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ABSTRACT

Purpose: Heat acclimation (HA) is recommended before competing in hot and humid conditions. HA has also been recently suggested to increase muscle strength, but its effects on human's muscle and tendon mechanical properties are not yet fully understood. This study investigated the effect of active HA on *gastrocnemius medialis* (GM) muscle-tendon properties. **Methods:** Thirty recreationally active participants performed 13 low-intensity cycling sessions, distributed over a 17-days period in hot (HA: ~38°C, ~58% relative humidity [RH]; n = 15) or in temperate environment (CON: ~23°C, ~35% RH; n = 15). Mechanical data and high-frame rate ultrasound images were collected during electrically-evoked and voluntary contractions pre- and post-intervention. Shear modulus was measured at rest in GM and vertical jump performance was assessed. **Results:** Core temperature decreased from the first to the last session in HA (-0.4 ± 0.3°C; P = 0.015), while sweat rate increased (+0.4 ± 0.3 $L.h^{-1}$; P = 0.010), suggesting effective HA; whereas no changes were observed in CON (both $P \geq 0.877$). Heart rate was higher in HA *vs*. CON and decreased throughout intervention in groups (both $P \leq 0.008$), without an interaction effect (P = 0.733). Muscle-tendon unit properties (*i.e.*, maximal and explosive isometric torque production, contractile properties, voluntary activation, joint and fascicular force-velocity relationship, passive muscle and active tendon stiffness) and vertical jump performance did not show training ($P \geq 0.067$) or group × training interaction ($P \geq 0.232$) effects. **Conclusions:** Effective active heat acclimation does not alter muscle-tendon properties. Preparing hot and humid conditions with active heat acclimation can be envisaged in all sporting disciplines without the risk of impairing muscle performance.

Key Words: REPEATED HOT EXPOSURE, EXERCISE, STRENGTH, FORCE-VELOCITY PROPERTIES, STIFFNESS, PERFORMANCE

INTRODUCTION

Exercising in the heat induces cardiovascular and neuromuscular impairments which may in turn limit exercise capacity (1, 2). However, repeated passive or active heat exposures, known as heat acclimation (HA), elicit specific physiological adaptations that may mitigate these alterations, improving exercise capacity in the heat (3-5) and potentially in temperate conditions (4). It is therefore recommended to train (or to be exposed) in the heat for 60-90 min per day (5, 6) for 1 to 2 weeks (2, 7) before competing in the heat. HA notably increases sweat rate (8) and plasma volume (9), and decreases sweat sodium concentration (10), core temperature (T_{core}) (8) and heart rate (3).

Moreover, passive HA appears to protect the central nervous system in the heat (11), and to increase electrically-evoked and voluntary force-generating capacity of plantar flexor muscles (12). To the best of our knowledge, few studies have investigated the effects of HA on such properties, and the underlying mechanisms are unknown. Interestingly, using repeated local heat exposure, muscle force was increased in humans (13, 14), and induced muscle hypertrophy in both animals and humans (13, 15). It was reported that repeated passive heat exposures may promote the activation and inhibition of the hypertrophic and atrophic signaling pathways, respectively (16, 17). Altogether, these previous studies may suggest that repeated heat exposure leads to increased force production, due to muscular adaptations. However, the muscle-tendon adaptations accompanying these increases in muscle force are unknown. Notably, they have not been investigated after an active HA, despite the fact that changes in muscle-tendon properties may impact athletic performance, especially the force-velocity relationship.

The anatomical structures of the calf can be used as a predictor of athletic ability (18), therefore a potential modification of these structures should be considered when investigating performance. Muscle size is considered to be dependent of fascicle architecture (19). Thus, muscle hypertrophy, potentially inducing structural changes, could have effects on muscle mechanical properties. For example, it was reported that maximum shortening velocity is increased with longitudinal fascicle growth (20). Although these phenomena are difficult to investigate *in vivo*, muscle-tendon unit properties could in turn be impacted. Explosive force production, commonly measured through the rate of torque development (RTD), is dependent of maximum voluntary force production (21). Therefore, RTD might be increased by repeated heat exposure inducing muscle hypertrophy which increases force-generating capacity. HA, possibly enhancing force production, could also impact force-velocity properties, by inducing a potential rightward shift of the force-velocity relationship. Recently, an inverse relation was found between *vastus lateralis* muscle fiber diameter and normalized stiffness (22). Whether a potential reduction in muscle stiffness may be elicited by such heat-mediated hypertrophy remains yet to be investigated. The relationship between contractile material, muscle typology and stiffness remain complex, with effects not always univocal.

This study, therefore, aimed to determine the mechanical adaptations of *gastrocnemius medialis* (GM) muscle-tendon unit and their subsequent impact on motor performance following active HA (*i.e.*, training in the heat) *vs.* a similar training in temperate conditions. Based on a previous study (12), it was hypothesized that HA would improve skeletal muscle contractility and enhance multi-joint performance during vertical jumps. Nevertheless, the effect on muscle-tendon unit properties were exploratory since, to the best of our knowledge, there is no

investigation exploring the effect of such intervention on tissue stiffness and joint and fascicle force-velocity relationship.

METHODS

Participants

Thirty volunteers participated in the study and were separated into two intervention groups: HA group or control group (CON), each group being composed of fifteen participants (8 males and 7 females). Both groups presented similar anthropometrical profile (HA: 27 ± 5 yrs [range: 21-35], 172 ± 9 cm, 67 ± 8 kg, 4.6 ± 2.9 h of training per week; CON: 26 ± 4 yrs [range: 20-34], 174 ±12 cm, 69 ± 15 kg, 4.3 ± 2.4 h of training per week). A non-inferiority sample size calculation based on peak twitch from a previous study (12) was used (G-Power, software version 3.1.9.7) to determine the sample-size required. With significance and power criteria set at 0.05 and 80%, respectively, a minimum of 8 participants was requested. Considering potential drop-out, males and females' inclusion, and other parameters measured, we choose to increase participants' recruitment to 15 in each group. Participants were healthy and recreationally active in endurance but not involved in resistance training. Exclusion criteria comprised illness, musculoskeletal injuries, recent temperature-manipulation program, pregnancy, be under 18 or over 45 yrs old. Participants were informed about the nature, aims and risk associated with the experimental procedure before giving their written informed consent. This study was approved by the Sud-Ouest et Outre-mer III ethics committee (reference: 3849) and conformed to the standards of the Declaration of Helsinki.

Experimental design

Two to four days after a familiarization session, participants were tested before (*i.e.*, 3-4 days; PRE) and after (*i.e.*, ~48 h; POST) 13 low-intensity cycling sessions (Fig. 1A). According to their group assignment, participants performed the cycling sessions either in a hot (HA: 38.1 ± 0.4°C, 57.9 ± 2.3% relative humidity [RH]) or a temperate environment (CON: 23.0 ± 0.7°C, 35.3 ± 3.8% RH). All neuromuscular testing was performed in temperate condition (22.8 ± 1.7°C, 34.3 ± 8.0% RH). Environmental conditions in HA group were based on previous studies reporting effective active HA with conditions ranging 37-40°C and 55-65% RH (23, 24), while temperate environment corresponded to the ambient laboratory conditions (*i.e.*, ~23°, ~35% RH).

Training protocol

Cycling sessions. Both HA and CON groups performed 13 training sessions of 1 h each, on an ergocycle (Wattbike Pro, Nottingham, United Kingdom), distributed over a 17-days period, the weekend being off (Fig. 1A). Pedaling intensity was adjusted for each participant, according to body mass, and varied from one session to another (between 1.3 and 2.5 $W.kg^{-1}$), except for the first and the last session which were identical (1.4 $W.kg^{-1}$ during 1 h; Supplemental Table, Supplemental Digital Content, Exercise prescription for the cycling sessions performed in hot and thermoneutral environment, http://links.lww.com/MSS/C790).

Physiological monitoring. Before starting the intervention, urine specific gravity (USG) was collected, using a calibrated refractometer (PAL-10S, Atago, Tokyo, Japan), to check the level of hydration of the participants (USG = 1.009 ± 0.008). If USG ≥ 1.020 participants had to drink 500 mL of water before starting the session. Physiological responses were measured in each

group during the first and the last cycling session (Fig. 1B). T_{core} was monitored rectally using an electronic capsule (e-Celsius, BodyCap, Caen, France) self-inserted by the length of a gloved finger. Mean skin temperature (T_{skin}) was calculated from four data loggers (iButtons, Maxim Integrated, San Jose, United-States) as: 0.3 × chest temperature + 0.3 × arm temperature + 0.2 × thigh temperature + 0.2 × shin temperature. Muscle temperature (T_{musc}) was measured in a subsample of participants from each group (n = 8 in HA group and n = 7 in CON group) immediately at the end of a single cycling session. It was measured in the middle of GM belly, using a needle intramuscular thermistor (MKA08050-A, Ellab, Roedovre, Denmark) inserted at ~1.5 cm from below the skin under local anesthesia (Xylocaïne, 3mL). Heart rate was measured with a chest strap (Garmin, Olathe, United-States), and sweat rate was calculated from changes in body mass corrected for the amount of water consumed over cycling sessions. Perceptual responses were also collected during the first and the last cycling session. We monitored thermal sensation (TS), thermal comfort (TC) and rate of perceived exertion (RPE) every 10 min and then averaged to obtain a value for each session. Heart rate and perceptual ratings were monitored, and experimenters supervised participants throughout each cycling session in order to consistently check their health and safety.

