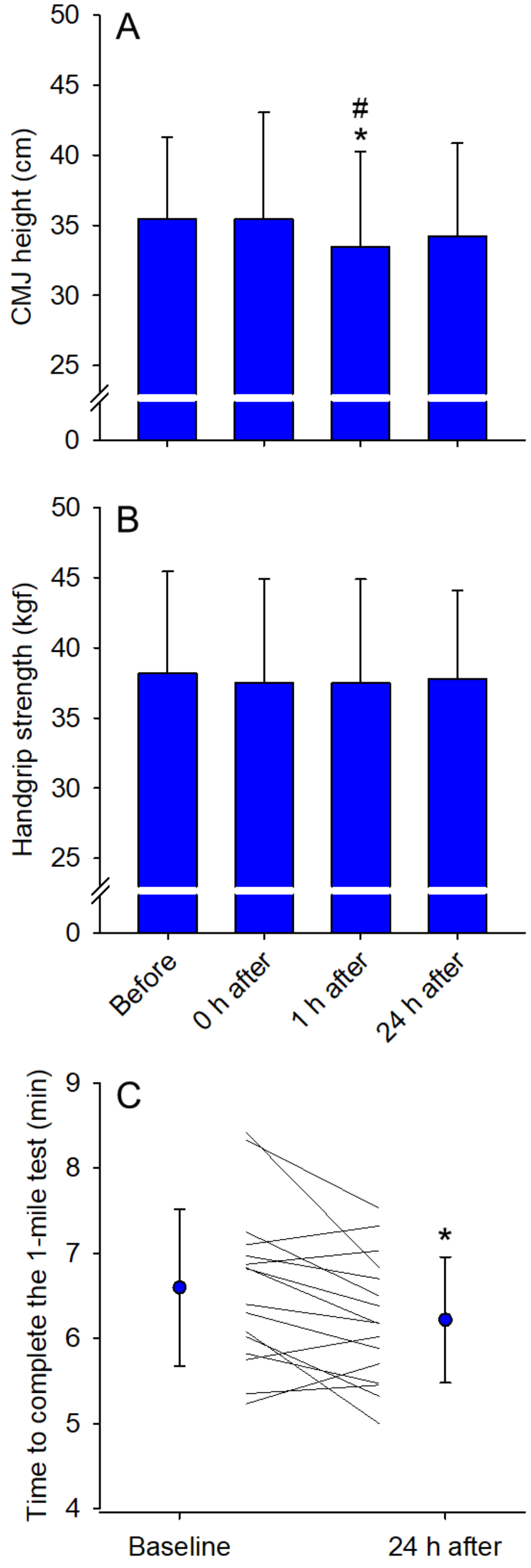

A
CMJ height (cm)
50
45
40
35
30
25
0
#
*
B
Handgrip strength (kgf)
50
45
40
35
30
25
0
Before
0 h after
1 h after
24 h after
C
Time to complete the 1-mile test (min)
9
8
7
6
5
4
*
Baseline
24 h after

Figure 4

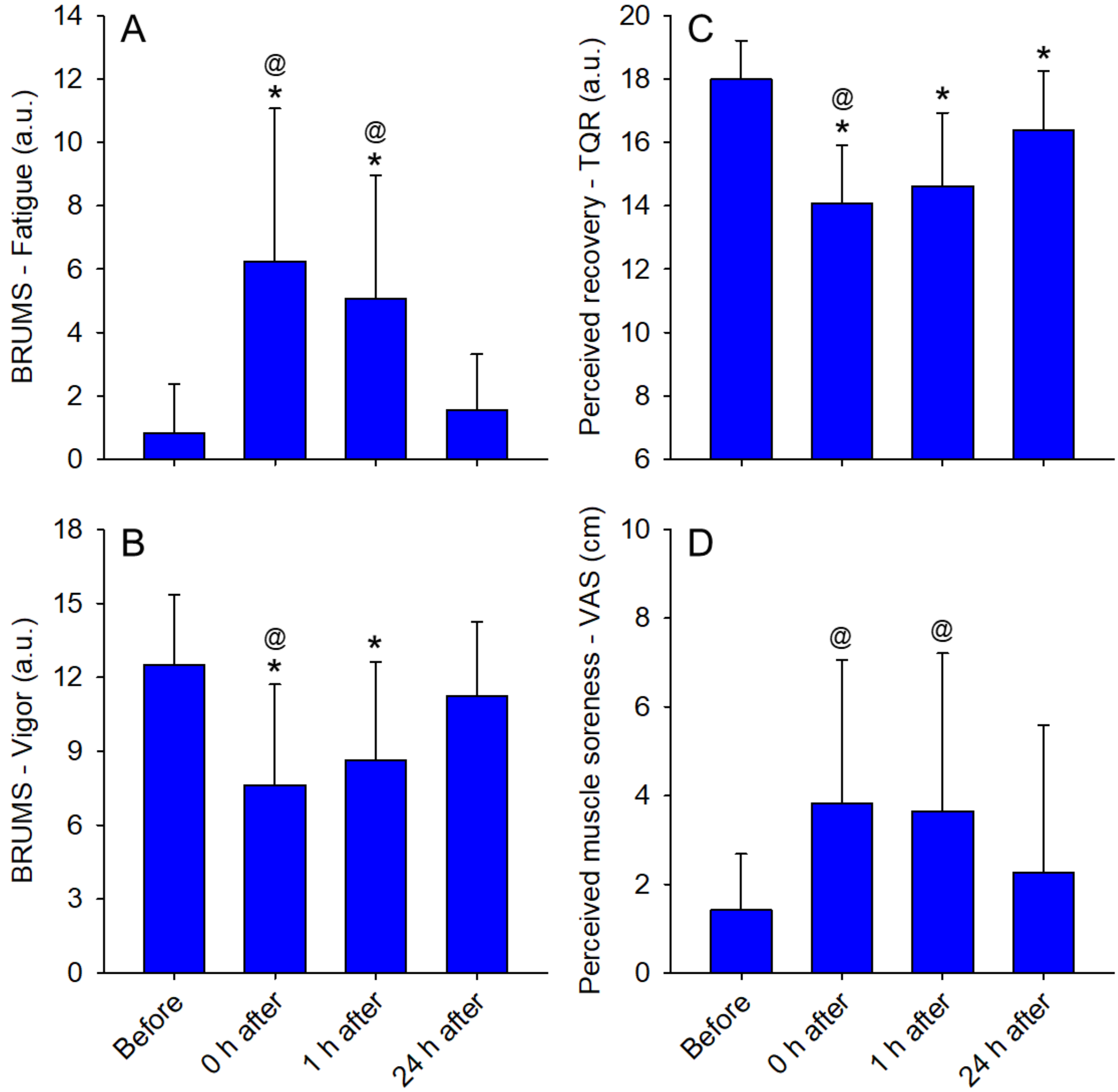

A
BRUMS - Fatigue (a.u.)
14
12
10
8
6
4
2
0
@
*
B
BRUMS - Vigor (a.u.)
18
15
12
9
6
3
0
C
Perceived recovery - TQR (a.u.)
20
18
16
14
12
10
8
6
D
Perceived muscle soreness - VAS (cm)
10
8
6
4
2
0
Before
0 h after
1 h after
24 h after

