# Heat acclimation has a protective effect on the central but not peripheral nervous system

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**Running head:** Neural and cognitive adaptations to heat acclimation

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**ABSTRACT**

This study aimed to clarify the pathway mediating hyperthermia-induced alterations in neural drive transmission, and determine if heat acclimation protects voluntary muscle activation and cognitive function in hyperthermic humans. Electrically evoked potentials (H-reflex and M-wave), executive function (special planning and working memory) and maximal voluntary isometric contractions (120 s) were assessed in fourteen participants in control condition (CON, 24ºC, 40% RH) and hyperthermic states (HYP, 44-50ºC, 50% RH), on consecutive days in a counterbalanced order. Thereafter, Participants were passively heat acclimated for 11 days (1 h per day, 48-50ºC, 50% RH) before repeating the initial assessments. Heat acclimation decreased rectal temperature in CON (-0.2ºC, $p<0.05$), but participants were maintained at ~39ºC in HYP. Heat acclimation increased the time required to reach 39ºC (+9 min), along with sweat rate (+0.7 l.h-1) and serum eHSP72 (+20%) in HYP ($p<0.05$). M-wave and H-reflex amplitudes were lower in HYP than CON ($p<0.05$) and were not protected by heat acclimation. Nerve conduction velocity was faster in HYP than CON ($p<0.05$) without being influenced by heat acclimation. These results suggest that peripheral neural drive transmission in the hyperthermic state is primarily affected by axonal conduction velocity rather than synaptic failure. Executive function, voluntary activation, and the ability to sustain torque were impaired in HYP ($p<0.05$). However, despite no perceptual changes ($p>0.05$), heat acclimation restored executive function, whilst protecting the ability to sustain voluntary activation and torque production during a prolonged contraction in hyperthermia ($p<0.05$). Ultimately, heat acclimation induces beneficial central but not peripheral neural adaptations.

## NEW & NOTEWORTHY

- Heat acclimation restores planning accuracy and working memory in hyperthermic humans, together with the supraspinal capacity to sustain motor drive during a sustained maximal voluntary contraction.
- Electrically evoked potential data (M-wave, H-reflex) indicate that heat acclimation does not protect against hyperthermia-induced impairments in peripheral neural drive transmission.
- Heat acclimation induces beneficial central but not peripheral neural adaptations.

**Key words:** Hyperthermia, Temperature, Acclimatization, Cognitive function, Electromyography, Exercise

## INTRODUCTION

The nervous system is vulnerable to hyperthermia (18). For example, hyperthermia has been shown to decrease neural drive transmission at both the level of the peripheral nervous system and the spinal cord (25, 26). However, there is contention regarding the mechanism(s) altering neural transmission at high temperatures. On one side, a negative linear correlation has been observed between peripheral (i.e. skin) temperature and the amplitude, duration, area and latency of a compound action potential (5). This suggests a shortening of the time that the voltage-gated sodium channels remain open with increasing temperature, leading to a decrease in the amplitude, duration and area of a single axon potential (34). Such a mechanism may be considered a side effect of the relationship between temperature and the rate of a chemical reaction, and is likely dependent on the absolute temperature only. Conversely, recent *in vitro* studies suggest that the decrement in neural transmission with hyperthermia could be partly linked to synaptic failure (16, 17). Importantly, such a failure in synaptic transmission was shown to be reversed by heat acclimation (17). Therefore, determining if heat acclimation protects transmission of the neural drive would allow to clarify the mechanism by which hyperthermia affects neural transmission in humans.

In addition, hyperthermia also decreases humans capacity to sustain voluntary activation (VA) for more than a few seconds (23, 26, 37). This reduction occurs independently of peripheral and spinal alterations in neural drive transmission (26), and is therefore likely due to a perturbation of supraspinal neural drive generation. The effect of hyperthermia on cerebral function is also visible through a decrease in cognitive function (9, 12, 13, 21). Cognitive impairment has been attributed to the

alliesthesial responses to hot environments (8), limiting the cognitive resources available to perform complex tasks (11, 13). There is currently no evidence that heat acclimation can protect the supraspinal generation of neural drive or cognitive function from the effects of hyperthermia. Given however, that heat acclimation might improve thermal comfort (19), we hypothesized that repeated passive heat exposure could partly protect the generation of neural drive and/or cognitive function from the effects of hyperthermia.