Testing protocol

Data collection and processing. PRE and POST neuromuscular tests were performed at the same time of day, with POST tests performed ~48 h after the thirteenth cycling session (*i.e.*, the fifteenth day) to exclude potential acute session effect. The testing sessions consisted of electrically-evoked, explosive and maximal voluntary isometric contractions, ballistic and isokinetic contractions, ramp contractions, passive shear wave elastography measurements and

verticals jumps, performed in the same order (Fig. 1C). Participant lay prone with the knees fully extended (0°) and the right ankle flexed at 90° (foot perpendicular to the tibia). The right foot was firmly fixed to the mechatronic ergometer footplate (Eracles-Technology, Compiègne, France) for the isometric and isokinetic contractions, and to a specific rotational footplate of a customized ergometer (Bio2M, Compiègne, France) for the ballistic contractions. Mechanical data were digitized at 4000 Hz, recorded synchronously by a 12-bit analog to digital converter (DT9800, Data Translation, Marlboro, USA), and were analyzed using custom scripts (Origin 2021, OriginLab Corporation, Northampton, USA and Matlab 2021b, The Mathworks, Natick, USA). The reliability of the collected biomechanical variables was evaluated in a preliminary pilot study reporting coefficient of variation ranging from 1.7% to 7.2%.

Muscle stiffness. An ultrafast ultrasound scanner (Aixplorer, v. 6, Supersonic Imagine, Aix en Provence, France) coupled with a linear transducer array (4-15 MHz, SuperLinear 15-4, Vermon, Tours, France), was used in shear wave elastography mode (musculoskeletal preset) to measure (three measurements) the shear modulus (*i.e.*, index of muscle stiffness) of the GM at rest (25). The probe was encapsulated into a custom cast over the right GM muscle belly, at 30% of the distance between the popliteal fossa and the center of the lateral malleolus. The probe location was considered appropriate when fascicles and aponeuroses were clearly visible across the image (Fig. 1Ci). Shear wave elastography image processing converted the colored map into shear elastic modulus values (Fig. 1Cii). The region of interest was inspected to exclude non-muscular structures and artefacts. The five successive maps that resulted in the lowest standard deviation (SD) of the shear modulus were averaged across trials.

Electrically-evoked contractions. The tibial nerve was electrically stimulated using a constant current stimulator (DS7AH, Digitimer, Letchworth Garden City, UK), delivering a single electrical pulse (200 µs, 400 V) through a cathode placed in the popliteal cavity and an anode placed distally to the patella (Fig. 1Ci). The intensity was adjusted for each participant by a progressive increase in amperage until plantar flexor force reached a plateau. Five stimulations were delivered at 150% of the electrical intensity required to elicit peak force (26). The mechanical response to the five electrically evoked stimulations was low-pass filtered (50 Hz, zero lag 3rd order Butterworth), analyzed and averaged to determine peak twitch amplitude (PT) and contraction time (CT). The electrically-evoked RTD was calculated as PT/CT (Fig. 1Ciii).

Voluntary isometric contractions. Participants performed five explosive rapid contractions (~1 s) to measure the RTD. The contractions were interspaced by ~30 s rest and the participants were instructed to contract *"as fast and hard as possible"* without any countermovement or pre-tension prior to the contraction onset (27). The onset of voluntary RTD was determined manually (28, 29). Briefly, torque signal was displayed with a constant y-axis scale of ~1 Nm and an x-axis scale of 500 ms. Then, a vertical cursor was placed on torque onset, displayed with a higher resolution to verify its position (29). The torque signal was low-pass filtered (150 Hz, zero-lag 3rd order Butterworth). The two trials resulting in the highest RTD from contraction onset to 200 ms (RTD_{0-200}) were averaged for analysis. RTD was calculated for specific time periods as the change in force divided by the time windows from 0 to 100 ms (RTD_{0-100}), 0 to 200 ms (RTD_{0-200}) and 100 to 200 ms ($RTD_{100-200}$). Thereafter, participants performed three 5-s isometric maximal voluntary contractions (MVC) interspaced by a 2 min rest period. MVC peak torque corresponded to the maximal force value obtained over a 500 ms moving window (Fig. 1Civ),

after filtering torque signal (150 Hz, zero lag 3rd order Butterworth). Voluntary activation (VA) was assessed during two additional MVC using an interpolated twitch evoked during the force plateau and a potentiated twitch evoked 4 s after the end of the contraction (doublet, 100 Hz; Fig. 1Cv). VA was determined as (%) as (1 – superimposed twitch / potentiated twitch) × 100. The highest VA value obtained was selected.

Active tendon stiffness. Participants linearly increased their isometric plantar flexor torque from 0 to 90% of the MVC peak torque within 9-s (Fig. 1Cvi). The participants followed the same force visual feedback for both sessions based on the PRE MVC peak torque. The task was performed twice (3 min apart) at each test session. The trial resulting in the lowest dispersion of produced torque signal according to the targeted torque during ramp contraction was considered for analysis. The displacement of the insertion of GM fascicle on the deep aponeurosis was tracked and recorded every 5% of MVC torque (sampling frequency: 100 Hz) corresponding to targeted torque level. The ankle joint torque measured by the ergometer was converted to tendon force (Ft) as $Ft = T/m_g$ (30) where m_g is the moment arm length of GM at 90° of ankle joint and knee fully extended, which was estimated from the limb length of each participant (31, 32). The active stiffness of tendon corresponded to the ratio between the change in force (in N) and the displacement of the aponeurosis (in mm) between 50 and 80% of MVC torque (33).

Muscle architecture. Ultrasonic images recorded in resting condition, ankle angle at 90°, were analyzed (Image J, National Institutes of Health, Bethesda, USA) to determine GM muscle architecture: fascicle length (L_F), pennation angle and muscle thickness.

Joint and fascicle force-velocity relationship. Participants performed maximal plantar flexors contractions over a 50° range of motion (*i.e.*, from 110 to 60°) at three isokinetic angular velocities (30, 200 and 400°.s^{-1}; three trials each) in a randomized order (similar between PRE and POST tests for each participant), with 1 min of rest between each trial. The participants were instructed to contract *"as strong and as fast as possible"* (34). Then, participants performed maximal plantar flexions from 110° to 60° of ankle flexion on the Bio2M ergometer, equipped with an angle sensor to provide ankle angle measurements, as previously described (33, 34). Two conditions (1 min rest) were tested in a randomized order (similar between PRE and POST): with 0 (five trials) or 2.6 kg (three trials) attached to the pedal. The torque measured by the isokinetic dynamometer was corrected for inertia and gravity to obtain external torque at the ankle joint. Angular velocity and plantar flexion torque were low-pass filtered (100 Hz, zero lag 3rd order Butterworth). For ballistic contractions, angular velocity was computed as the derivative of low-pass filtered (100 Hz, zero lag 3rd order Butterworth) ankle angle over time and total plantar flexion torque was calculated as the moment of inertia (*i.e.*, foot, footplate and additional loads) multiplied by the acceleration of the ankle angle (34). Ultrasound sampling frequency was adapted to the condition (500 Hz, 1000 Hz and 2000 Hz for angular velocity at 30°.s^{-1}, 200-400°.s^{-1} and high-velocity conditions, respectively). Changes in GM L_F and pennation angle were assessed on three fascicles using the UltraTrack method (28, 33, 35). Fascicle shortening velocity (V_F) was computed as the first-time derivative of L_F (low-pass filtered: 50 Hz, zero lag 3rd order Butterworth). Fascicle force was calculated from the GM muscle force, calculated from the torque divided by the Achilles tendon lever arm (36), [*i.e.*, 20.9% of total plantar flexion force (37)], divided by the cosine of the pennation angle. Joint velocity, V_F, torque and fascicle force were averaged from 100 to 70° to obtain the joint and fascicle force-velocity relationship

(Fig. 1Cvii and Cviii) (33, 34). The two trials resulting in the highest mean joint velocity were averaged. Joint and fascicle force-velocity relationships were determined using the hyperbolic equation proposed by Hill (38): $V = b \times (F_0 - F)/(F + a)$, where F_0 is maximal theoretical force, F is force, V is velocity, a and b are coefficients, determined for each participant, using a fit with F and V values obtained during ballistic and isokinetic contractions. The maximal theoretical velocity (V_0) was considered as the x-intercept of the F-V curve.