Figure 5

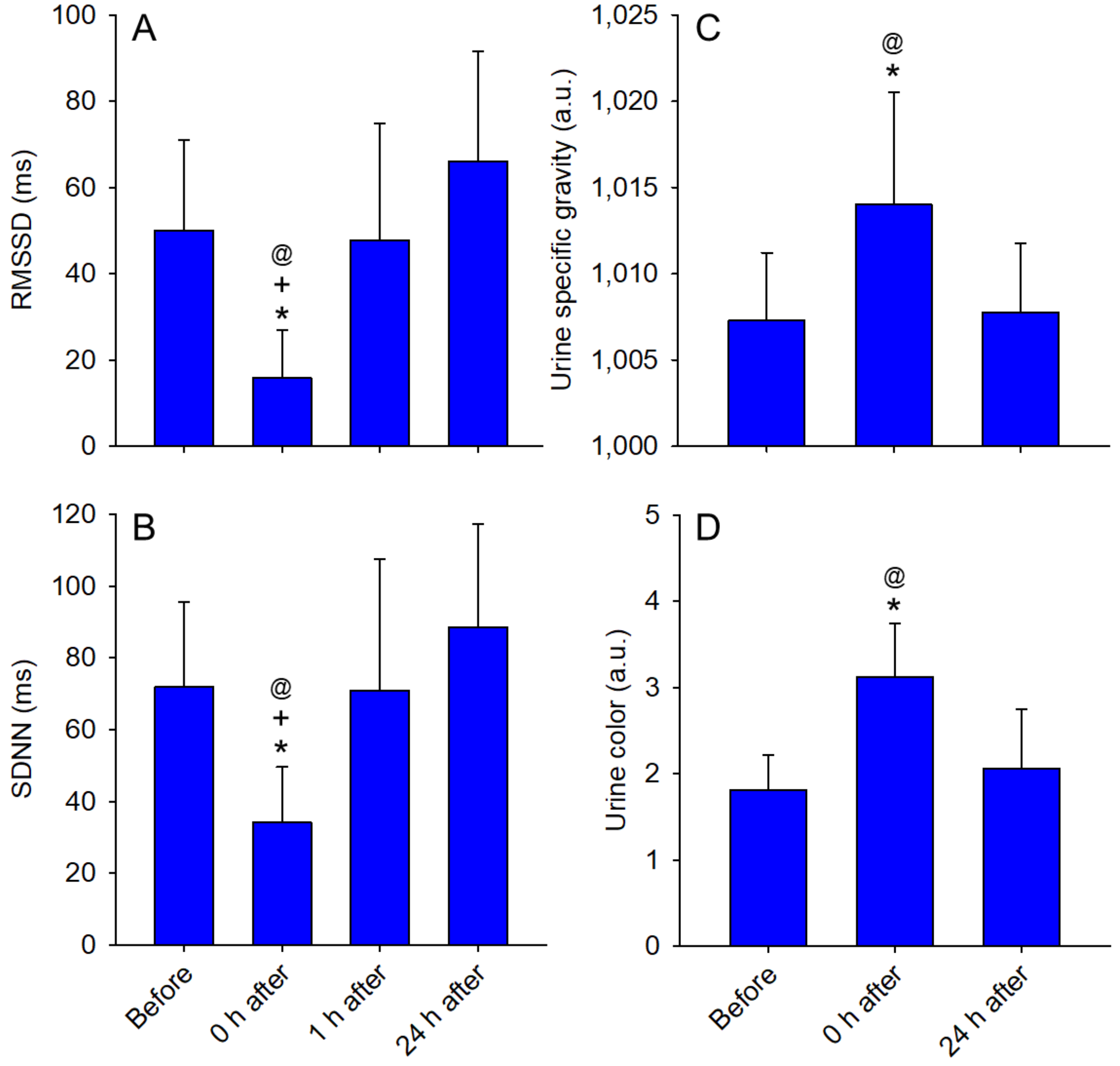
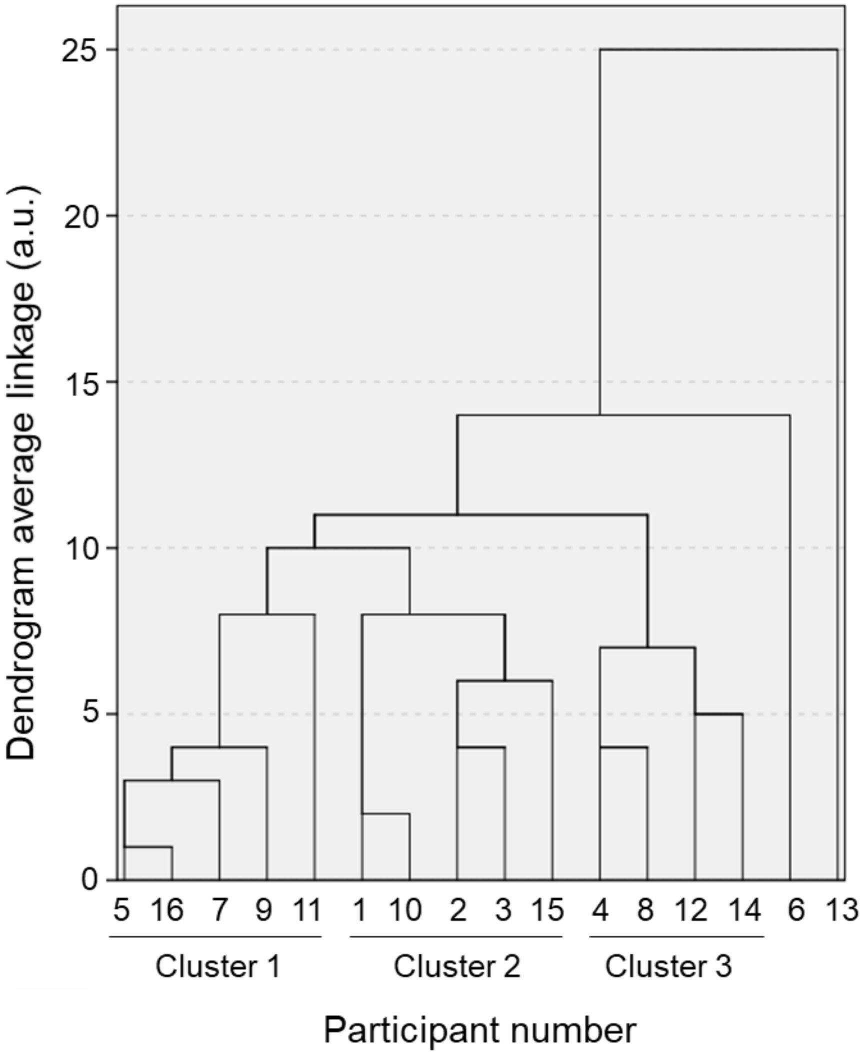
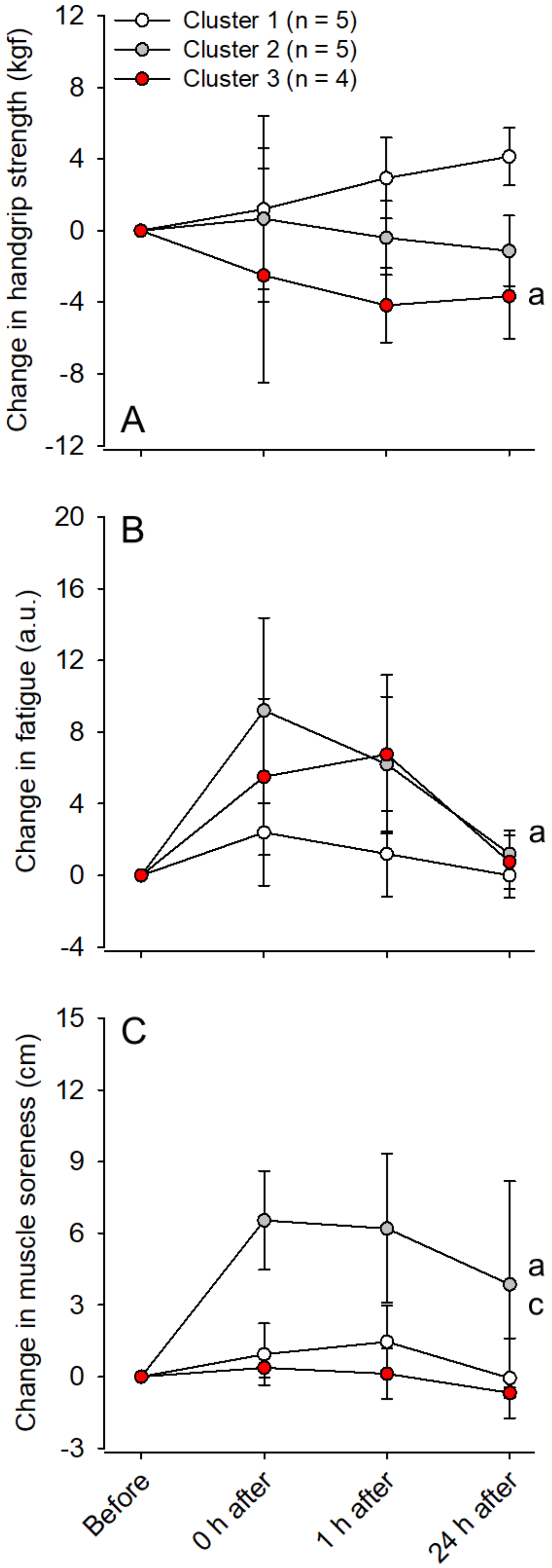

A
RMSSD (ms)
0
20
40
60
80
100
@
+
*
C
Urine specific gravity (a.u.)
1,000
1,005
1,010
1,015
1,020
1,025
@
*
B
SDNN (ms)
0
20
40
60
80
100
120
@
+
*
D
Urine color (a.u.)
0
1
2
3
4
5
@
*
Before
0 h after
1 h after
24 h after
Before
0 h after
24 h after

Figure 6



25
20
15
10
5
0
Dendrogram average linkage (a.u.)
5 16 7 9 11 1 10 2 3 15 4 8 12 14 6 13
Cluster 1
Cluster 2
Cluster 3
Participant number

Figure 7


Cluster 1 (n = 5)
Cluster 2 (n = 5)
Cluster 3 (n = 4)
Change in handgrip strength (kgf)
A
a
Change in fatigue (a.u.)
B
a
Change in muscle soreness (cm)
C
a
c
Before
0 h after
1 h after
24 h after

5 ESTUDO 3

Short-term heat acclimation has minimal impact on post-exercise recovery following self-paced 10 km run in the heat

Abstract

Heat acclimation (HA) improves physical performance and mitigates physiological and perceptual strain during exercise in hot conditions. However, whether HA modifies post-exercise recovery is unknown. This study investigated the effects of a short-term HA protocol on the recovery of physical performance, physiological, and perceptual variables after a self-paced 10 km treadmill run in the heat. Sixteen recreational runners (two women) were randomly assigned to the HA or control (CON) group. The HA group was subjected to a controlled hyperthermia HA protocol that consisted of five exercise sessions at an ambient temperature of 40.1 ± 0.4°C and relative humidity of 46.8 ± 7.4%. The CON group completed similar procedures but at 24.5°C ± 0.62 e 48.8 ± 9.8%. Before and after the interventions, the athletes performed self-paced 10 km runs at 35°C. Recovery was assessed using physical performance measures (countermovement jump height, handgrip strength, and 1-mile time), physiological markers [heart rate variability and urine specific gravity (USG)], and perceptual variables (fatigue, vigor, and muscle soreness) at multiple points before and after the exercise. As expected, the short-term HA protocol improved running performance, as evidenced by a shorter time to complete the 10 km. In contrast, HA had minimal effects on recovery time course of most variables analyzed. The exceptions were the impaired cardiac parasympathetic reactivation during the first hour after run and small effects on USG values and perceived fatigue in the HA group. In conclusion, despite increasing running performance in the heat, HA has minimal effects on the recovery time of physiological, perceptual, and physical performance variables following a prolonged exercise in hot conditions.

Keywords: acclimation, athletic performance, autonomic nervous system, dehydration, fatigue, hot temperature, post-exercise recovery, thermoregulation

INTRODUCTION

Climate change has intensified over recent decades, directly affecting human health and physical performance (González-Gaudiano; Meira-Cartea, 2019). The ongoing climate change is characterized, among other undesirable effects, by global warming and more frequent, severe, and prolonged heat waves (Bell; Gasparrini; Benjamin, 2024; Bitencourt; Fuentes; Maia; Amorim, 2016), forcing athletes to train and compete often under adverse environmental conditions. Athletes engaged in outdoor sports face increased risks (Abu-omar; Gelius; Messing, 2020; Cunningham; MCCULLOUGH; HOHENSEE, 2020; EVANS, 2019), including those engaged in long-distance running, which is one of the most popular sports in urban environments due to its low cost, easy accessibility, and various health and quality-of-life benefits (Doğusan; Koçak, 2024; Shipway, 2010).

The combination of heat exposure and prolonged physical exercise triggers a range of adverse responses, especially in non-heat acclimated individuals, who experience augmented cardiovascular strain, body temperatures, and ratings of perceived exertion, alongside accelerated fatigue and a higher risk of heat illnesses (Bharadwaj; Chaudhary; Gupta; Kumar, 2024). In this sense, understanding the effectiveness and utility of mitigation strategies is essential to maximize performance and secure a safe practice during long-distance runs under environmental heat stress.

Heat acclimation (HA) is accepted as the most effective heat mitigation strategy (Gibson; James; Mee; Willmott *et al.*, 2020). Heat-acclimated individuals demonstrate beneficial physiological and perceptual adaptations during exercise-heat stress, such as improved thermotolerance, increased sweat rate, and lower body core temperature, heart rate, perceived exertion, and thermal sensation scores (Chalmers; Esterman; Eston; Bowering *et al.*, 2014b; Racinais; Alonso; Coutts; Flouris *et al.*, 2015). These adaptations allow heat-acclimated athletes to perform better in hot conditions, as evidenced by faster running speed or higher power output performance during self-paced exercises (Lorenzo; Halliwill; Sawka; Minson, 2010) and greater tolerance to fatigue during fixed-intensity exercises (Astokorki; Mauger, 2017).

In most sports, athletes follow training schedules with daily sessions and often face congested competition schedules that challenge their ability to recover and, therefore, to perform (Wanner; Wilke; Duffield, 2016). Recovery is a multifactorial phenomenon involving physiological and psychological aspects to restore homeostasis, repair muscle tissue, and rebalance the autonomic nervous system (Kallus; Kellmann, 2016; Tomlin, Dona L; Wenger, Howard A, 2001). Post-exercise recovery is improved by training, as evidenced by a lower perception of fatigue, an augmented vigor, and an attenuated decrement in countermovement (CMJ) jump height after 10 weeks of specific sports training (Wilke; Wanner; Penna; Maia-Lima *et al.*, 2021). However, no study has yet investigated how HA protocols influence recovery dynamics. This knowledge is essential to optimize training and recovery prescription, mainly because of the ongoing climate change and the increasing popularity of HA training to improve performance and induce beneficial adaptations. As stated earlier, HA allows athletes to exercise at faster speeds or higher power outputs, generating higher external training and competitive loads that may slow recovery.

This study investigated the effects of short-term HA on the recovery timeline of performance, physiological, and perceptual variables after a self-paced 10 km treadmill run in the heat. We hypothesized that despite running the 10 km at a faster pace, heat-acclimated individuals would have improved recovery compared to non-acclimated control individuals.