The first aim of this study was to determine the pathway(s) responsible for impaired neural drive transmission in hyperthermic humans. Based on animal studies suggesting a synaptic failure reversible by heat acclimation, heat acclimation was used as a model to inform on these pathways. The second aim of this study was to ascertain if heat acclimation can attenuate the influence of heat stress on voluntary activation during a sustained contraction and cognitive function during an executive function. It was hypothesized that heat acclimation would improve both voluntary neural drive and cognitive function in hyperthermic humans.

## METHODS

### Ethical approval

The project was approved by the Aspetar Scientific Committee (CMO/000033/fj) and by an external (Anti-Doping Laboratory Qatar) ethics committee (F2013000004). The procedures complied with the Declaration of Helsinki regarding human experimentation. Written informed consent was obtained from all participants prior to the beginning of testing.

## Participants

From an initial group of sixteen volunteers, 14 male participants (age: 33 ±8 yr, body mass: 74 ±7 kg and height: 177 ±7 cm) completed the study. The sample size was based on previous studies from our group showing an effect of inducing hyperthermia with the selected protocol on the parameters measured in the current study(25, 26). The study was conducted at the end of the winter (average temperature range 14 to 27ºC) to avoid heat acclimatization. Participants completed a Medical History Questionnaire and Physical Activity Readiness Questionnaire (PAR-Q) before being admitted to the study. None of the participants suffered from neural or muscular pathologies at the time of the experiment. None reported a history of heat-related illness. The two participants who withdrawn from the study did it for personal reason.

## General procedure

*Experimental design*

Following a familiarization session, participants completed testing trials in a normothermic state in temperate conditions (CON, 24ºC and 40% relative humidity (RH)), and in a hyperthermic state in hot ambient conditions (HYP, 44-50ºC and 50% RH) on consecutive days, at the same time-of-day, in a counter-balanced order. Following these trials, participants were passively heat acclimated for 11 days, 1 h per day (48-50ºC and 50% RH; see *Passive heat acclimation*). Participants then repeated the two initial testing trials (i.e. CON and HYP) in the same order and at the same time-of-day post-acclimation. All trials were performed in an environmental chamber (Tescor, Warminster, PA, USA).

*Familiarization session*

Several days (~1 week) before the experiments, participants were familiarized to the complete test procedure in a temperate environment, including the subjective scales, cognitive assessment, nerve stimulation procedure and performing maximal voluntary isometric contractions (MVC). All procedures were first explained and then practiced before being completed during the experimental sessions. Of note, the software used for the cognitive assessment (*see below*) provides a short familiarization repeated at the beginning each test. For the MVCs, participants were familiarized to performing isometric contractions of the plantar flexors until they were able to produce 3 MVCs with less than 3% variation in torque. Thereafter, participants performed a sustained 120 s MVC. The same equipment, positions and procedures were used for all testing trials. The dynamometric pedal was calibrated before each test session in the conditions (i.e. CON or HYP) of the experiment.

*Control normothermic trial (CON)*

Following instrumentation (i.e. rectal probe and skin temperature sensors), participants rested for 30 min in the environmental chamber before performing the cognitive assessment. Thereafter, participants were equipped with surface and intra-muscular electrodes (*see below*) and were positioned on the dynamometric chair. The electrical intensity required to elicit $M_{max}$ and $H_{max}$ was determined before evoking 6 electrical stimulations at each of these intensities at rest, followed by 6 electrical stimulations with a constant background muscle activity (i.e. 10% of maximal force, *see below*). A venous blood sample was then drawn. Thereafter, participants

performed three brief (5 s) MVCs to determine their maximal torque before performing a sustained (120 s) MVC.

*Hyperthermic trial (HYP)*

During the initial resting period, the temperature and relative humidity were set at 50ºC and 50%, respectively. Once participants reached a rectal temperature of 39ºC, the environmental temperature was adjusted between 44-50ºC to ensure rectal temperature remained at ~39ºC for the testing procedure. The testing procedure was the same as in CON.