Vertical jumps performance. After the neuromuscular tests and a standardized warm-up, three squat jumps (SJ), three counter-movement jumps (CMJ) and three multi-rebound jumps (MRJ; Fig. 1Civ) (39, 40) were performed in a randomized order (similar between PRE and POST). Participants were instructed to jump *"as high as possible"*. All jumps were recorded (flight times for SJ, CMJ and MRJ and ground contact for MRJ) using a sensor system (Optojump Next, Microgate, Bolzano, Italy). Then, participants were required to perform repeated CMJ, for 15 s (41). Participants were instructed to keep their hands on their hips throughout the preparatory and jump phases. Jump height from the best SJ and CMJ trials was retained for analysis. Mean jump height and jump decrement were calculated from the 15-s period of repeated CMJ. Lower limb stiffness (K_N in $N.m^{-1}$), was measured from MRJ as:

$$K_N = \frac{M \times \pi(T_f + T_c)}{T_c{}^2\left(\frac{T_f + T_c}{\pi} - \frac{T_c}{4}\right)}$$

where M is the total body mass (kg), T_c is the ground contact time and T_f the flight time (39). From repeated CMJ mean jump height and jump height decrement were computed (41).

Statistical analysis

Statistical analyses were performed with Jamovi [2.0.0.0, The jamovi project (2021)]. The assumption of normality of the data were verified using the Kolmogorov–Smirnov test. Values were reported as mean ± SD unless otherwise stated. T_{musc} was compared between the groups using a Student paired t-test. Two-way ANOVAs (group × training) were used to assess the effect of the group (HA, CON) and training protocol (Session 1, Session 13) on physiological parameters and perceptual ratings measured during training sessions: T_{core}, heart rate, sweat rate, TS, TC and RPE. Non-parametric tests were performed to observe the effect of group (Kruskal-Wallis) and repeated training session (Friedman) on T_{skin} (non-normalized data). Two-way ANOVAs (group × training) were used to assess the effect of the group (HA, CON) and training protocol (PRE, POST) on: electrically-evoked characteristics (PT, CT, HRT, RTD), MVC, VA, RTD voluntary measures ($RTD_{0\text{-}100}$, $RTD_{0\text{-}200}$ and $RTD_{100\text{-}200}$), muscle architecture (L_F, pennation angle and muscle thickness), GM shear modulus, tendon active stiffness and vertical jump performance (SJ, CMJ, lower limb stiffness, repeated CMJ jump height and jump height decrement). On joint and fascicle force-velocity relationship, three-way ANOVAs (group × training × load) were used to determine the potential effect of group (HA, CON), training protocol (PRE, POST) and load (ballistic and isokinetic contractions) on joint velocity, joint torque, fascicle shortening velocity and fascicle force. When the sphericity assumption in repeated measures ANOVAs was violated (Mauchly's test), a Geisser-Greenhouse correction was used. When appropriate, post-hoc analyses were performed using a Bonferroni correction. Effect sizes were described in terms of partial eta-squared (η_p^2, with $\eta_p^2 \geq 0.06$ representing a moderate effect and $\eta_p^2 \geq 0.14$ a large effect). Statistical significance was set at $P < 0.05$.

RESULTS

An initial statistical analysis considering the sex of the participants was performed, using three-way ANOVAs (group × training × sex), to assess the effects of group (HA, CON), training protocol (Session1/PRE, Session 13/POST) and sex (female, male) on the physiological and biomechanical parameters. Analyses revealed that sweat rate and several parameters including neuromuscular responses (*i.e.*, PT amplitude, MVC peak force, RTD voluntary measures), muscle architecture (*i.e.*, fascicle length, muscle thickness) and jump performance (*i.e.*, SJ, CMJ, lower limb stiffness and jump decrement), were higher in males than females ($P \leq 0.032$, $\eta_p^2 \geq 0.085$). However, the analyses did not show any training × sex interactions ($P \geq 0.062$, $\eta_p^2 \leq 0.084$). The following results are therefore analyzed and reported irrespective of sex.

Protocol compliance

All participants completed all cycling sessions except one from HA group who replaced 3 cycling sessions by fast treadmill walking due to cycling discomfort and one from CON group who missed the eleventh cycling session, and so completed 12 cycling sessions. Two participants from CON group performed a 1-week break during the training protocol due to COVID-19 contact case declaration, the experimental design was shifted one week to complete the 13 cycling sessions. Compliance with the protocol was 98.5% and 94.4% for HA and CON groups, respectively.

Thermoregulatory responses

There was a main effect of group, training and an interaction effect on T_{core} (all $P \leq 0.037$, $\eta_p^2 \geq 0.08$) due to a higher T_{core} in HA group *vs*. CON group during the first session (+0.6

± 0.3°C; $P < 0.001$, $\eta_p^2 = 0.37$) and a decreased in T_{core} in HA group following intervention (-0.4 ± 0.3°C; $P = 0.015$, $\eta_p^2 = 0.22$), leading to similar average T_{core} for each group during the thirteenth session ($P = 0.879$, $\eta_p^2 = 0.06$; Fig. 2A). Average T_{skin} was higher in HA group *vs*. CON group during the first and the thirteenth cycling session (+3.8 ± 0.6°C and +3.4 ± 0.6°C; both $P < 0.001$, $\eta_p^2 \geq 0.81$), decreased in HA group following intervention (-0.4 ± 0.3°C; $P < 0.001$, $\eta_p^2 = 0.82$) and was not impacted by training in CON group ($P = 0.405$, $\eta_p^2 = 0.02$; Fig. 2B). T_{musc} measured at the end of a single cycling session was higher in HA group *vs*. CON group [38.4 ± 0.5 ($n = 8$) *vs.* 37.3 ± 1.1°C ($n = 7$); $P = 0.017$, $\eta_p^2 = 0.36$]. Heart rate was higher in HA group *vs*. CON group from first to thirteenth session ($P < 0.001$, $\eta_p^2 = 0.32$) and decreased throughout intervention ($P = 0.008$, $\eta_p^2 = 0.12$), without an interaction effect ($P = 0.733$, $\eta_p^2 < 0.01$; Fig 2C). Sweat rate was higher in HA group first to thirteenth session ($P < 0.001$, $\eta_p^2 = 0.58$) and increased along intervention ($P = 0.035$, $\eta_p^2 = 0.08$) with an interaction effect ($P = 0.014$, $\eta_p^2 = 0.10$) due to a higher sweat rate in HA group *vs*. CON group from the first to the thirteenth session (+0.5 ± 0.3 $L.h^{-1}$ and +0.8 ± 0.3 $L.h^{-1}$; both $P < 0.001$; $\eta_p^2 \geq 0.53$) and to an increasing sweat rate following intervention in HA group (+0.4 ± 0.3 $L.h^{-1}$; $P = 0.010$, $\eta_p^2 = 0.19$; Fig. 2D). In line with these physiological responses, perceptual ratings were higher in HA group *vs.* CON group ($P \leq 0.004$; $\eta_p^2 \geq 0.140$), and decreased throughout intervention ($P \leq 0.001$; $\eta_p^2 \geq 0.262$), without presenting an interaction effects ($P \geq 0.167$; $\eta_p^2 \leq 0.034$; Table 1).

Neuromuscular responses

Neither effect of group (all $P \geq 0.299$, $\eta_p^2 \leq 0.02$) or training (all $P \geq 0.225$, $\eta_p^2 \leq 0.03$) nor interaction (all $P \geq 0.659$, $\eta_p^2 < 0.01$) were revealed for PT, CT, HRT and RTD in electrically-evoked contractions, RTD voluntary measures, MVC torque production and VA

(Table 2). Joint velocity revealed a main effect of group (P = 0.032, η_p^2 = 0.02), which was higher in HA group, and a main effect of load (P < 0.001, η_p^2 = 0.79), without a training or an interaction effect (all $P \geq 0.232$, $\eta_p^2 \leq 0.01$; Fig. 3 A and B). There was no significant effect of group and training on joint torque (both $P \geq 0.195$, $\eta_p^2 \leq 0.01$), but a main effect of load (P < 0.001, η_p^2 = 0.79) on joint torque, which decreased with decreasing load. Any interactions were found on joint torque (all $P \geq 0.479$, $\eta_p^2 \leq 0.01$; Fig. 3 A and B; Supplemental Figure, Supplemental Digital Content, Joint and fascicle force-velocity relationship obtained and after intervention in HA and CON groups, http://links.lww.com/MSS/C790).

Muscle-tendon unit properties

Two-way ANOVA revealed neither an effect of group (all $P \geq 0.213$, $\eta_p^2 \leq 0.03$), training (all $P \geq 0.583$, $\eta_p^2 \leq 0.01$) nor interaction (all $P \geq 0.793$, $\eta_p^2 \leq 0.01$) on L_F, pennation angle and muscle thickness (Table 3).