METHODS

Ethical care

The current study received approval from the Research Ethics Committee of the Universidade Federal de Minas Gerais (protocol number: CAAE 67163323.7.0000.5149) and followed the principles outlined in the 1964 Declaration of Helsinki and its subsequent amendments, ensuring that all experimental procedures were conducted with the highest ethical standards. All participants were informed about the study's objectives, procedures, and any potential risks and benefits associated with participation. Subsequently, they signed an informed consent form confirming their voluntary participation in the study.

Participants

The participants were 16 long-distance recreational runners (2 women), tropical natives living in São Luís, a city located in the northeast region of Brazil (latitude: 2° 31′ 51″ South, longitude: 44° 18′ 24″ West). The monthly average ambient temperature (T_A) and relative humidity (RH) values in São Luís correspond to 26.6 ± 2.2°C and 82.3 ± 11.5%, respectively (INMET, 2025; data calculated based on the period from 2020 to 2024).

Participants met the following inclusion criteria: 1) they responded “no” to all questions of the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas; Reading; Shephard, 1992); 2) trained regularly for at least two years; 3) maintained a weekly training frequency of at least five days; 4) were free of lower limb injuries within the six months before the experiments; and 5) completed a 10 km run outdoor in less than 50 min (men) or 55 min (women) within the six months prior to the experiments. This performance could have been achieved during training sessions or competitions. The female participants were using monophasic oral contraceptives and were tested during the active pill phase, specifically from the 2nd to the 21st day of use (Andrade; Nunes-Leite; Bruzzi; Souza *et al.*, 2023; SIMS; Heather, 2018). Moreover, the participants abstained from using medications and nutritional supplements during the experiments.

Experimental protocol

The present study was conducted at the Cardiovascular Adaptations to Exercise Laboratory (LACORE) at the Universidade Federal do Maranhão (UFMA), under controlled environmental conditions and with continuous monitoring of variables of interest.

The participants were randomly assigned to one of the two groups: heat acclimation (HA; n = 8) or control (CON; n = 8). The experimental protocol was divided into three phases (*i.e.*, pre-intervention, acclimation or control intervention, and post-intervention), totaling ten laboratory visits for each participant.

PRE INTERVENTION

The pre-intervention phase consisted of three visits. During the first visit, participants signed the informed consent form, and their body mass and height were measured using a digital scale coupled to a stadiometer (Welmy, model W200), with resolutions of 50 g and 0.5 cm, respectively. These measurements were used to calculate body mass index and surface area according to the equations proposed by (KEYS; FIDANZA; KARVONEN; KIMURA *et al.*, 2014) and (DUBOIS, 1916), respectively. Body fat percentage was estimated using a bioimpedance device (OMRON, model HBF– 514C). The participants were then familiarized with using the perceptual scales and the handgrip and with performing countermovement jumps (CMJ). Next, participants performed a 1-mile (1.6-kilometer) treadmill run to estimate their maximal oxygen consumption (VO_{2MAX}) under temperate conditions: T_A = 24.4 ± 0.4°C and RH = 55 ± 4%. Upon finishing this test, they completed an additional 8.4 km at 70% of the average speed of their best self-reported 10 km performance (*i.e.*, 9.7 ± 1.3 $km.h^{-1}$); this extra distance was covered at the same T_A as the 1-mile test and aimed to familiarize the athletes with running for a prolonged period on a treadmill.

The second visit took place 24 h after the first. Participants completed 10 km at a self-paced intensity under hot and humid environmental conditions (T_A = 35.4 ± 0.4°C, RH = 60 ± 8%). Physiological (*i.e.*, heart rate, aural canal temperature, and skin temperature) and perceptual variables (RPE, thermal comfort, and thermal sensation) were measured at every km. To assess post-exercise recovery, additional variables were collected before, immediately after, 1 h after, and 24 h after the treadmill run. These included CMJ height, handgrip force, heart rate variability, urine specific gravity, urine color, mood status, perceived recovery, and muscle soreness. The measurements taken 24 h after the treadmill run corresponded to the participants' third visit to the laboratory. During this last visit, they also completed a 1-mile test. These initial experiments have been described elsewhere.

INTERVENTION PHASE

The intervention phase took place between the fourth and eighth visits. Participants in the HA group completed five consecutive daily sessions of controlled hyperthermia, following a protocol adapted from de Castro and collaborators (De Castro Magalhães; Amorim; Passos; Fonseca *et al.*, 2010). Each session consisted of 45 minutes of treadmill running at 8 km/h, followed by 15 minutes of walking at 6 km/h, in a controlled environment with T_A = 40.1 ± 0.4°C and RH = 46.8 ± 7.3%. Participants in the CON group performed the same five sessions under temperate conditions (T_A = 24.4 ± 0.6°C and RH = 48.8 ± 9.8%).

Auditory canal temperature was monitored during the exercise using an infrared thermometer (model AOJ-20A, AOJ Medical, China) with a display resolution of 0.1°C. In the HA group, the acclimation sessions were designed to increase auditory canal temperature by 1.5°C above baseline during the initial 45 minutes of running and maintain this hyperthermia level during the final 15 minutes of walking. The HA protocol was conducted over five days at T_A = 40.1 ± 0.4°C, which was approximately 5°C above the temperature recorded during the time trial (T_A = 35.4 ± 0.1°C), as recommended in the literature (Guy; Deakin; Edwards; Miller *et al.*, 2015b; Périard; Travers; Racinais; Sawka, 2016).

POST INTERVENTION

The post-intervention phase included the ninth and tenth visits, during which the 10 km self-paced run and all recovery assessments performed during the second and third visits were repeated in full. This allowed for direct pre- and post-intervention comparisons of physical, physiological, and perceptual recovery markers.

Procedures

1-mile test

This test was performed to estimate the participants' VO_{2MAX} (on the first visit) and analyze their recovery (on the third and tenth visits). The athletes were instructed to run one mile as fast as possible on a treadmill (Matrix model T1X, Johnson Health Tech) and had no feedback regarding their performance while exercising; they could only visualize the distance covered. Following the recommendations by the American College of Sports Medicine for maximal testing (Mahler; Froelicher; Miller; York, 1995), the 1-mile (1,600-kilometer) test was interrupted in the event of equipment failure, at a participant's request or if a participant showed an unusual lack of breathing or signs of malaise, such as pale appearance of the skin or cyanosis (signs of poor perfusion). None of these conditions were observed in the current study.

VO_{2MAX} was estimated using the following equation, specifically developed for physically active young Brazilians (almeida; Campbell; Pardono; Sotero *et al.*, 2010) $VO_{2MAX} = 8.101 + 0.177 \times S_{AVG}$, where S_{AVG} is the average speed in m.min^{-1} to complete one mile.

Familiarization with the handgrip test and CMJs

Initially, the participants ran 10 min at 70% of the mean speed obtained during their best 10 km performance to increase lower limb muscle temperature, which is known to enhance performance in high-intensity, short-duration exercises (CARMO; GOULART; CABIDO; MARTINS *et al.*, 2023).

After the standardized warm-up, the athletes were familiarized with the CMJs by completing 3 sets of 3 repetitions, with a 30-s rest between them, as previously described (CLAUDINO; MEZÊNCIO; SONCIN; FERREIRA *et al.*, 2012). The participants started the jump standing, with their feet positioned parallel on a contact mat (CEFISE, BRAZIL), measuring 1000 x 600 x 8 mm, connected to the Jump System® software (Cefise Ltda, São Paulo, Brazil, accuracy of 0.1 cm) and both hands on the waist to avoid the influence of the upper limbs on the jump height. At the experimenter's signal, the individuals performed a continuous movement that consisted of quickly flexing the knees to 90° (eccentric action) and then jumping (concentric action) as high as possible (CLAUDINO; MEZÊNCIO; SONCIN; FERREIRA *et al.*, 2012).

The participants were then familiarized with the procedures to assess right handgrip strength using a hand dynamometer (model SH5001, Saehan Corporation, South Korea). The handgrip test was conducted according to the recommendations of the American Society of Hand Therapists, with the volunteer sitting on a chair with the shoulder adducted, the elbow flexed at 90°, the forearm in a neutral position, and the wrist position varying from 0 to 30° of extension (BELLACE; HEALY; BESSER; BYRON *et al.*, 2000). The participants repeated the test until three consecutive consistent measurements of handgrip strength were recorded.

Assessment of recovery timeline

Physical performance, physiological, and perceptual variables were measured at four different time points to assess how participants recovered following exercise-heat stress. These time points were as follows: pre-exercise, immediately after, 1 h after, and 24 h after the 10 km run. The exceptions were the urine specific gravity and color that were not measured immediately after and the 1-mile test that was only performed 24 h after.

Measurements before the 10 km run

The experimental procedures were always carried out between 2:30 and 6:00 p.m. The 10 km runs were initiated with less than 1 h difference between participants to control for circadian rhythm.

Upon arrival at the laboratory, participants remained at rest for 20 minutes in a room with controlled T_A at ~25 °C to allow for physiological baseline measurements. Hydration status was assessed using a portable optical refractometer (model RTP-20 ATC, Instrutherm®), previously calibrated with deionized water. A urine specific gravity equal to or less than 1.029 indicates an euhydrated state (ARMSTRONG; MARESH; CASTELLANI; BERGERON *et al.*, 1994). If a participant was found dehydrated, he/she was provided with 500 mL of water, and his/her urine specific gravity was tested again 30 to 60 min later.

Resting heart rate was measured for 10 min while the participants were lying to determine their heart rate variability (HRV). After that, they answered three scales to assess the mood state, perceived recovery, and perceived muscle soreness. Mood state was assessed through the Brunel Mood Scale (BRUMS), translated and validated in Portuguese (Rohlfs; Rotta; Luft; Andrade *et al.*, 2008). The BRUMS has six sub-scales (i.e., anger, confusion, depression, fatigue, tension, and vigor), each composed of four items. Each item is preceded by the question “How do you feel right now?” and is answered on a 5-point scale (from 0 to 4). We only assessed the fatigue and vigor sub-scales, usually affected by a single exercise session (Wilke; Fernandes; Martins; Lacerda *et al.*, 2019a).

Muscle soreness was determined using a visual analog scale (VAS) (Miller; Duncan; Brown; Sparks *et al.*, 2003). A 10-centimeter line was drawn; 0 corresponded to no muscle soreness, while 10 corresponded to the maximum muscle soreness imaginable. The experimenter palpated the thigh and calf regions, and then the athlete marked a point in the straight line, indicating their current perceived muscle soreness. Perceived muscle soreness was determined separately for the tight and calf, and these scores were summated to indicate lower limb muscle soreness. Perceived recovery was determined using the 15-point total quality of recovery (TQR) scale (Kenttä; Hassmén, 1998), ranging from 6 (worse than very, very poor recovery) to 20 (better than very, very good recovery).

The athletes performed a treadmill warm-up for 10 minutes at a speed corresponding to 70% of the average velocity obtained in their best 10-km race time. Subsequently, they completed five countermovement jumps (CMJs) and three repetitions of the handgrip force test. For the CMJs, the highest and lowest values were excluded, and the arithmetic mean of the remaining three measurements was used for analysis. Handgrip strength was determined by the arithmetic mean of the three repetitions. After these procedures, the athletes were weighed and directed to a heated room, where the 10-km run was performed.