*Passive heat acclimation*

Passive heat acclimation consisted of remaining seated in environmental conditions set to 50ºC and 50% RH for the first 10 min and then adjusted to 48ºC and 50% RH for the remaining 50 min. Based on pilot testing, this adjustment was necessary for the participants to tolerate the 60 min daily heat exposure, especially at the beginning of the acclimation period. Each participant performed the 11 sessions. In 4 instances (i.e. 3% of the total sessions), a session was terminated after 50 to 56 min due to participant discomfort. Each participant reached a rectal temperature above 38.5ºC in at least 9 of their 11 sessions. In addition, the acclimation session of day 3, day 6 and day 9 was slightly prolonged until the participant reached a rectal temperature of 39ºC (average duration 66 ±8 min).

**Testing procedure**

*Thermoregulatory responses.* Core temperature was monitored using a rectal probe inserted 15 cm beyond the anal sphincter (Ellab, Hilleroed, Denmark). Local skin

temperatures (i.e. chest, arm, thigh and lower leg) and heart rate were measured telemetrically (Equivital, Cambridge, UK). Mean skin temperature was calculated as 0.3 x chest temperature + 0.3 x arm temperature + 0.2 x thigh temperature + 0.2 x lower leg temperature(32). Sweat rate was calculated from changes in nude body mass and corrected from the amount of water consumed over the trial duration.

*Perceptual and emotional responses*

Thermal sensation(1) and thermal comfort(3) were measured using 1-7 Likert-type scales. Values were obtained before and after the cognitive assessment, the electrically evoked potentials and the sustained MVC, and subsequently averaged to represent the response during the testing trial. Before starting the cognitive assessment, the participants completed the Positive and Negative Affect Schedule (PANAS)(40). The PANAS is a 20-item self-report psychometric scale developed to measure the largely independent constructs of positive (PA) and negative (NA) affects as both states and traits(40). PANAS has a high internal consistency and displays good test-retest reliability across different time frames, as well as good external validity for general psychological stress and depressed affect (40). High-NA reflects subjective distress and unpleasurable engagement, and low NA the absence of these feelings. By contrast, PA represents the extent to which an individual experiences pleasurable engagement with the environment. The positive and negative affects scores were computed and the negative/positive affect ratio was calculated.

*Cognitive assessment*

Participants completed the One Touch Stockings of Cambridge test procedure (OTS) from the Cantab software (CANTABeclipse, Cambridge Cognition, Cambridge, UK).

This is a test of executive function, based upon the Tower of Hanoi test. It assesses both the spatial planning and the working memory subdomains. Briefly, participants were shown two displays containing three colored balls. The displays were presented in such a way that they could be perceived as stacks of colored balls held in stockings suspended from a beam. Subjects had to mentally calculate the minimum number of moves required to make the bottom display match the upper display and then to select the corresponding answer between 1 and 7 at the bottom of the screen. The outcome measures were the latency of response and the number of problems solved on the first choice (i.e. accuracy) for the highest level of complexity (i.e. OTS-6, requiring six moves), as this model was shown to be sensitive to the effect of hyperthermia (10, 11). Each measure was obtained by averaging the score obtained over four trials. In order to minimize a potential training effect throughout the protocol, each participant was first familiarized to the complete test a week before the experiment. In addition, each test was preceded by a short practice (requiring successively 1, 2, 3 and 4 moves) to ascertain a subsequent maximal performance.

*Electrically evoked potentials*

Participants were seated with ankle and knee angles of 90° and 100° respectively, and the right foot securely strapped to a dynamometric pedal (Captels, St Mathieu de Treviers, France). Percutaneous stimulations (400 V, rectangular current pulse of 0.2 ms) were delivered by a constant current stimulator (Digitimer DS7AH, Digitimer, Hertfordshire, England) to the tibial nerve. The cathode (diameter = 9 mm, Ambu Blue sensor T, Ambu A/S, Denmark) was located in the popliteal fossa (with constant pressure supplied by a strap) and the anode (5 x 9 cm) located slightly distal to the patella. Initially, the current was progressively increased in small increments (5 to 10