Three-way ANOVA revealed a main effect of group (P = 0.007, η_p^2 = 0.02) on V_F, which was higher in HA group, and a main effect of load (P < 0.001, η_p^2 = 0.71). No effect of training or interactions (group × training, group × load, training × load or group × training × load) were shown (all $P \geq 0.370$, $\eta_p^2 \leq 0.02$; Fig. 3 C and D). There was no significant effect of group and training (both $P \geq 0.067$, $\eta_p^2 \leq 0.01$) but a main effect of load (P < 0.001, η_p^2 = 0.83) on fascicle force. No interactions (group × training, group × load, training × load or group × training × load) were shown on fascicle force (all $P \geq 0.458$, $\eta_p^2 \leq 0.01$; Fig. 3 C and D; Supplemental Figure, Supplemental Digital Content, http://links.lww.com/MSS/C790).

Shear modulus measured in GM at rest showed an effect of group ($P = 0.012$, $\eta_p^2 = 0.11$). Shear modulus was lower in HA group, with neither an effect of training ($P = 0.454$, $\eta_p^2 = 0.01$) nor interaction ($P = 0.508$, $\eta_p^2 = 0.01$; Table 3). Active Achilles tendon stiffness was not affected by group ($P = 0.172$; $\eta_p^2 = 0.04$) or training ($P = 0.920$; $\eta_p^2 < 0.01$) with no interaction effect ($P = 0.923$; $\eta_p^2 < 0.01$; Table 3).

Jump performance

There was no significant effect of group (all $P \geq 0.244$, $\eta_p^2 \leq 0.03$), training (all $P \geq 0.332$, $\eta_p^2 \leq 0.02$) or interaction (all $P \geq 0.899$, $\eta_p^2 \leq 0.03$) on SJ and CMJ height, lower limb stiffness, repeated CMJ mean jump height or jump height decrement (Table 4).

DISCUSSION

The present study investigated for the first time the effect of an active HA on the muscle-tendon unit properties. While the current 13-session active HA elicited effective physiological adaptation (*i.e.*, decrease in T_{core} and heart rate, and increase in sweat rate from the first to the last session), none of the muscle-tendon unit properties (*i.e.*, force-velocity properties, tissue stiffness, voluntary activation) and multi-joint dynamic performance were affected, positively or negatively, from PRE to POST. These findings improve the understanding of human adaptations of motor performance to repeated heat exposure and provide relevant mechanistic understanding that may help to update current HA prescription in reference to sport-specific neuromuscular requirements. Active HA, essential to induce physiological adaptations, is not detrimental to motor performance and force-velocity properties and can therefore be used by all athletes

preparing competition in hot environments, whatever their sporting discipline, without fear of altering their muscle-tendon unit properties.

HA phenotype

While the CON group showed a decrease in heart rate (-10 ± 7 bpm) with training, they did not show any changes in T_{core}, T_{skin} or sweat rate (Fig. 2). Conversely, the HA group showed a decrease in T_{core} and T_{skin} (both, -0.4 ± 0.3°C), a decrease in heart rate (-13 ± 11 bpm) and an increase of sweat rate (+0.4 ± 0.3 $L.h^{-1}$) from the first to the last cycling session. These results reflect a reduced physiological strain and better thermoregulatory abilities while exercising in the heat after intervention (6, 8). Reduced T_{core} and increased sweat rate, together with faster sweating onset at lower threshold (42) are indicative of central and peripheral physiological adaptations (5) to heat. These results are in line with the existing literature on human HA (5) and the fact that HA is the primary countermeasure recommended before competing in the heat (6). Accordingly, perceptual ratings were higher in the HA group and decreased with training, showing that acclimation was also perceptually effective.

However, recent observations reported that athletes requiring explosive strength are less likely to use HA; with only 16 of 50 elite sprinters, jumpers and throwers (*i.e.*, 32%) adopting HA before the 2019 IAAF World Championships in Doha (unpublished data), while the proportion is double (*i.e.*, 63%) in road-race endurance athletes using HA (43). Thus, the current study investigating the effect of HA on muscle-tendon unit mechanical properties is likely to support those athletes in taking an informed decision by determining the positive or negative effect of active HA on sport-specific muscle capabilities.

Active HA does not impact single-joint performance

In line with Racinais et al. (12), contraction time and half-relaxation time inferred from electrically-evoked contractions were unchanged following intervention in HA and CON groups. However, those authors noted an increase in peak twitch amplitude and MVC torque from PRE- to POST-HA, that were not observed in the current study. Importantly, despite analyzing the same muscle, the HA protocol was different (*i.e.*, passive *vs.* active). Using localized heat therapy (*i.e.*, hot pack application increasing *vastus lateralis* T_{musc} from 34.9 ± 0.5°C to 38.3 ± 0.1°C), others studies have reported an increase of knee extensors maximum torque after 10-weeks [8 h/day, 4 days a week (13)] or after 8-weeks of application [90 min/day, 5 days a week (14)]. Nevertheless, 6-weeks of local heat therapy on plantar flexors (*i.e.*, 8 h/day using heat pads, 5 days/week) induced no effect on strength and contractile properties of plantar flexors in active participants (44). Active short-term HA also showed no impact on knee extensor maximal force and VA (45). Recent studies also showed that adding local repeated heat stress during a long period (*i.e.*, 10-12 weeks) of resistance training had no effect on muscle strength production (46, 47). Therefore, repeated heat exposure may not further increase muscle force in participant already exposed to mechanical, and metabolic, stress elicited by strength training (46, 47).

The current study adds to these previous observations that joint force-velocity properties, including maximal theoretical joint torque and joint velocity, were unchanged in HA and CON groups following intervention (Fig. 3). Moreover, the absence of effects described above during voluntary and electrically-evoked isometric contractions was also observable during ballistic and isokinetic contractions, leading to no changes in velocity indices. Taken together, the current data confirm that repeated heat exposure is not detrimental to muscle force production.

Active HA does not impact muscle-tendon interactions

Muscle-tendon unit properties were also unaffected from PRE to POST in HA and CON groups. Muscle architecture (*i.e.*, fascicle length, pennation angle and muscle thickness) remained unchanged at the end of the intervention ($P \geq 0.583$), while we might have expected an increase in muscle thickness in the HA group, based on the findings from a previously mentioned study. Indeed, an increase in cross-sectional areas of *vastus lateralis* and *rectus femoris* was reported after 10-weeks of localized heat therapy (13), suggesting that heat stress might stimulate the intracellular signaling(s) responsible for protein synthesis and therefore muscle hypertrophy. However, the 6-weeks protocol, investigating the same muscle group as in the current study, did not induce change neither plantar flexors MVC peak force nor *gastrocnemius lateralis* cross-sectional area (44). As described above, adding local repeated heat stress during a resistance training protocol has not consistently shown beneficial effects on muscle hypertrophy, depending on the dose of heat exposure and the activity of the participants beside the heat exposures (46, 47). The present lack of change in muscle thickness after HA suggested an absence of hypertrophy and therefore may explain unchanged strength production, this absence of effect is in accordance with the unaffected single-joint performance results, following HA. While aforementioned studies used long-term and partial repeated heat exposure (*i.e.*, between 6 and 10 weeks, and local heat application), it differs from our 13 sessions of total active heat exposure, potentially insufficient to induces similar adaptations. The different heating methods could thereafter lead to different effects.

T_{musc} measured at the end of a single cycling session was higher in the HA group *vs*. CON group (38.4 ± 0.5 *vs*. 37.3 ± 1.1°C), however, T_{musc} was probably not sufficiently high enough to induce changes in hypertrophy-related skeletal muscle signaling. Indeed, Ihsan et al. (16) showed that increasing *vastus lateralis* T_{musc} to 38.8 ± 0.5°C enhanced anabolic signaling through the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway, while an increase of T_{musc} to 38.1 ± 0.6°C induced none of these changes. This is in line with an activated Akt/mTOR signaling pathways, a crucial mediator of protein synthesis and hypertrophy, reported in animals' study after heat stress (17), with higher responses at 41°C, and might suggest that a larger dose of heat stress may be required to observe the aforementioned effects. Thus, the present findings could highlight an insufficient T_{musc} increase in response to heat to activate anabolic signaling pathways involved in muscle hypertrophy. However, it is an assumption that should be investigated since muscle groups and temperatures range were different. Moreover, the current data showed that active HA did not alter maximal theoretical fascicle force and shortening velocity, or fascicle force-velocity relationship of GM muscle (Fig. 3C). To the best of our knowledge, these muscle-tendon unit properties have been investigated, for the first time *in vivo* in humans. Thereby, irrespectively of the methodological aspect discussed above (*i.e.*, activity of the participants, level of temperature, muscle group investigated) the current data showed that repeated heat exposure has neither advantageous, nor detrimental effect on fascicle force-velocity properties.