10 km run

The exercise took place in a small room (3.0 m in length x 2.5 m in width x 2.2 m in height), and the ambient temperature was increased with the help of three electrical heaters (Britânia, model AB1100N). The athletes were instructed to run the 10 km as fast as possible and again had no feedback about their performance. They could drink refrigerated water *ad libitum*.

The following variables were measured during the 10 km runs to ensure that heat acclimation occurred following the five sessions of exercise heat-stress: the time to complete the 10 km run, average running speed, the rate of increase in ear canal temperature, and sweating rate.

Measurements after the 10 km

Body mass was measured immediately after the exercise. Thereafter, measurements were made as previously described and in the following order: HRV, CMJ height, handgrip force, BRUMS, TQR, and muscle soreness. The athletes could not provide a urine sample at this time point due to dehydration caused by exercise-heat stress.

One hour after the exercise, procedures for determining HRV were carried out, and then the participants were subjected to another warm-up session. Additional measurements were made in the following order: CMJs, handgrip force, BRUMS, TQR, and muscle soreness. Similar procedures were made 24 h after exercise, except a 1-mile test that was conducted as the final assessment before the participants were cleared to leave the laboratory.

Calculated variables

Heart rate variability (HRV), an indirect measure of autonomic cardiac activity, was obtained by recording R-R intervals of the resting heart rate. Data were collected using an H-10 chest strap and transmitted to a smartphone equipped with the Kubios HRV software (Elite HRV, Asheville, NC, USA). The following time-domain HRV variables were analyzed: mean R-R interval (named R-R interval for simplicity), the root mean square of successive differences between R-R intervals (RMSSD), and the standard deviation of normal R-R intervals (SDNN). RMSSD and SDNN indicate the parasympathetic cardiac activity in short-term recordings (Shaffer; Ginsberg, 2017).

The volume of water ingested was calculated by determining the difference between the amount of water available before and after the exercise. Whole-body sweat loss was calculated as the difference between the pre-exercise (BM_{PRE}) and post-exercise body mass (BM_{POST}), corrected by the volume of water ingested, according to the following equation: sweat loss = $BM_{PRE} - BM_{POST}$ + water ingested. This equation generated results in mg that were converted to mL. Of note, no participant asked to urinate between the two body mass measurements. Sweat rate ($L \cdot h^{-1}$) was calculated by dividing whole-body sweat loss by the time to complete the 10 km. The percentage change in body mass (%ΔBM) was determined using the following equation: $\%\Delta BM = [(BM_{PRE} - BM_{POST}) / BM_{PRE}] \times 100$.

Statistical analysis

Data normality was verified using the Shapiro-Wilk test. Data are presented as means ± standard deviations unless otherwise stated. Statistical analysis was performed using SigmaPlot 11.0. Statistical significance was set at $\alpha = 0.05$ for all analyses.

In the pre-intervention phase, the anthropometric characteristics of the participants, the variables measured during the 10 km run, and performance in the 1-mile test were compared between groups using unpaired Student's t-tests. Mixed-model two-way ANOVAs were used to compare the following variables between groups and recovery time points: CMJ height, handgrip strength, mean RR, RMSSD, SDNN, urine specific gravity, urine color, fatigue, vigor, and perceived soreness. Tukey's post hoc tests were used whenever applicable.

Next, two analyses were conducted to understand whether heat acclimation influences post-exercise recovery. First, we subtracted the values obtained in the second 10 km run from those obtained in the first run for each participant, averaged the calculated data for each group, and then compared the values between groups. The changes in variables measured during the 10 km run and the changes in performance in the 1-mile test were compared between groups using unpaired Student's t-tests. Mixed-model two-way ANOVAs were used to compare the changes in recovery variables between groups and recovery time points. Again, Tukey's post hoc tests were used whenever applicable.

In the other analysis, we compared data between the first and second 10 km run exclusively in the HA group. Repeated-measures two-way ANOVAs were used to compare the recovery variables between experimental phases (*i.e.*, before and after HA) and time points. Then, we subtracted the values recorded 1 h after from those recorded immediately after and compared the resulting values between experimental phases using paired Student's t-tests to assess short-term post-exercise recovery. Moreover, we subtracted the values recorded 24 h after from those recorded immediately after and compared the resulting values between experimental phases using paired Student's t-tests to assess long-term post-exercise recovery.

3. RESULTS

3.1. Participants

Table 1 presents the participants' characteristics at baseline. Each experimental group included one female participant and seven male participants. No significant intergroup differences were observed for any of the nine variables analyzed.

Table 1. Characteristics of the participants.

Variable	CON (n = 8)	HA (n = 8)	*P-value*
Age (years)	35.4 ± 11.1	31.3 ± 9.7	0.456
Body mass (kg)	65.9 ± 5.6	69.6 ± 10.5	0.401
Height (cm)	169.1 ± 8.1	169.9 ± 9.5	0.867
BMI (kg/m²)	23.1 ± 2.5	24.0 ± 2.2	0.469
Body surface area (m²)	1.8 ± 0.1	1.8 ± 0.2	0.532
Body fat (%)	18.9 ± 7.8	23.5 ± 6.1	0.209
Training experience (years)	4.0 ± 1.5	4.8 ± 2.9	0.523
VO_{2MAX} ($mL \cdot kg^{-1} \cdot min^{-1}$)	47.8 ± 9.6	45.4 ± 10.3	0.636

Legend: BMI = body mass index; CON = control; HA = heat acclimated; VO_{2MAX} = maximum rate of oxygen uptake. Data are expressed as means ± SD. Unpaired Student's t-tests were used to generate the *P*-values reported in the table.

3.2. Physical performance and post-exercise recovery after the first 10 km run in the heat

We first compared the physical performance and key physiological and perceptual variables between the two experimental groups during the first 10 km run (*i.e.*, at pre-intervention); no significant differences were observed between these groups (Table 2). Of note, at this point, the participants had not been exposed to any experimental intervention, and therefore, the lack of differences was important to ensure that the two groups were similar before the interventions.

Table 2. Physical performance and key physiological and perceptual variables during the first 10 km run (*i.e.*, at pre-intervention).

Variable	CON (n = 8)	HA (n = 8)	*P-value*
Time to complete 10 km (min)	54.5 ± 5.6	57.3 ± 8.7	0.464
Average running speed (km/h)	11.1 ± 1.2	10.7 ± 1.7	0.576
End - Ear canal temperature (°C)	39.2 ± 0.5	39.0 ± 0.4	0.321
End - Heart rate (bpm)	175 ± 13	174 ± 15	0.848
End - RPE (a.u.)	18 ± 1	18 ± 2	0.744

Legend: CON = control; HA = heat acclimated; RPE = rating of perceived exertion. End means that variables were measured when the participants finished the predetermined distance. Data are expressed as means ± SD. Unpaired Student's t-tests were used to generate the *P*-values reported in the table.

Next, we analyzed the recovery time course of performance, physiological, and perceptual variables between the two groups, which were later assigned to the HA and CON protocols. The mixed-model two-way ANOVAs revealed no significant interactions between group and time in all variables analyzed (Figure 1), except for the handgrip force ($F = 3.44$, $p = 0.025$). Of note, the post hoc test did not identify significant differences in pairwise comparisons of handgrip force between the HA and CON groups. In addition, no significant main effect of group was observed for the variables analyzed, except for the USG ($F = 8.07$, $p = 0.013$), which was consistently higher in the HA than in the CON group.

As expected, most variables showed temporal changes during the post-exercise recovery, except for the handgrip force, for which a significant main effect of time was not observed ($F = 0.19$, $p = 0.906$). CMJ height ($F = 4.56$, $p = 0.007$) was lower 1 h after the run compared to pre-exercise and immediately after. Mean RR ($F = 40.86$, $p < 0.001$) and RMSSD ($F = 16.11$, $p < 0.001$) were lower immediately after compared to all other time points, while mean RR was also higher at 24 h after than at 1 h after. Urine specific gravity ($F = 19.58$, $p < 0.001$) was higher immediately after the run compared to pre-exercise and 24 h after. Vigor ($F = 17.34$, $p < 0.001$) and fatigue ($F = 15.10$, $p < 0.001$) were, respectively, reduced and augmented immediately after and 1 h after compared to pre-exercise and 24 h after. Finally, muscle soreness ($F = 6.25$, $p < 0.001$) was higher immediately after and 1 h after compared to pre-exercise. The physical performance in the 1-mile test, assessed 24 h after the 10 km run, was not different between the two groups ($t = -0.0575$, $p = 0.956$).

INSERT FIGURE 1

Figure 1. Recovery time course after the 10 km treadmill run in the heat for the following variables: countermovement jump (CMJ) height (panel A), handgrip force (B), 1-mile performance (C), fatigue (D), vigor (E), muscle soreness (F), urine specific gravity (USG, G), average time interval between consecutive heartbeats (mean RR; H), and root mean square of successive differences (RMSSD; I). The data for control (CON) and heat-acclimated (HA) groups are presented in blue and red, respectively. Most variables were analyzed at the following time points: before, immediately after, 1 h after, and 24 h after. Data are expressed as means ± SD. * $p < 0.05$ vs. before the 10 km run; # $p < 0.05$ vs. immediately after the 10 km run; + $p < 0.05$ vs. 1 hour after the 10 km run; & $p < 0.05$ CON group (main effect of intervention).

3.3. Effects of short-term heat acclimation on physical performance and recovery after a 10 km run in the heat

During the short-term heat acclimation protocol, expected differences in physiological and perceptual variables, indicative of effective acclimation, were observed. When comparing the first with the fifth day of acclimation, the ear canal temperature was reduced under resting conditions (37.62 ± 0.75°C vs. 38.00 ± 1.02°C, $p < 0.05$), while heart rate and RPE were reduced during exercise-heat stress (heart rate: 131 ± 12 bpm vs. 139 ± 12 bpm; RPE: 9.7 ± 0.5 vs. 10.2 ± 0.5, $p < 0.05$).

Table 3 presents the pre- to post-intervention differences in variables related to physical performance and physiological and perceptual responses measured during the 10 km run. HA produced a significant reduction in the time to complete the 10 km and a significant increase in average running speed. The changes in ear canal temperature and RPE almost reach significance in the comparisons between groups; therefore, the end-ear canal temperature tended to be high and the end-RPE tended to be low in the HA group compared to the CON group. No intergroup differences were observed in the end-heart rate.

Table 3: Changes in the performance and physiological and perceptual responses between the first and second 10 km runs in the control and heat-acclimated groups.

Variable	CON (n = 8)	HA (n = 8)	*P-value*
Δ Time to complete 10 km (min)	2.9 ± 6.2	-5.4 ± 4.2*	0.007
Δ Average running speed (km/h)	-0.5 ± 1.1	1.0 ± 0.7	0.005
Δ End - Ear canal temperature (°C)	-0.4 ± 0.7	0.3 ± 0.5	0.060
Δ End - Heart rate (bpm)	11 ± 20	3 ± 14	0.567
Δ End - RPE (a.u.)	0 ± 2	-2 ± 2	0.063

Legend: CON = control group; HA = heat acclimation; RPE = rating of perceived exertion. Changes were calculated by subtracting the values of the second 10 km run from those of the first 10 km run. End means that variables were measured when the participants finished the predetermined distance. Unpaired Student's t-tests were used to generate the *P*-values reported in the table. * $p < 0.05$ vs. CON group.