mA) until there was no further increase in the peak-to-peak amplitude of the electrophysiological M-wave. This intensity was subsequently increased by 50% to ascertain a plateau in M-wave amplitude ($M_{max}$)(28). Thereafter, the current was adjusted in 1 to 2 mA increments to determine the maximal peak-to-peak amplitude of the H-reflex ($H_{max}$). The M-wave and H-reflex were recorded via bipolar surface electrodes (Ambu Blue sensor T, Ambu A/S, Denmark; recording diameter 9 mm; inter-electrode distance 3 cm) over the muscle belly of the *soleus* (SOL) and *gastrocnemius medialis* (GM). M-waves were also recorded via intra-muscular electrodes (IM) inserted into the GM in 9 participants using fine wire electrode (30 mm x 27 GA, Motion Lab System, Baton Rouge, LA, USA). All signals were recorded using MP35 hardware (Biopac Systems Inc., Santa Barbara, CA) and dedicated software (BSL Pro Version 3.6.7, Biopac Systems Inc., Santa Barbara, CA). The signal was amplified (gain = 1000), filtered (30-500 Hz) and recorded at a sampling frequency of 10 kHz. Before electrode placement, the skin was shaved and washed to remove surface layers of dead skin, hair and oil, and a reference electrode was placed over the patella. Maximal M-waves and H-reflexes were measured 6 times each, both at rest and during a submaximal contraction performed at a constant intensity (determined during the familiarization session) corresponding to 10% MVC to ensure a constant level of background muscle activity(26). The 6 recordings were averaged for analyzes. The stimulations at the intensity required to elicit $M_{max}$ were interspersed by 6 s and the stimulations at the intensity required to elicit $H_{max}$ were interspersed by 20 s.

*Sustained maximal voluntary isometric contraction*

Participants were seated in the same position and apparatus than for the electrically evoked potentials. The torque was displayed on a screen in front of them with the y-axis set as their maximal torque and the x-axis representing 120 s. They were instructed to produce a maximal effort from the beginning of the contraction (i.e. to reach their maximal torque) and to sustain it through the 120 s. Torque was recorded by the dynamometric pedal and analyzed as a percentage of the maximal torque. The maximal torque was re-assessed at each trial prior to the sustained MVC as the average of 3 brief MVCs (5 s) separated by 1 min of rest. In addition, superimposed twitches were evoked via electrically evoked stimulations (doublets, $M_{max}$ intensity) of the tibial nerve at 2, 30, 60, 90 and 119 s of the sustained MVC. The ratio of the amplitude of the superimposed twitches over the amplitude of a potentiated twitch evoked 4 s after the contraction was used to assess the level of voluntary activation (VA) as: VA (%) = (1-Superimposed Twitch/Potentiated Twitch) x 100.

*Heat shock protein 72*

Venous blood samples (10 mL) were drawn from an antecubital vein to evaluate the extracellular expression of heat shock protein 72 (HSP72). The samples were collected at rest in a seated position before entering the environmental chamber, and after 60 minutes at a core temperature of 39ºC. Serum was collected from blood collection tubes (BD Vacutainer, Oxford, England) with an acrylic-based gel and a spray-dried clot activator coating. The blood samples were centrifuged in a swinging bucket rotor (Multifuge 1S/1S-R, Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 3000 rpm within 15 min of collection. The serum was stored at -80ºC for further analyses. HSP72 concentration was analyzed from enzyme-linked immune sorbent assays (ELISA) using a commercial kit (Cusabio Biotech Co., Baltimore,

MD, USA), an automatic ELISA microplate reader (Infinite® 200 PRO NanoQuant, Tecan, Mannedorf, Switzerland) and Magellan Standard software (version 7.1). The limit of sensitivity was ≤0.78 ng/ml.

**Statistical analyzes**

Data were coded in SPSS 21.0 (SPSS, Chicago, IL, US). The effects of thermal state (CON vs. HYP) and acclimation (pre- vs. post-acclimation) were analyzed for each variable by two-way analyses of variance (ANOVA) for repeated (two-sided) measures (thermal state x acclimation status). Least Squared Difference (LSD) were used for *post-hoc* pairwise comparisons. A three-way ANOVA for repeated measures (thermal state x acclimation status x 5 times) was used to analyze voluntary activation during the sustained MVC. Sidak correction for multiple comparison were applied for *post-hoc* pairwise comparisons. ANOVA assumptions were verified preceding all statistical analyzes and Greenhouse-Geisser corrections were applied where appropriate. The effect of thermal state and acclimation on the force production during the sustained MVC was analyzed by linear mixed models. Beside thermal state, its interaction with time (i.e. fatigue) was included as fixed factors and intercept with time were included as random factors. The covariance structure of the random effects was set at unstructured and all models converged. Thermal state was also categorized as CON and HYP categories and a similar analysis was conducted with condition instead of thermal state. Effect-sizes are described in terms of partial eta-squared ($\eta 2$, with $\eta 2 \geq 0.06$ representing a moderate effect and $\eta 2 \geq 0.14$ a large effect). The level of statistical significance was set at $p<0.05$. Data are reported as mean ± SD.