Soft tissues stiffness, measured through passive GM and active Achilles tendon stiffness, was also unaffected from PRE to POST in HA and CON groups. Although our recent study reported an acute decrease in passive GM muscle and active Achille tendon stiffness at the end

of a passive acute heat exposure (33), no study has yet reported the effects of active HA on soft tissues stiffness. These findings suggest that changes in tissues stiffness induced by acute heat exposure are transient and do not translate into chronic adaptations after repeated active HA sessions. Unaffected muscle and tendon stiffness suggest no impact on the metabolic cost, at least linked to soft tissues stiffness (48), reinforcing the non-alteration of muscle-tendon unit interactions and their absence of negative effect on performance following active HA.

Active HA does not impact multi-joint dynamic performance

In accordance with the previous sections, the absence of HA effect at the muscle and fascicle level is similar at multi-joint level. Vertical jump performance (*i.e.*, SJ and CMJ height, repeated CMJ mean jump height and jump height decrement) and lower limb stiffness measured during MRJ were unchanged in HA and CON groups following intervention. Peak vertical height during SJ and CMJ was also unchanged following 5-day active HA, cycling at high- or low-intensity at 32°C (49). These data reinforce the fact that active HA does not impact coordination and muscle control and their potential impact on motor performance, given that multi-joint dynamic movements, closer to *in situ* situations encountered in many sports activities and related to performance, were also unchanged.

Significance and Perspectives

Even if muscle architecture was unchanged in the current study, it is however important to keep in mind that the morphological adaptations at the origin of muscle hypertrophy remain difficult to generalize (50). Moreover, although the absence of changes in two-dimensional architecture suggest an absence of modifications in three-dimensional, it would be interesting to

investigate the effects of repeated heat exposure, with precedent protocol inducing muscle hypertrophy, using three-dimensional shape and architecture of human muscles from *in vivo* imaging data.

This study includes novel practical information potentially useful to the coaches and athletes, who may be reluctant to use HA in sporting disciplines requiring explosive movements. Our findings showed that 13 low-intensity cycling sessions performed in hot and humid environment is beneficial to acclimate athletes from a physiological point of view without altering muscle-tendon mechanical properties, nor muscle and multi-joint performance. Therefore, the current data suggest that active HA before competing in the heat will not alter isometric and dynamic strength capacities, joint velocity, explosivity, soft tissues stiffness and vertical jump performance. Reluctant practitioners and athletes from explosive sporting disciplines may therefore use active HA without altering muscle performance. It also opens up a perspective: it would be interesting to understand why some coaches and athletes, especially in explosive activities, are reluctant to use HA although the benefits from a physiological point of view are well known, and whether our study could help the more reluctant to use HA. It would also be interesting to investigate the effect of active HA in a more practical context, in a view to better implement such strategy on ballistic performance in a field sport context, for example.

Of note, the effects described in the current study are resulting from testing performed in temperate environment; and it remains unclear how this absence of effects may impact, or not, the acute muscle-tendon unit properties responses to heat exposure during exercise, especially since we have recently demonstrated a modification of some of these properties [*i.e.*, faster early

RTD, rightward shift of the joint force-velocity relationship and reduced soft tissues stiffness following an acute passive heat exposure (33)]. An interesting perspective from this work would be to investigate the effects of an active HA on the acute responses of a low-intensity cycling session performed in hot and humid environment.

CONCLUSIONS

This study explored for the first time the *in vivo* changes in muscle-tendon properties and interactions elicited by active heat acclimation. Low-intensity cycling sessions protocol performed in heat (~38°C and 58% relative humidity) induced heat acclimation, as evidenced by a decrease in physiological stress/strain from the first to the last session. However, active heat acclimation did not impact muscle-tendon unit properties (*i.e.*, electrically-evoked contractile properties, maximal voluntary peak and explosive torque production, voluntary activation, joint force-velocity relationship, muscle architecture, passive muscle and active Achille tendon stiffness and fascicle force-velocity relationship) and did not modified dynamic movements such as vertical jump performance. These findings may contribute to refine recommendations dealing with HA in reference to sport-specific muscle and tendon mechanical properties. This may further reassure coaches and athletes preparing for hot and humid environments that an active HA, necessary to induce physiological adaptations and to cope with the heat, will not impact muscular properties and sport-specific related performance.

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Conflict of Interest

None of the authors has any conflicts of interest to declare with companies or manufacturers who will benefit from the results of the present study. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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FIGURES LEGENDS

Figure 1. Experimental design: general overview (A), physiological monitoring during cycling sessions (B) and data collection during testing sessions (C). Neuromuscular testing (Ci), shear modulus of the *gastrocnemius medialis* (GM) (Cii), PT, CT, HRT and RTD from electrically-evoked contractions (Ciii), MVC peak torque, voluntary RTD (Civ), VA (Cv), ramp contraction performed from 0 to 90% of MVC peak torque (Cvi), joint and fascicle force-velocity relationships (Cvii, Cviii) and vertical jumps (Cix) performed before (PRE) and after (*i.e.*, 15^{th} day, POST) intervention in heat acclimation (HA) and control (CON) groups.

Figure 2. Mean core temperature [T_{core}; (A)], mean skin temperature [T_{skin}; (B)], mean heart rate (C) and sweat rate (D) measured during the first (Session 1) and the last (Session 13) cycling session in heat acclimation (HA) and control (CON) groups. The bold trace represents the mean change of the whole sample (n = 15 for each parameter in each group, except for T_{skin}: n = 14 in each group), box charts correspond to SD and dashed traces connect individual values. * Significant difference between HA and CON, † significant difference between session 1 and session 13, ‡ significant difference between HA and CON for the same cycling session, $P < 0.05$.

Figure 3. Joint (A, B) and fascicle (C, D) force-velocity relationship obtained before (PRE) and after (POST) intervention in heat acclimation (HA) and control (CON) groups. Filled area represents SD and circles represent mean data obtained during isokinetic contractions (30, 200 and 400°.s^{-1}) and ballistic contractions (0 and 2.6 kg). * Significant differences between HA and

CON groups on joint and fascicle shortening velocity, $P < 0.05$. $n = 15$ in each group for panel A and B; $n = 14$ for panel C and $n = 13$ for panel D.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplemental Digital Content.docx

Supplemental Table: Exercise prescription for the cycling sessions performed in hot and thermoneutral environment.

Supplemental Figure: Joint and fascicle force-velocity relationship obtained before and after intervention in HA and CON) groups.