We calculated the changes in the recovery time course by subtracting the values measured before and after the second 10 km run from those measured before and after the first 10 km run. Then, these changes were compared between groups and over time to determine whether short-term heat acclimation has influenced recovery.

The mixed-model two-way ANOVAs revealed no significant group x time interactions and no significant main effect of time for the changes in the recovery time course (Figure 2). No significant main effect of group was observed for the changes in all variables analyzed, except for fatigue ($F = 5.08$, $p = 0.051$) and USG ($F = 5.35$, $p = 0.036$), which were lower in the HA than in the CON group.

The changes in physical performance during the 1-mile test, assessed 24 h after the 10 km run, were not different between the two groups ($t = -0.468$, $p = 0.654$). Values were negative in both groups, indicating better performance in the 1-mile test after the second 10 km.

INSERT FIGURE 2

Figure 2. Intervention-induced changes in the recovery time course after the 10 km treadmill run in the heat for the following variables: countermovement jump (CMJ) height (panel A), handgrip force (B), 1-mile performance (C), fatigue (D), vigor (E), and muscle soreness (F), urine specific gravity (USG, G), average time interval between consecutive heartbeats (mean RR; H), and root mean square of successive differences (RMSSD; I). The data for control (CON) and heat-acclimated (HA) groups are presented in blue and red, respectively. Most variables were analyzed at the following time points: before, immediately after, 1 h after, and 24 h after. Data are expressed as means ± SD. & $p < 0.05$ CON group (main effect of intervention).

Our last analysis compared pre- and post-intervention data in the HA group. Considering that our inter-group (*i.e.*, between-subject) analyses could not identify consistent effects induced by heat acclimation on recovery, we felt it important to confirm these findings through within-subject analyses specific to the HA individuals (Figures 3 to 5). The values measured at post-exercise were subtracted from those measured at pre-exercise to understand the exercise-induced changes in the variables analyzed, and then the results between the first and second 10 km runs (*i.e.*, at pre- and post-intervention) were compared (panels B, F, and J of Figures 3 to 5). No significant differences were observed in the nine variables analyzed.

Second, we subtracted the values measured 1 h after the exercise from those measured immediately after to assess short-term recovery and then compared the results between the two 10 km runs (panels C, G, and K) of Figures 3 to 5). RMSSD was the only variable with a significant effect ($t = 4.10$, $p = 0.005$; Figure 5). The change in RMSSD during the 1 h after the exercise was higher at pre-intervention than at post-intervention. No significant differences were observed in the other seven variables analyzed (*i.e.*, USG was not analyzed at this point).

Third, we subtracted the values measured 24 h after the exercise from those measured immediately after to assess recovery during a longer time frame and then compared the results between the two 10 km runs (panels D, H, and L of Figures 3 to 5). No significant differences were observed in the other nine variables analyzed.

INSERT FIGURES 3, 4, and 5

Figure 3. Recovery time course after the 10 km treadmill run in the heat for the following variables: countermovement jump (CMJ) height (panel A), handgrip force (E). The recovery time course was also assessed by calculating the changes between immediately after and before exercise (B and F), between 1 h after and immediately after (C and G), and between 24 h after and immediately after (D and H). The analyses in this figure included only individuals of the heat-acclimated (HA) group. The data for the first 10 km run (pre-acclimation) and the second 10 km (post-acclimation) groups are presented in white and red, respectively. Data are expressed as means ± SD.

Figure 4. Recovery time course after the 10 km treadmill run in the heat for the following variables: fatigue (panel A), vigor (E), and muscle soreness (I). The recovery time course was also assessed by calculating the changes between immediately after and before exercise (B, G, and J), between 1 h after and immediately after (C, G, and K), and between 24 h after and immediately after (D, H, and L). The analyses in this figure included only individuals of the heat-acclimated (HA) group. The data for the first 10 km run (pre-acclimation) and the second 10 km (post-acclimation) groups are presented in white and red, respectively. Data are expressed as means ± SD.

Figure 5. Recovery time course after the 10 km treadmill run in the heat for the following variables: urine specific gravity (USG; A), average time interval between consecutive heartbeats (mean RR; panel E), and root mean square of successive differences mean RR (RMSSD; I), and. The recovery time course was also assessed by calculating the changes between immediately after and before exercise (B, G, and J), between 1 h after and immediately after (G and K), and between 24 h after and immediately after (D, H, and L). The analyses in this figure included only individuals of the heat-acclimated (HA) group. The data for the first 10 km run (pre-acclimation) and the second 10 km (post-acclimation) groups are presented in white and red, respectively. Data are expressed as means ± SD.

4. DISCUSSION

The short-term HA protocol improved physical performance during the 10 km self-paced treadmill run under hot conditions but had minimal effect on the recovery time course for almost of most variables. This finding is relevant from an applied perspective because running at faster average speeds after HA reflects an increased external load during physical exertion, which could contribute to impairing recovery time course in heat-acclimated individuals. This impaired recovery was only noticed for RMSSD during the 1 h subsequent post exercise in the within-subject analyses. In contrast, between-group comparison analyses provided evidence for an HA-mediated beneficial effect on USG and perceived fatigue.

As expected, short-term HA significantly improved endurance performance during self-paced running in the heat, corroborating previous findings (Benjamin; Sekiguchi; Struder; Szymanski *et al.*, 2021; Racinais; Alonso; Coutts; Flouris *et al.*, 2015; Tyler; Reeve; Sieh; Cheung, 2024). HA individuals completed the 10 km 5.4 ± 4.2 min faster while maintaining a 0.9 ± 0.6 km/h faster average speed. No differences in performance were observed in the CON individuals, indicating that the performance improvements were due to the HA protocol rather than a learning effect, possibly caused by repeating the 10 km in the heat (Wardenaar; Ortega-Santos; Vento; Beaumont *et al.*, 2021). These findings emphasize the effectiveness of HA protocols based on controlled hyperthermia in improving performance (Benjamin; Sekiguchi; Struder; Szymanski *et al.*, 2021; Guy; Deakin; Edwards; Miller *et al.*, 2015b). Moreover, only five sessions were required to produce meaningful benefits, indicating that short-term HA can a practical experience for preparation for competitions under environmental heat stress.

In the between-subject analyses, evidence was observed for an HA-mediated beneficial effect on USG and perceived fatigue, and an impaired recovery was noticed for RMSSD during the 1 h after the 10 km run in the within-subject analyses. However, it should be emphasized that HA influenced the recovery timeline of a few of the several variables investigated and that these changes were not reproduced in the different data analyses employed by the current study. The beneficial effects of heat acclimation (HA) on urine specific gravity (USG) and perceived fatigue were not observed in the within-subject analyses, while the reduction in RMSSD was also not confirmed in the between-subject analyses. Although the punctual changes induced by HA will be discussed in the following paragraphs, our interpretation of the current data is that short-term HA barely affects the time course of physiological, perceptual, and performance variables during post-exercise recovery.

Short-term HA seemed to slow cardiac parasympathetic reactivation following exercise-heat stress. RMSSD, an index of parasympathetic cardiac activity (Asarcikli; Hayiroglu; Osken; Keskin *et al.*, 2022), showed an attenuated increase during the 1 hour that followed the second 10 km run (post-intervention) compared to the first run (pre-intervention). Notably, exercise intensity influences the cardiac autonomic response recorded following exercise (Kannankeril et al., 2004; Michael et al., 2017). In this sense, the slow recovery after HA may reflect the faster running speed (*i.e.*, greater physiological load) during the second 10 km treadmill run. Importantly, HA did not significantly influence the changes 24 hours after the exercise, indicating that HA effects on RMSSD were short-lived and that one day represented enough time for complete parasympathetic reactivation, regardless of the acclimation status.

Five HA sessions lowered the scores of perceived fatigue compared to the CON protocol, but did not change perceived vigor and muscle soreness. Investigating the role of HA on post-exercise recovery was a novelty of the current study. Therefore, our findings will be discussed in the light of physical training, an intervention that, like HA, also induces performance, physiological, and perceptual adaptations. Our findings partially agree with a previous study showing that 10 weeks of preseason training improved the perception of fatigue and vigor (i.e., the latter was not observed in the current experiments) following a high-intensity futsal training session (WILKE et al., 2021). An improved perception of fatigue following exercise-heat stress could contribute to a faster recovery and readiness for a subsequent training or completion stimulus; however, HA did not influence performance recovery, as indicated by the three tests we conducted: CMJ, handgrip, and one-mile run. These findings are suggestive that recovery of perceptual and physical performance variables follows different time courses and reinforce the idea that different recovery profiles may exist among athletes (Wilke et al., 2019).

As reported for perceived fatigue, short-term HA lowered USG values compared to the CON protocol. Much evidence supports that HA improves fluid-electrolytic balance during exercise-heat stress, contributing to defending plasma volume in conditions of marked water losses through sweating. Indeed, HA expands plasma volume, thus allowing a greater sweat output but still avoiding severe dehydration (Lorenzo et al., 2010; (BUONO; KOLDING; LESLIE; MORENO *et al.*, 2018). Evidence supports that HA decreases the core temperature threshold for the onset of sweating and pronounced sudomotor thermosensitivity — adaptive mechanisms that enhance evaporative heat loss (Sekiguchi; Benjamin; Dion; Manning *et al.*, 2021). Of note, we did not observe differences in sweat rate between the HA and CON groups in the current experiments, suggesting that 5 days of HA may not be enough to generate apparent sudomotor adaptations.

It should be noted that the individuals subjected to HA had consistently higher USG values during pre-intervention testing. Therefore, the current data may suggest that HA corrected rather than improved fluid balance in acclimated individuals. Alternatively, we cannot rule out that these individuals adhered better to the experimenters' recommendations post-intervention than pre-intervention. The reduced USG values at post-exercise recovery following HA should be further confirmed.

HA individuals decided to run the 10 km at faster speeds, meaning that they exercised at a higher external (*i.e.*, mechanical) load. Despite this higher load, recovery was not impaired, except for the RMSSD, which increased less during the 1 hour but not during the 24 hours following exercise-heat stress. Considering that runners usually rest for more than 1 hour between training sessions, even those who train two times a day (Bernat-Adell; ColLADO-BOIRA; Moles-julio; Panizo-González *et al.*, 2021), this impaired parasympathetic reactivation is possibly of minor practical significance. Conversely, heat acclimation (HA) did not consistently improve the recovery time course of the variables assessed. However, the fact that recreational athletes subjected to HA were able to perform at a higher workload while maintaining a recovery profile similar to the control group suggests a potential benefit of HA in supporting performance under heat stress conditions. This higher workload may have imposed greater physiological strain, which could partially explain the absence of recovery improvements. Even so, these findings are valuable for coaches and strength and conditioning professionals, as they highlight the importance of considering performance capacity alongside recovery when prescribing training in hot environments.

This study is not free of limitations. First, the participants could recover from the treadmill run under moderate ambient conditions; therefore, their exposure to the heat was terminated with the end of the exercise. The fact that the participants were not exposed to environmental heat stress following exercise has possibly accelerated the recovery of performance, physiological, and perceptual variables and may have hampered us from reporting further pieces of evidence of an improved recovery in the HA group.

Second, the ear canal temperature was measured to estimate core body temperature. Although the ear canal temperature significantly deviates from other core temperature indices, the agreement between the ear canal and rectal temperatures is stronger during exercise in the heat (MORAES et al., *data not published*). Third, no blood biomarkers were measured after the 10 km run in the heat. Even though these biomarkers (*e.g.*, creatine kinase, lactate dehydrogenase, and c-reactive protein) can help assess objectively the level of fatigue/muscle damage induced by exercise (Bird; Linden; Hawley, 2014; Takayama; Aoyagi; Takahashi; Nabekura, 2018), they are not often measured in track-and-field training settings. Moreover, the changes in these biomarkers induced by a 10 km run in the heat are minor (VINHAS, 2013). Lastly, only two women volunteered to participate in the current experiments. Although we committed to reducing women's underrepresentation (Hutchins et al., 2021) in our studies, recruiting women who meet our inclusion criteria has been challenging.

In conclusion, the present study provides further evidence that a short-term HA protocol effectively improves performance and reduces physiological and perceptual strain during exercise-heat stress. However, HA acclimation effects on the recovery of performance, physiological, and perceptual variables were scarce and inconsistent between the multiple data analyses we performed. The few effects observed following HA were restricted to an impaired RMSSD recovery during the 1 h after exercise-heat stress and the improved USG values and perceived fatigue. The current findings also indicate that, despite increasing the running speed (external load), HA minimally affects the post-exercise recovery time course of physiological, perceptual, and physical performance variables.

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Figure 1

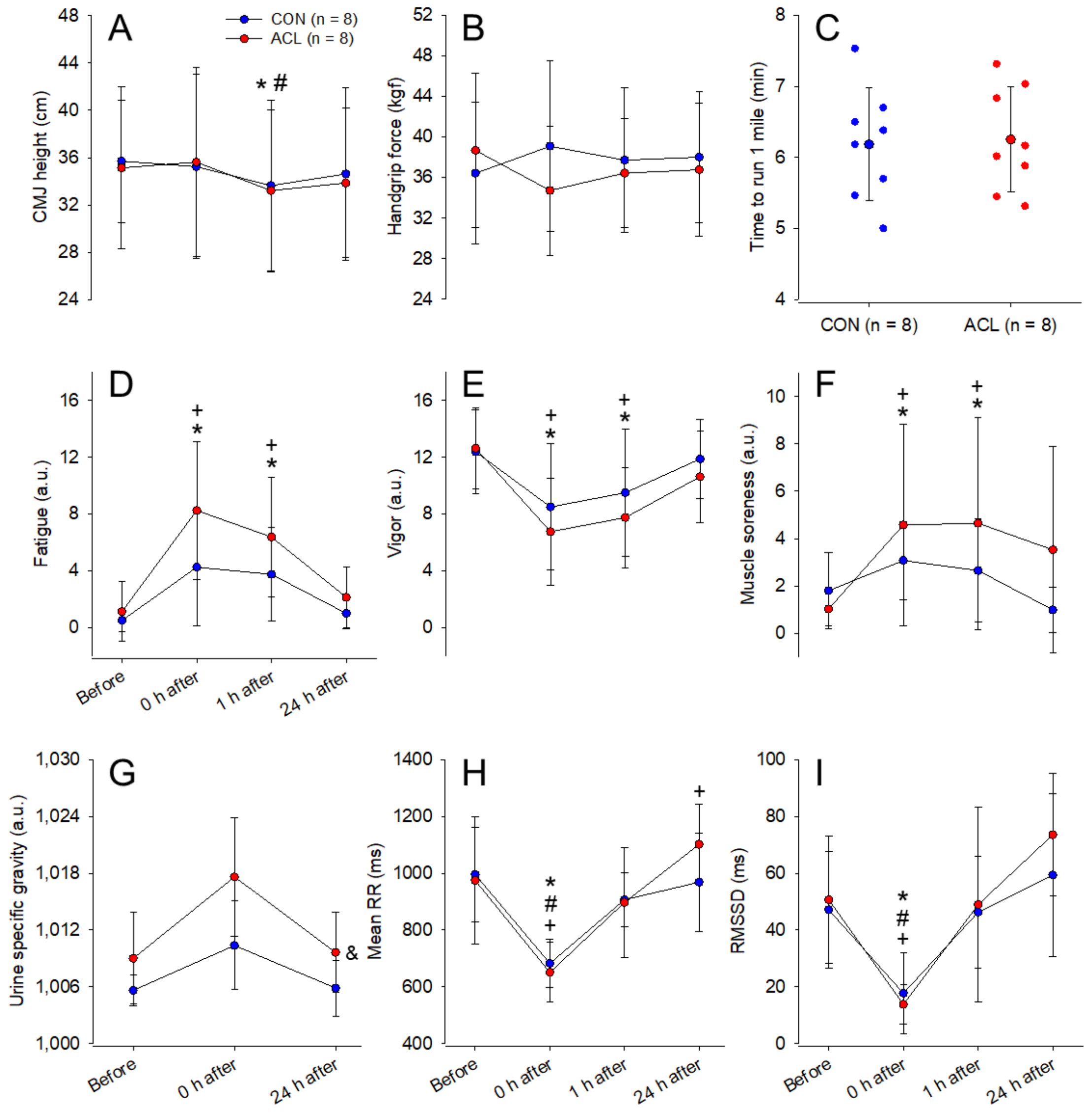
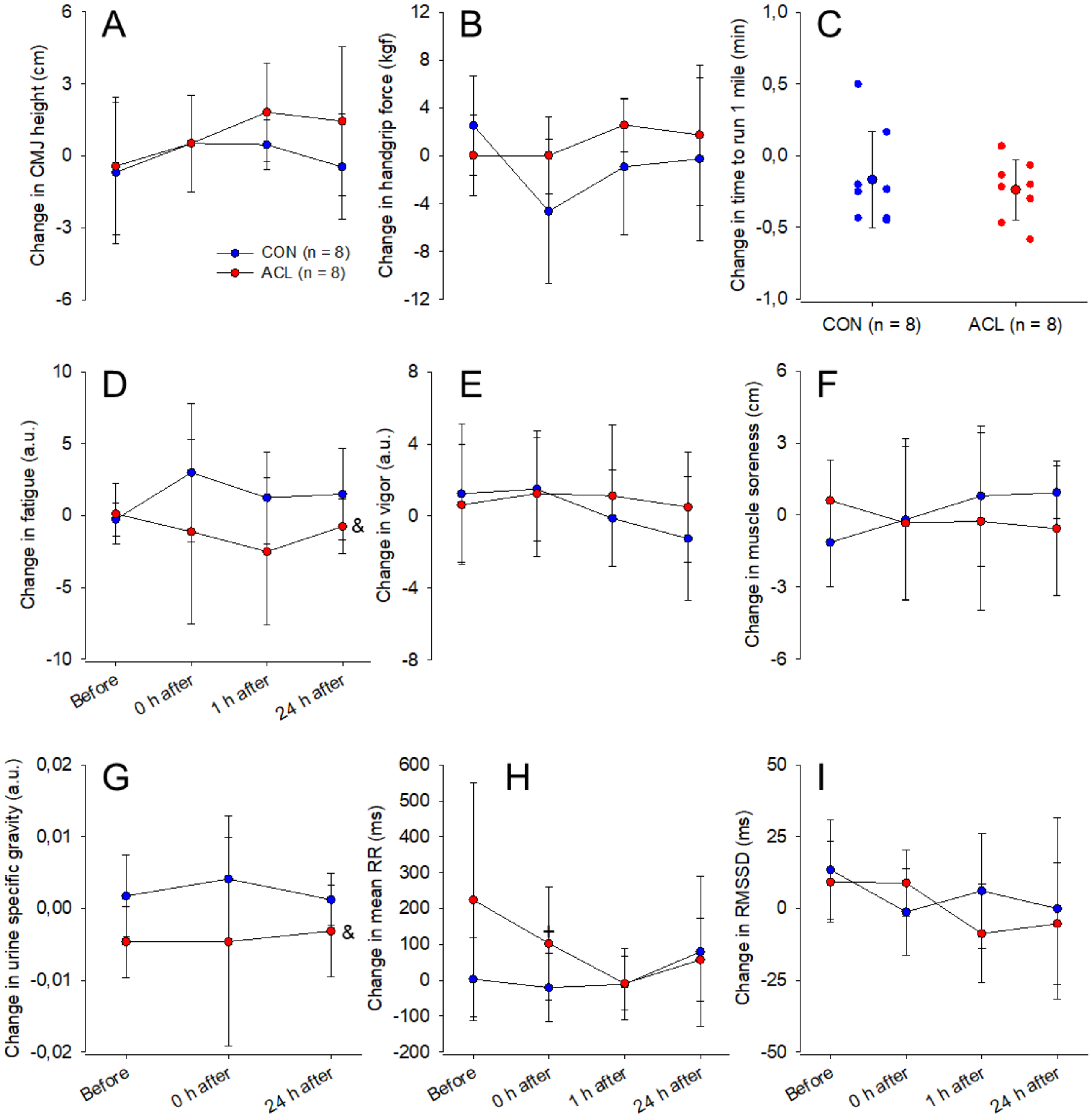


Figure 2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CON (n = 8)
ACL (n = 8)
B
Change in handgrip force (kgf)
C
Change in time to run 1 mile (min)
CON (n = 8)
ACL (n = 8)
D
Change in fatigue (a.u.)
&
E
Change in vigor (a.u.)
F
Change in muscle soreness (cm)
Before
0 h after
1 h after
24 h after
G
Change in urine specific gravity (a.u.)
&
H
Change in mean RR (ms)
I
Change in RMSSD (ms)