Figure 1

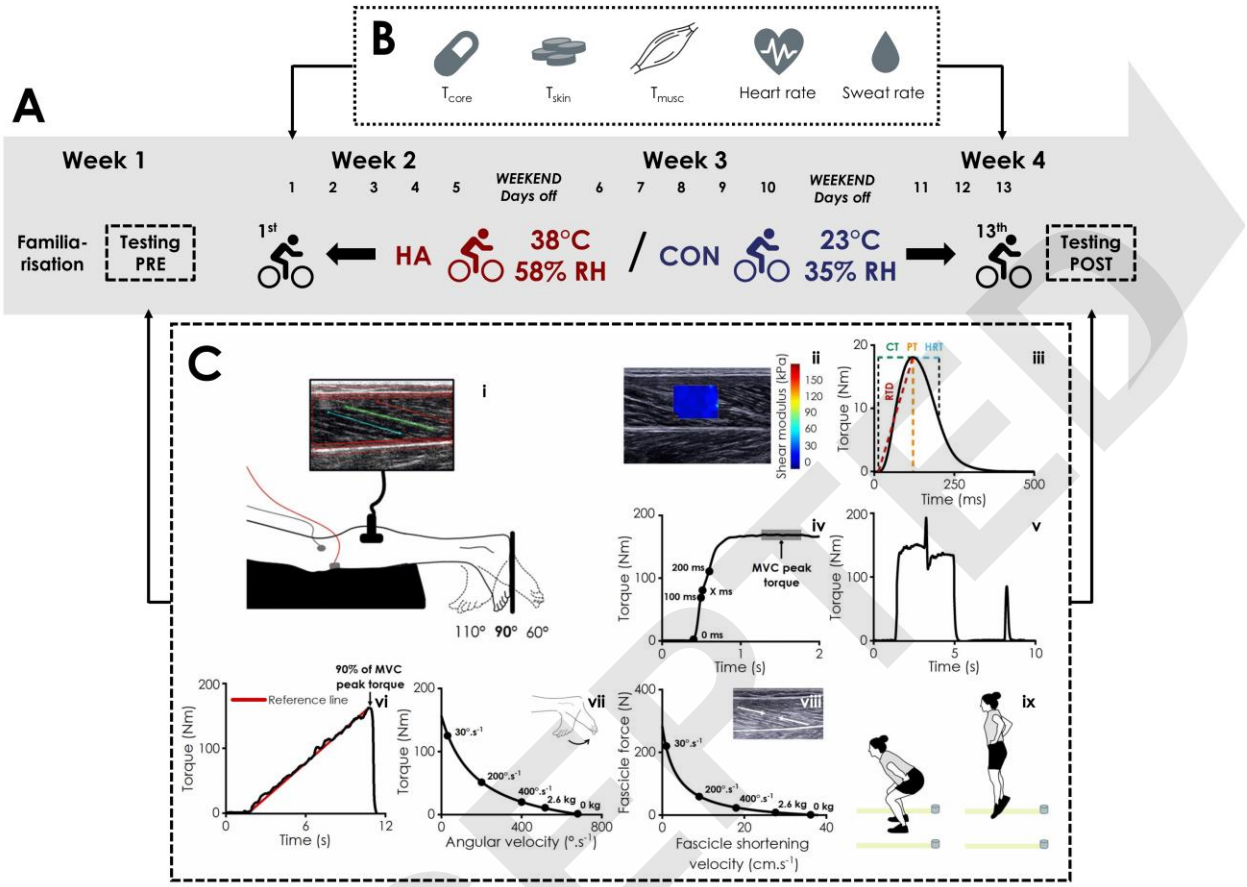


Figure 2

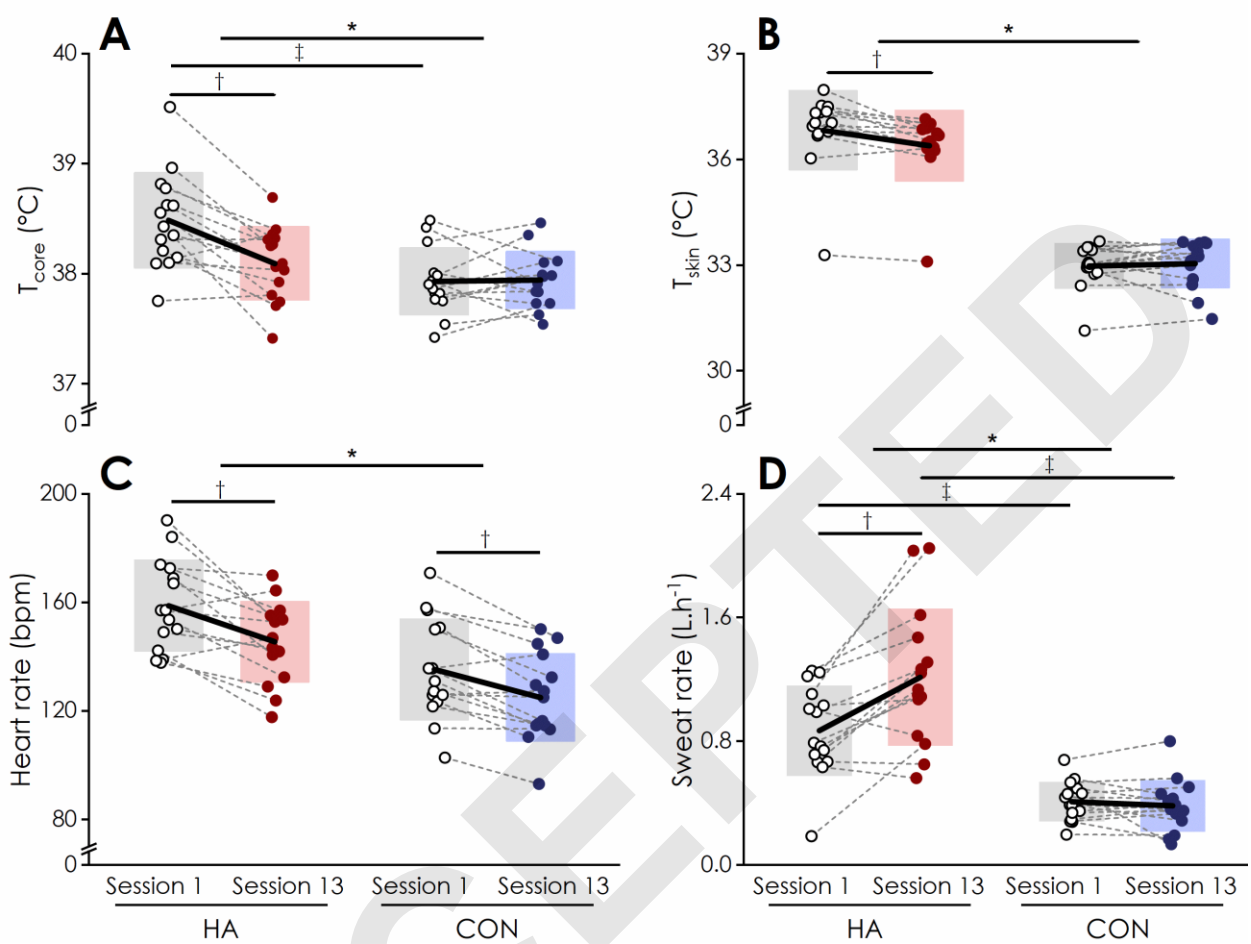


Figure 3

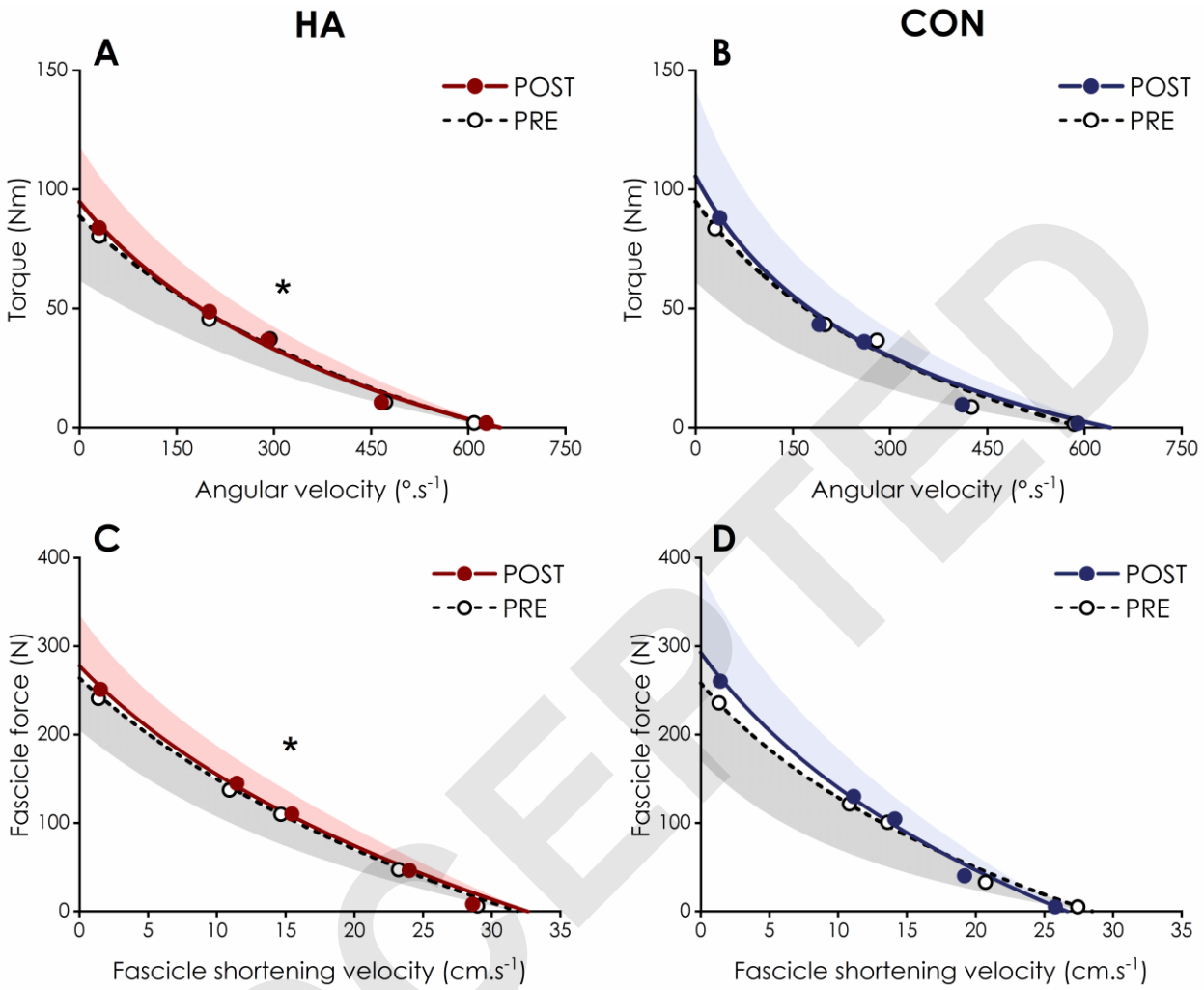


Table 1. **Mean perceptual responses measured during the first (Session 1) and the thirteenth (Session 13) cycling session of the training protocol in heat acclimation (HA) and control (CON) group.**