Figure 3

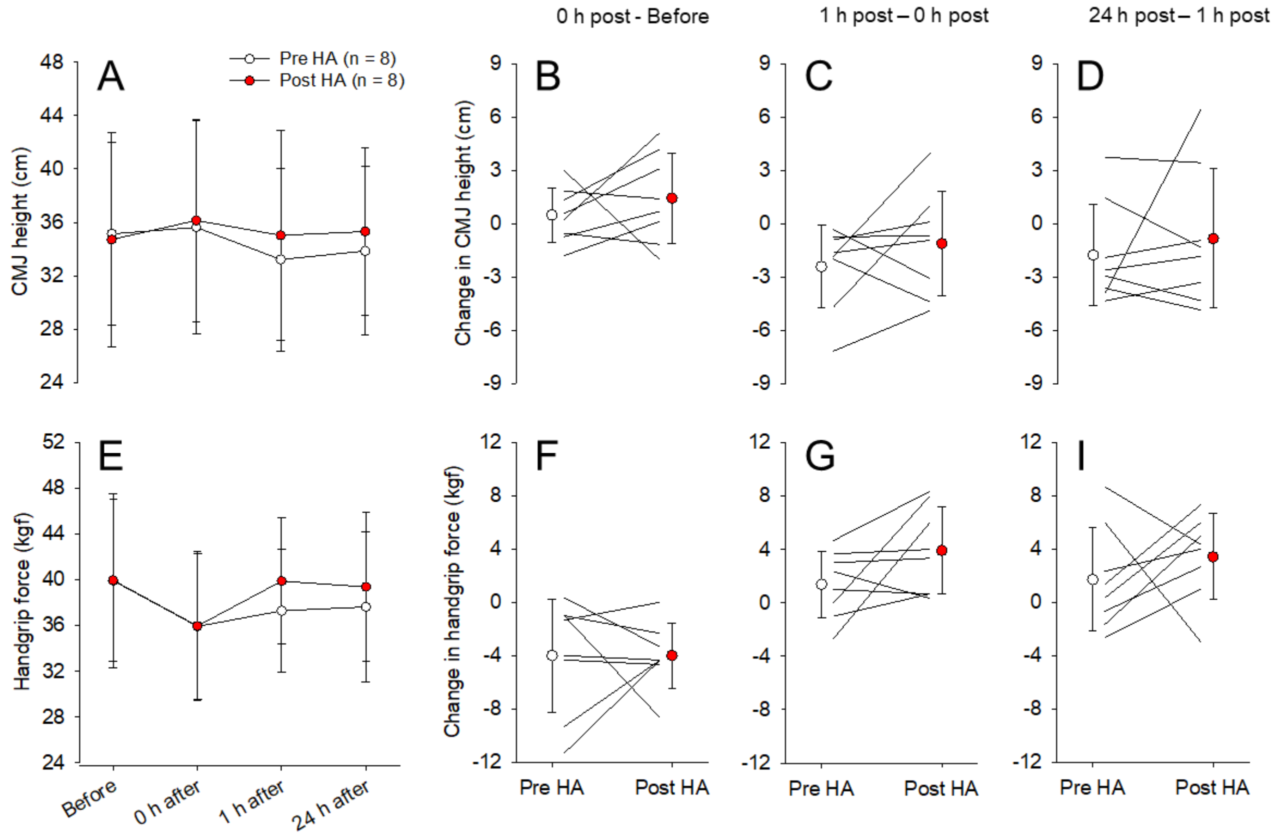
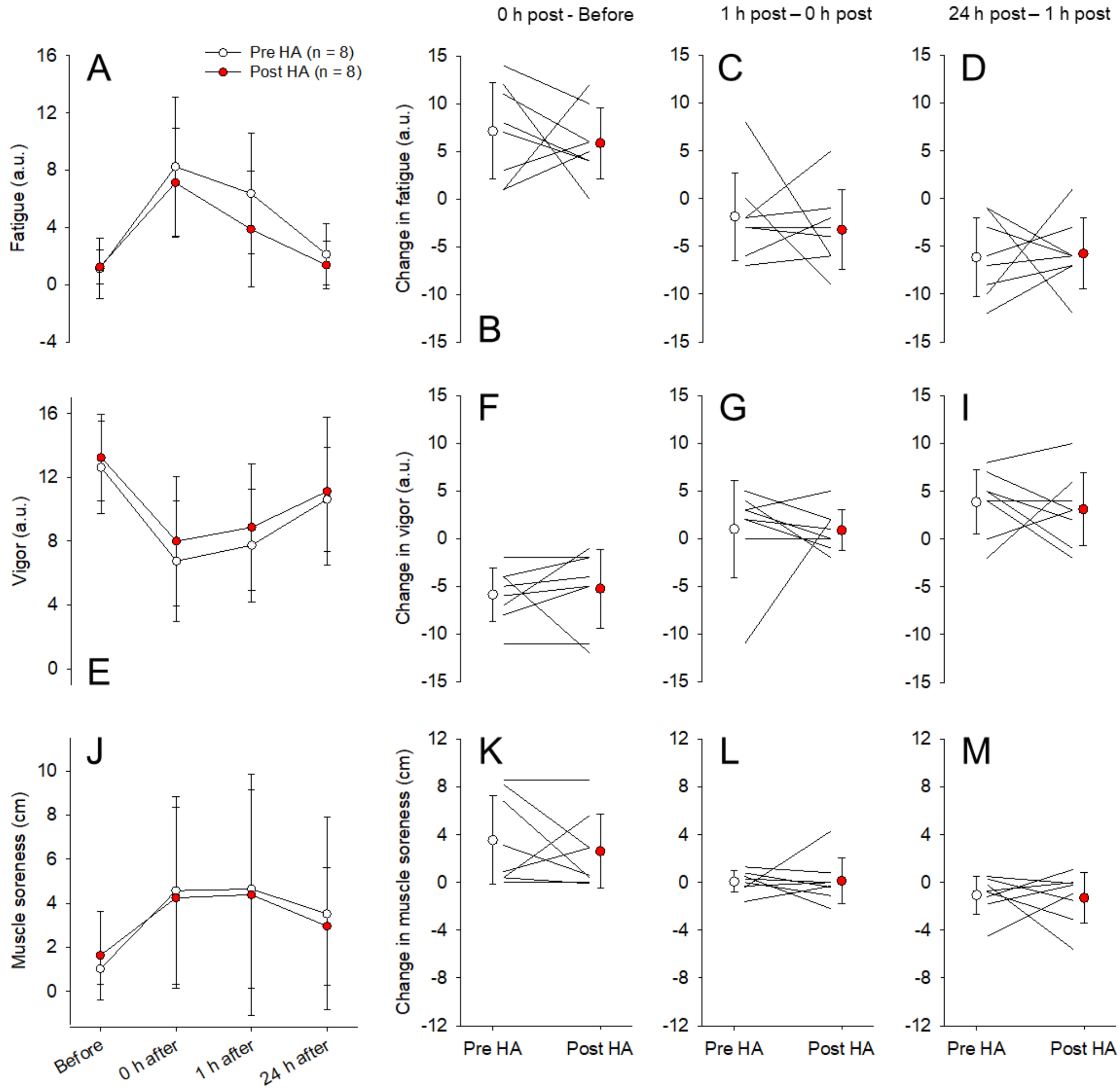

0 h post - Before
1 h post – 0 h post
24 h post – 1 h post
A
B
C
D
E
F
G
I
Pre HA (n = 8)
Post HA (n = 8)
CMJ height (cm)
Change in CMJ height (cm)
Handgrip force (kgf)
Change in handgrip force (kgf)
Before
0 h after
1 h after
24 h after
Pre HA
Post HA

Figure 4


0 h post - Before
1 h post – 0 h post
24 h post – 1 h post
A
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J
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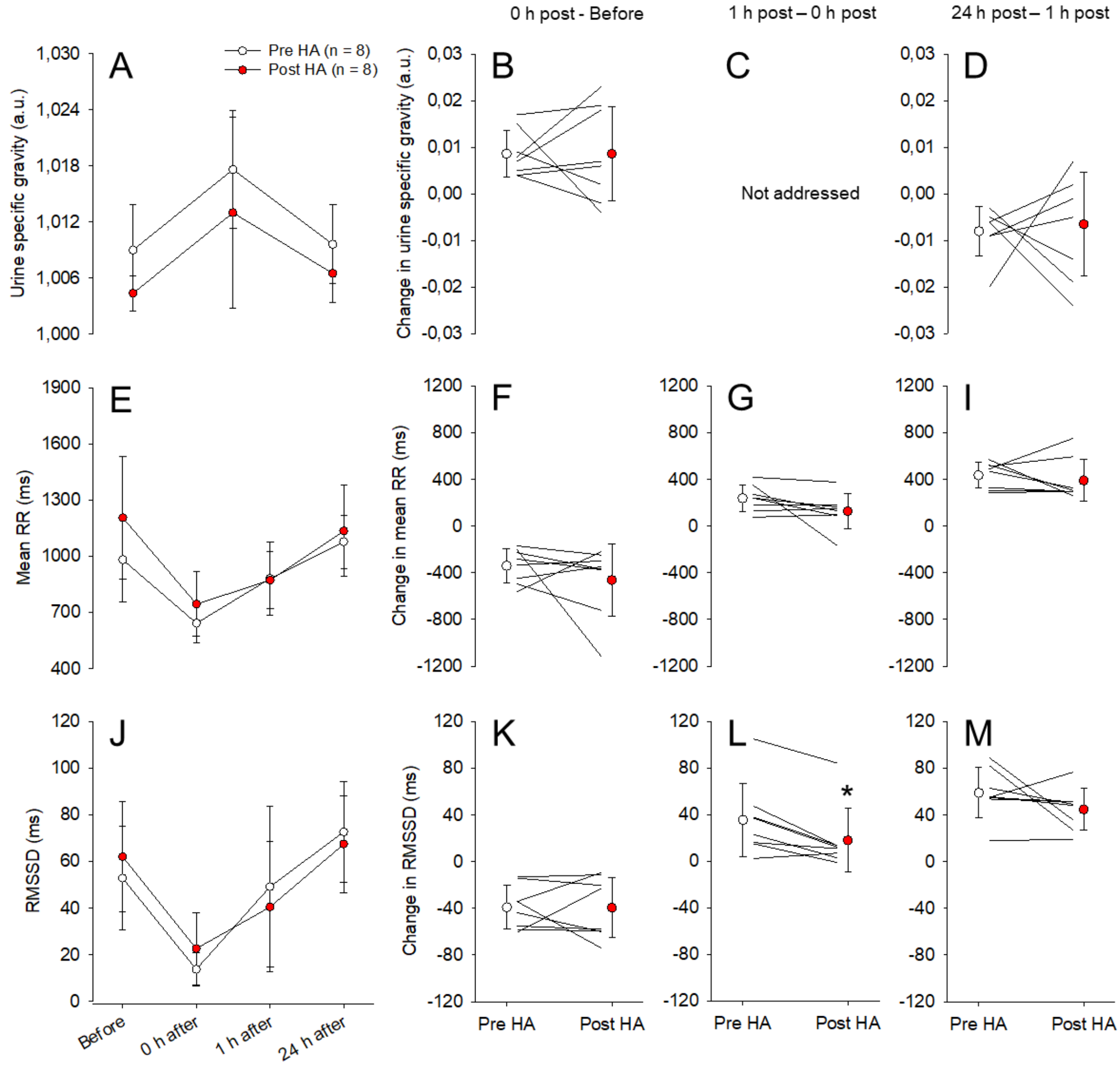
M
Pre HA (n = 8)
Post HA (n = 8)
Fatigue (a.u.)
Change in fatigue (a.u.)
Vigor (a.u.)
Change in vigor (a.u.)
Muscle soreness (cm)
Change in muscle soreness (cm)
Before
0 h after
1 h after
24 h after
Pre HA
Post HA

Figure 5


0 h post - Before
1 h post – 0 h post
24 h post – 1 h post
A
B
C
D
Pre HA (n = 8)
Post HA (n = 8)
Urine specific gravity (a.u.)
Change in urine specific gravity (a.u.)
Not addressed
E
F
G
I
Mean RR (ms)
Change in mean RR (ms)
J
K
L
M
RMSSD (ms)
Change in RMSSD (ms)
Before
0 h after
1 h after
24 h after
Pre HA
Post HA
*

6 CONSIDERAÇÕES FINAIS

O aquecimento global tem aumentado a frequência, a severidade e a duração das ondas de calor, expondo mais pessoas a condições ambientais extremas. Entre os mais afetados, estão os praticantes de atividades esportivas, como os corredores de rua, que frequentemente enfrentam condições adversas durante seus treinos e competições. Esse cenário impõe desafios significativos à saúde e ao desempenho físico dos corredores, especialmente em regiões tropicais, cujo clima é caracterizado por elevados valores de temperatura e umidade relativa do ar (Bierlier; Scantamburlo, 2022).

A prática de atividades físicas, realizada de forma recreativa ou competitiva, em ambientes termicamente estressantes tem se tornado cada vez mais frequente em diversas regiões do mundo. Durante os Jogos Olímpicos de Tóquio 2020, muitos atletas enfrentaram condições ambientais extremas, com temperaturas acima de 30°C e umidade relativa superior a 70%. Foram registrados 50 casos de intercorrências relacionadas ao calor, sendo 7 casos (14%) ocorrendo nos locais de realização da maratona e da marcha atlética. No total, 100 dos 567 atletas (18%) e 125 dos 541 membros das delegações (23%) necessitaram de atendimento médico em clínicas especializadas no tratamento de complicações induzidas pelo calor (Tanaka; Tanaka; Yokota; Otomo *et al.*, 2023). Situação semelhante foi observada no Campeonato Mundial de Atletismo de 2019, realizado em Doha, no Catar, sob condições climáticas adversas. Na ocasião, entre os atletas das provas de maratona e marcha atlética, foram relatados 20 casos de colapso associado ao exercício, 16 episódios de cãibras musculares induzidas pelo esforço físico e 18 ocorrências de exaustão pelo calor. Ademais, apenas 40 das 69 competidoras conseguiram concluir a maratona feminina (Racinais; Havenith; Aylwin; Ihsan *et al.*, 2022).

A realidade brasileira não é diferente, sendo que o número de provas de corrida realizadas ao ar livre (*outdoor*), sob condições quentes e úmidas, vem aumentando. Em 2024, por exemplo, mais de 100 provas foram realizadas na cidade de São Luís, muitas delas em condições desafiadoras, com temperaturas acima de 30°C e umidade relativa superior a 70%. Essas provas, com distâncias que variam de 5 km a 21 km e largadas entre às 5:30 e 6:00 h da manhã, desafiam principalmente os atletas amadores, que tendem a passar mais tempo expostos ao estresse térmico ambiental, em comparação aos atletas de elite, que concluem as provas em menos tempo.

Nessas situações, fatores ambientais como calor intenso, umidade elevada, baixa circulação do ar e radiação solar direta dificultam a dissipação do calor corporal gerado a partir do metabolismo aumentado, sobrecarregando o sistema termorregulador. Essa sobrecarga pode levar a uma redução de 3% a 20% no desempenho físico e aumentar consideravelmente o risco de complicações médicas graves. O calor, inclusive, está entre as principais causas naturais de morte em atletas, superando outras etiologias comuns em ambientes esportivos (Aylwin; Havenith; Cardinale; Lloyd *et al.*, 2023), como a doença aguda da montanha, (Winter; Bjorkman; Miller; Nichols *et al.*, 2021) ou hipotermia (Noakes, 2000).

As complicações relacionadas ao calor compreendem desde manifestações leves, como cãibras musculares, até quadros potencialmente fatais, como a insolação por esforço (também conhecida como choque hipertérmico). A insolação por esforço é uma emergência médica caracterizada por valores de temperatura corporal interna superiores a 40,5 °C, acompanhados de disfunções neurológicas como confusão mental, ataxia, perda de equilíbrio, apatia e irritabilidade (Armstrong; Casa; Millard-Stafford; Moran *et al.*, 2007). Sua ocorrência exige resposta rápida e protocolos bem definidos para garantir a sobrevivência dos atletas acometidos (Hosokawa; Casa; Racinais, 2020).

Para mitigar os riscos associados ao estresse térmico em grandes eventos esportivos, recomenda-se a adoção de três procedimentos: (1) monitorar de forma sistemática a ocorrência de complicações relacionadas ao calor durante as competições; (2) validar e utilizar índices de estresse térmico ambiental adequados à realidade de cada esporte; e (3) implementar estratégias efetivas de mitigação, como alteração dos horários das provas, criação de pontos de hidratação, aplicação de técnicas de resfriamento corporal e inclusão de protocolos de aclimatação durante os treinamentos.

Diante desse contexto, propusemos investigar a fadiga neuromuscular de membros inferiores após o exercício físico realizado em duas condições ambientais. Investigamos também o curso temporal da recuperação de diversas variáveis (fisiológicas, perceptivas e de desempenho físico) após corrida em ambiente quente. Por fim, buscamos analisar os efeitos de um protocolo curto de aclimatação ao calor, com duração de cinco dias, a fim de verificar se, além dos benefícios já descritos na literatura (Guy; Deakin; Edwards; Miller *et al.*, 2015b), esse processo também exerce influência positiva sobre a recuperação pós-esforço de atletas recreativos.

No primeiro estudo, observamos que as temperaturas corporais e os valores de percepção subjetiva do esforço e percepção térmica foram mais elevados durante a corrida de 10 km em ambiente quente em comparação ao ambiente temperado. Maiores sobrecargas fisiológica e perceptiva foram observadas a 35°C, ainda que os atletas tenham mantido uma menor velocidade média, ou seja, tenham tido pior desempenho em comparação a 25°C. Esses achados corroboram a literatura que aponta o estresse térmico ambiental como um fator limitante do desempenho aeróbico (Racinais; Alonso; Coutts; Flouris *et al.*, 2015), uma vez que a prática esportiva realizada em ambiente quente, entre outros aspectos, impõe sobrecarga adicional aos mecanismos de termorregulação, favorecendo a elevação das temperaturas corporais e, consequentemente, antecipando a fadiga.

No que se refere à fadiga neuromuscular de membros inferiores, observou-se uma redução na altura do salto com contramovimento (SCM) uma hora após a corrida, em ambas as condições ambientais. Em média, essa diminuição foi de 7,0% (2,3 cm) em relação aos valores obtidos no pré-exercício. A redução da altura do salto com contramovimento não foi diferente 1 hora após o exercício nas duas condições ambientais, sugerindo que a maior carga externa (velocidade mais rápida) a 25°C compensa os efeitos de respostas perceptivas mais significativas a 35˚C na indução de fadiga neuromuscular.

No segundo estudo, os resultados obtidos com os 16 voluntários revelaram aumentos na fadiga, na dor muscular percebida e na gravidade específica da urina, além de reduções no vigor, na percepção de recuperação e na modulação parassimpática cardíaca após 10 km de corrida em temperatura ambiente de 35°C. Também foi observada uma redução na altura do salto com contramovimento (SCM) uma hora após o exercício. A maioria dessas alterações mostrou-se transitória, retornando aos valores basais após 24 horas, com excessão da percepção de recuperação, que permaneceu reduzida.

Análises secundárias desse estudo revelaram que a redução da altura do salto com contramovimento 1 hora após a corrida não se correlacionou com as alterações em varivéis perceptivas, indicando que as alterações nas variáveis de desempenho físico e perceptivas seguem diferentes cursos temporais após o exercício. Por fim, análises de cluster conseguiram identificar a existência de três clusters de atletas com diferentes perfis de recuperação. Um dos cluster apresentou melhor recuperação que os demais, conforme evidenciado por aumentos na força de preensão manual e menores escores de fadiga após o exercício. Além disso, um segundo cluster apresentou maior percepção de dor muscular que os demais.

No terceiro estudo, investigamos os efeitos de um protocolo de aclimatação ao calor no tempo de recuperação de variáveis fisiológicas, perceptivas e de desempenho físico após uma corrida realizada em ambiente quente e úmido. Observamos que o protocolo de aclimatação ao calor de curto prazo (5 dias) foi suficiente para melhorar o desempenho físico durante uma corrida de 10 km realizada em esteira, em intensidade autorregulada e sob condições ambientais quentes, corroborando estudos anteriores (Guy; Deakin; Edwards; Miller *et al.*, 2015b; Wardenaar; Ortega-Santos; Vento; Beaumont *et al.*, 2021). Esse achado reforça a efetividade da aclimatação ao calor como uma estratégia prática para melhorar o desempenho de atletas que se exercitam em ambientes quentes, permitindo a manutenção de velocidades médias mais elevadas. Por exemplo, o grupo aclimatado concluiu a prova simulada 5,4 ± 4,2 minutos mais rapidamente e mantiveram uma velocidade média 0,9 ± 0,6 km/h superior em comparação ao momento pré intervenção.

Apesar da melhora no desempenho aeróbico, a aclimatação ao calor não influenciou de forma significativa o curso temporal de recuperação para a maioria das variáveis fisiológicas, perceptivas e de desempenho físico avaliadas. Trata-se de um achado importante pois, mesmo com o aumento da carga externa imposto pela maior velocidade selecionada pelos atletas, houve poucas diferenças na recuperação pós-exercício. Como aspectos positivos, foram observados efeitos benéficos da aclimatação ao calor sobre a percepção de fadiga e sobre a gravidade específica da urina. Como aspecto negativo, observou-se uma recuperação mais lenta da modulação parassimpática cardíaca nos indivíduos aclimatados ao calor. Embora esteja associada a uma maior sobrecarga mecânica em função do aumento do desempenho, a aclimatação ao calor promove adaptações que favorecem o equilíbrio hídrico e melhoram a percepção de fadiga, contribuindo para atenuar possíveis impactos negativos sobre a recuperação.

Os nossos achados revelam a importância de submeter atletas a protocolos de aclimatação ao calor antes de eventos esportivos realizados em ambientes com elevado estresse térmico. Essa estratégia resulta em melhor desempenho físico, além de reduzir a percepção de fadiga e melhorar os marcadores de hidratação, como a gravidade específica da urina, no período após o exercício. Algumas adaptações benéficas induzidas pela aclimatação ao calor podem ser alcançadas por meio protocolos curtos, caracterizados por 5 sessões de treino diárias, com duração de 60 minutos, em ambiente quente, seja natural ou artificialmente controlado.

Além disso, os resultados reforçam a importância de um monitoramento abrangente da fadiga e da recuperação pós-exercício. No caso de corredores que participam de modalidades de fundo, esse monitoramento deve ocorrer especialmente nas 24 horas seguintes ao esforço físico. A fadiga neuromuscular de membros inferiores foi evidente após os 10 km em ambas as condições ambientais, assim como outras alterações fisiológicas e perceptivas. De forma geral, quase todas as variáveis retornaram aos valores basais no dia seguinte à corrida de 10 km, o que está condizente com a elevada frequência semanal de treinamento de corredores de fundo. Contudo, cabe ressaltar que a percepção de recuperação ainda estava reduzida 24 horas após a corrida. Por isso, recomenda-se o uso de ferramentas acessíveis e práticas, como escalas de recuperação percebida (TQR) e de dor muscular (DOMS), medidas urinárias para avaliar o estado de hidratação e testes como o salto vertical (CMJ) para avaliar fadiga neuromuscular. Em conjunto, essas ferramentas auxiliam no acompanhamento da recuperação e no planejamento do treinamento (*e.g.*, quando de deve aplicar um novo estímulo).

5 CONCLUSÕES

1) O ambiente quente reduziu o desempenho físico e acentuou as percepções do esforço e térmica durante 10 km de corrida em intensidade autorregulada. As corridas nos dois ambientes causaram fadiga neuromuscular de membros inferiores, conforme evidenciado pela redução na altura do salto com contramovimento 1 hora após o exercício; contudo, não houve diferença estatística entre as duas condições.

2) A corrida de 10 km resultou em aumento da fadiga, dor muscular percebida e gravidade específica da urina, além de redução do vigor, recuperação percebida e atividade parassimpática; a maioria dessas alterações foi transitória, exceto a recuperação percebida, que permaneceu reduzida após 24 horas.

3) A aclimatação ao calor promoveu melhor desempenho físico durante os 10 km de corrida em ambiente quente, sem comprometer, de forma geral, a recuperação dos atletas, com destaque para benefícios na percepção de fadiga e na gravidade específica da urina, apesar de uma reativação parassimpática cardíaca mais lenta na 1 hora subsequente ao exercício.

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