	HA		CON		Main effect		
	Session 1	**Session 13**	**Session 1**	**Session 13**	**Group**	**Training**	**Interaction**
TS	6.4 ± 0.4	5.9 ± 0.4	5.3 ± 0.3	4.8 ± 0.4	***P* < 0.001** $\eta_p^2 = 0.612$	***P* < 0.001** $\eta^2_p = 0.262$	*P* = 0.859 $\eta^2_p < 0.001$
TC	6.1 ± 0.3	5.4 ± 0.4	4.9 ± 0.2	4.5 ± 0.4	***P* < 0.001** $\eta_p^2 = 0.279$	***P* < 0.001** $\eta_p^2 = 0.626$	*P* = 0.199 $\eta_p^2 = 0.029$
RPE	14.3 ± 1.9	12.1 ± 1.4	10.8 ± 1.5	10.0 ± 1.5	***P* = 0.004** $\eta_p^2 = 0.140$	***P* < 0.001** $\eta_p^2 = 0.362$	*P* = 0.167 $\eta_p^2 = 0.034$

Values are presented as mean ± SD. TS, thermal sensation (1 to 7, cold to hot); TC, thermal comfort (1 to 7; too cool to much too warm) and RPE, rate of perceived exertion (6 to 20, no exertion at all to maximal exertion). *n* = 15 in each group.

Table 2. **Mechanical parameters measured before (PRE) and after (POST) training protocol in heat acclimation (HA) and control (CON) group.**

	HA		CON		Main effect		
	PRE	**POST**	**PRE**	**POST**	**Group**	**Training**	**Interaction**
PT (Nm)	16.9 ± 4.4	16.2 ± 3.8	18.1 ± 4.2	17.5 ± 3.6	$P = 0.305$ $\eta_p^2 = 0.02$	$P = 0.926$ $\eta_p^2 < 0.01$	$P = 0.689$ $\eta_p^2 < 0.01$
CT (ms)	107 ± 8	103 ± 9	108 ± 8	105 ± 7	$P = 0.299$ $\eta_p^2 = 0.02$	$P = 0.558$ $\eta_p^2 < 0.01$	$P = 0.822$ $\eta_p^2 < 0.01$
HRT (ms)	92 ± 7	97 ± 9	95 ± 12	93 ± 12	$P = 0.848$ $\eta_p^2 < 0.01$	$P = 0.497$ $\eta_p^2 = 0.01$	$P = 0.976$ $\eta_p^2 < 0.01$
RTD electrically-evoked ($Nm.ms^{-1}$)	0.16 ± 0.05	0.16 ± 0.04	0.17 ± 0.04	0.17 ± 0.03	$P = 0.549$ $\eta_p^2 < 0.01$	$P = 0.898$ $\eta_p^2 < 0.01$	$P = 0.659$ $\eta_p^2 < 0.01$
$RTD_{0\text{-}100}$ ($Nm.s^{-1}$)	575 ± 152	574 ± 161	549 ± 147	548 ± 136	$P = 0.588$ $\eta_p^2 < 0.01$	$P = 0.983$ $\eta_p^2 < 0.01$	$P = 0.996$ $\eta_p^2 < 0.01$
$RTD_{0\text{-}200}$ ($Nm.s^{-1}$)	483 ± 110	511 ± 134	501 ± 116	509 ± 108	$P = 0.847$ $\eta_p^2 < 0.01$	$P = 0.643$ $\eta_p^2 < 0.01$	$P = 0.795$ $\eta_p^2 < 0.01$
$RTD_{100\text{-}200}$ ($Nm.s^{-1}$)	391 ± 111	448 ± 122	452 ± 99	469 ± 121	$P = 0.847$ $\eta_p^2 < 0.01$	$P = 0.643$ $\eta_p^2 < 0.01$	$P = 0.795$ $\eta_p^2 < 0.01$
MVC peak torque (Nm)	129 ± 20	136 ± 23	126 ± 32	131 ± 31	$P = 0.669$ $\eta_p^2 < 0.01$	$P = 0.508$ $\eta_p^2 < 0.01$	$P = 0.875$ $\eta_p^2 < 0.01$
VA (%)	96 ± 2	95 ± 4	93 ± 7	93 ± 5	$P = 0.778$ $\eta_p^2 < 0.01$	$P = 0.225$ $\eta^2_p = 0.03$	$P = 0.693$ $\eta_p^2 < 0.01$

Values are presented as mean ± SD. PT, peak twitch amplitude; CT, contraction time; HRT, half-relaxation time; RTD, rate of torque development; $RTD_{0\text{-}100}$, rate of torque development between contraction onset and 100 ms; $RTD_{0\text{-}200}$, rate of torque development between contraction onset and 200 ms; $RTD_{0\text{-}100}$, rate of torque development between 100 and 200 ms after contraction onset; MVC, maximal voluntary contraction; VA, voluntary activation. $n = 15$ in each group, except for VA ($n = 12$ in HA group and $n = 11$ in CON group).

Table 3. **Muscle architecture and tissues stiffness measured before (PRE) and after (POST) training protocol in heat acclimation (HA) and control (CON) group.**

	HA		CON		Main effect		
	PRE	**POST**	**PRE**	**POST**	**Group**	**Training**	**Interaction**
Muscle architecture							
Fascicle length (cm)	5.2 ± 0.5	5.2 ± 0.5	5.4 ± 0.5	5.5 ± 0.5	$P = 0.213$ $\eta_p^2 = 0.03$	$P = 0.743$ $\eta_p^2 < 0.01$	$P = 0.793$ $\eta_p^2 < 0.01$
Pennation angle (°)	16.4 ± 1.6	16.3 ± 2.3	16.6 ± 1.9	16.6 ± 2.1	$P = 0.667$ $\eta_p^2 < 0.01$	$P = 0.934$ $\eta_p^2 < 0.01$	$P = 0.922$ $\eta_p^2 < 0.01$
Muscle thickness (cm)	1.52 ± 0.20	1.55 ± 0.25	1.59 ± 0.24	1.65 ± 0.23	$P = 0.267$ $\eta_p^2 = 0.02$	$P = 0.583$ $\eta_p^2 < 0.01$	$P = 0.797$ $\eta_p^2 < 0.01$
Tissues stiffness							
GM shear modulus (kPa)	13.2 ± 1.6	13.1 ± 1.5	15.2 ± 2.3	14.3 ± 1.9	$\mathbf{P = 0.012}$ $\eta_p^2 = 0.11$	$P = 0.454$ $\eta^2_p = 0.01$	$P = 0.508$ $\eta_p^2 = 0.01$
Active Achilles tendon stiffness ($N.mm^{-1}$)	25.2 ± 7.6	25.2 ± 7.3	22.0 ± 5.4	22.4 ± 5.8	$P = 0.172$ $\eta_p^2 = 0.04$	$P = 0.920$ $\eta_p^2 < 0.01$	$P = 0.923$ $\eta_p^2 < 0.01$

Values are presented as mean ± SD. GM, *gastrocnemius medialis*. $n = 15$ in each group except for active tendon stiffness where $n = 12$ in CON group.

Table 4. **Jump performance measured before (PRE) and after (POST) training protocol in heat acclimation (HA) and control (CON) group.**

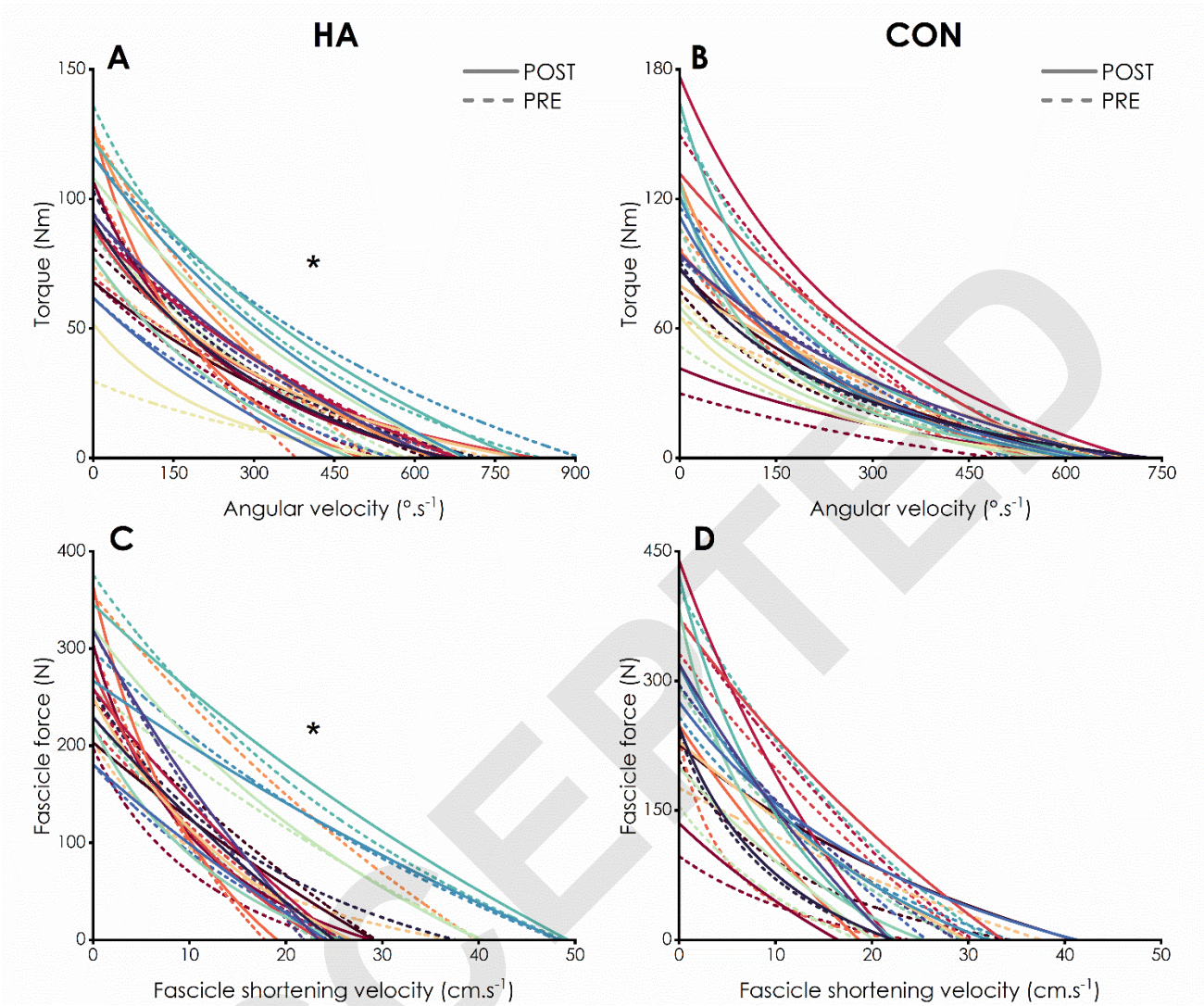
	HA		CON		Main effect		
	PRE	**POST**	**PRE**	**POST**	**Group**	**Training**	**Interaction**
SJ (cm)	27.6 ± 6.6	25.9 ± 6.4	26.6 ± 5.4	24.7 ± 4.5	$P = 0.529$ $\eta_p^2 < 0.01$	$P = 0.332$ $\eta^2_p = 0.02$	$P = 0.973$ $\eta^2_p = 0.03$
CMJ (cm)	29.6 ± 7.1	28.3 ± 6.8	28.6 ± 5.9	28.2 ± 5.1	$P = 0.889$ $\eta_p^2 < 0.01$	$P = 0.780$ $\eta_p^2 < 0.01$	$P = 0.899$ $\eta_p^2 < 0.01$
Lower limb stiffness ($kN.m^{-1}$)	27.1 ± 6.2	26.8 ± 6.0	25.1 ± 4.1	24.4 ± 4.0	$P = 0.244$ $\eta_p^2 = 0.03$	$P = 0.767$ $\eta_p^2 < 0.01$	$P = 0.904$ $\eta_p^2 < 0.01$
Repeated CMJ mean height (cm)	26.5 ± 6.9	25.7 ± 6.8	24.9 ± 5.0	24.6 ± 4.4	$P = 0.475$ $\eta_p^2 = 0.01$	$P = 0.741$ $\eta_p^2 < 0.01$	$P = 0.899$ $\eta^2_p = 0.03$
Jump decrement (%)	11.9 ± 3.9	12.4 ± 3.9	11.8 ± 3.2	11.4 ± 2.8	$P = 0.504$ $\eta_p^2 = 0.01$	$P = 0.770$ $\eta_p^2 < 0.01$	$P = 0.964$ $\eta^2_{p\,=} 0.03$

Values are presented as mean ± SD. SJ, squat jump; CMJ, counter movement jump; lower limb stiffness measured during multi-rebound jumps and jump decrement measured during repeated CMJ. In HA group $n = 14$ for SJ and CMJ and $n = 12$ for multi-rebound jumps and repeated CMJ. $n = 15$ in CON group.

Supplemental Table. Exercise prescription for the cycling sessions performed in hot and thermoneutral environment. Pedaling intensity was adjusted for each participant, according to body mass, and varied from one session to another.

	Time and corresponding target intensity ($W.kg^{-1}$)
Session 1	0 to 60 min: 1.4
Session 2	0 to 15 min: 1.3 / 15 to 45 min: 1.6 / 45 to 60 min: 1.4
Session 3	0 to 15 min: 1.3 / 15 to 25 min: 1.8 / 25 to 35 min: 1.4 / 35 to 45 min: 1.8 / 45 to 60 min: 1.3
Session 4	0 to 20 min: 1.4 / 20 to 40 min: 1.7 / 40 to 60 min: 1.5
Session 5	0 to 15 min: 1.4 /15 to 18 min: 2.2 /18 to 21 min: 1.1 / 21 to 24 min: 2.2 / 24 to 27 min: 1.1 /27 to 30 min: 2.2 /30 to 33 min: 1.1 / 33 to 36 min: 2.2 / 36 to 39 min: 1.1 / 39 to 42 min: 2.2 / 42 to 45 min: 1.1 / 45 to 60 min: 1.4
Session 6	0 to 20 min: 1.4 / 20 to 40 min: 1.7 / 40 to 60 min: 1.5
Session 7	0 to 15 min: 1.4 / 15 to 15 min 30: sprint / 15 min 30 to 20 min: 1.3 / 20 to 20 min 30: sprint / 20 min 30 to 25 min: 1.3 / 25 to 25 min 30: sprint / 25 min 30 to 30 min: 1.3 / 30 to 30 min 30: sprint / 30 min 30 to 35 min: 1.3 / 35 to 35 min 30: sprint / 35 min 30 to 40 min: 1.3 / 40 to 40 min 30: sprint / 40 min 30 to 45 min: 1.3 / 45 to 60 min: 1.4
Session 8	0 to 15 min: 1.3 / 15 to 45 min: 1.6 / 45 to 60 min: 1.4
Session 9	0 to 20 min:1.4 / 20 to 21 min: 2.5 / 21 to 23 min: 1.1 / 23 to 24 min: 2.5 / 24 to 26 min: 1.1 / 26 to 27 min: 2.5 / 27 to 29 min: 1.1 / 29 to 30 min: 2.5 / 30 to 32 min: 1.1 / 32 to 33 min: 2.5 / 33 to 35 min: 1.1 / 35 to 36 min: 2.5 / 36 to 38 min: 1.1 / 38 to 39 min: 2.5 / 39 to 41 min: 1.1 / 41 to 42 min: 2.5 / 42 to 44 min: 1.1 / 44 to 60 min: 1.4
Session 10	0 to 20 min: 1.4 / 20 to 30 min: 1.9 / 30 to 35 min: 1.5 / 35 to 45 min: 1.9 / 45 to 60 min: 1.4
Session 11	0 to 15 min: 1.4 / 15 to 17 min: 2.1 / 17 to 18 min: 1.1 / 18 to 20 min: 2.1 / 20 to 21 min: 1.1 / 21 to 23 min: 2.1 / 23 to 24 min: 1.1 / 24 to 26 min: 2.1 / 26 to 27 min: 1.1 / 27 to 29 min: 2.1 / 29 to 30 min: 1.1 /30 to 32 min: 2.1 / 32 to 33 min: 1.1 / 33 to 35 min: 2.1 / 35 to 36 min: 1.1 / 36 to 38 min: 2.1 / 38 to 39 min: 1.1 / 39 to 41 min: 2.1 / 41 to 42 min: 1.1 / 42 to 44 min: 2.1 / 44 to 45 min: 1.1 / 45 to 60 min: 1.4
Session 12	0 to 20 min: 1.4 / 20 to 40 min: 1.7 / 40 to 60 min: 1.5
Session 13	0 to 60 min: 1.4

For each cycling session the time period and the corresponding intensity are specified.



Supplemental Figure. Joint (A, B) and fascicle (C, D) force-velocity relationship obtained before (PRE; dashed lines) and after (POST; solid lines) intervention in heat acclimation (HA) and control (CON) groups. Each color represents an individual participant within HA and CON groups (but two different participants between HA and CON groups). * Significant differences between HA and CON groups on joint and fascicle shortening velocity, $P < 0.05$. $n = 15$ in each group for panel A and B; $n = 14$ for panel C and $n = 13$ for panel D.