311 **RESULTS**

312 **Thermoregulatory responses**

313 As displayed in Table 1, environmental temperature ($p<0.001$, $\eta^2=0.998$) and RH
314 ($p<0.001$, $\eta^2=0.967$) were higher in HYP than CON, but were similar pre- and post-
315 acclimation (temperature: $p=0.836$, $\eta^2=0.003$; RH: $p=0.903$, $\eta^2=0.001$) for a given
316 condition.

317 Rectal temperature ($p<0.001$, $\eta^2=0.976$), skin temperature ($p<0.001$, $\eta^2=0.994$), sweat
318 rate ($p<0.001$, $\eta^2=0.883$), heart rate ($p<0.001$, $\eta^2=0.920$), and eHSP72 concentration
319 ($p=0.008$, $\eta^2=0.428$) were higher in HYP than CON. Acclimation decreased mean
320 rectal temperature ($p=0.007$, $\eta^2=0.445$) during the testing sessions with a significant
321 decrease in CON ($p=0.026$), but not HYP ($p=0.152$) (interaction effect: $p=0.604$,
322 $\eta^2=0.021$). However, acclimation increased the duration required to reach the target
323 core temperature of 39ºC in HYP (68 ±13 vs 59 ±15 min, $p=0.005$, $\eta^2=0.469$).
324 Acclimation increased sweat rate ($p=0.001$, $\eta^2=0.583$) due to an increase in HYP
325 ($p<0.001$), but not CON ($p=0.730$) (interaction effect: $p<0.001$, $\eta^2=0.707$).
326 Acclimation decreased mean heart rate in HYP ($p=0.013$), but not CON ($p=0.419$)
327 (interaction effect: $p=0.297$, $\eta^2=0.083$). Acclimation largely increased eHSP72
328 ($\eta^2=0.255$) although not reaching significance ($p=0.055$) due to an increase in HYP
329 ($p=0.042$), but not CON ($p=0.319$) (interaction effect: $p=0.248$, $\eta^2=0.101$).

330

331 **Perceptual and affective responses**

332 Thermal sensation ($p<0.001$, $\eta^2=0.943$) and thermal discomfort ($p<0.001$, $\eta^2=0.937$)
333 were higher in HYP than CON (Table 1), but were not affected by acclimation
334 ($p=0.196$, $\eta^2=0.135$, and $p=0.252$, $\eta^2=0.108$; respectively), nor was an interaction
335 effect displayed ($p=0.384$, $\eta^2=0.064$, and $p=0.585$, $\eta^2=0.026$; respectively). Positive

affects were lower ($p=0.021$, $\eta^2=0.346$) and negative affects higher ($p<0.001$, $\eta^2=0.674$) in HYP than CON, without an effect of acclimation ($p=0.384$, $\eta^2=0.059$, and $p=0.365$, $\eta^2=0.064$; respectively), nor was an interaction effect displayed ($p=0.909$, $\eta^2=0.001$, and $p=0.962$, $\eta^2<0.001$; respectively). Consequently, the negative/positive affects ratio was higher in HYP than CON ($p=0.001$, $\eta^2=0.607$), without an effect of acclimation ($p=0.816$, $\eta^2=0.004$) or an interaction effect ($p=0.901$, $\eta^2=0.001$).

**Cognitive responses**

Accuracy during OTS-6 was improved by acclimation ($p=0.028$, $\eta^2=0.321$) with an interaction effect ($p=0.007$, $\eta^2=0.437$). The interaction effect was due to a significant impairment in accuracy in HYP pre-acclimation (-0.786 [-1.543;-0.029], p=0.043) that was recovered post-acclimation (+0.214 [-0.301;+0.730], p=0.385). This was associated to an improvement in accuracy from pre- to post-acclimation in HYP (+1.143 [+0.508;+1.778], p=0.002), but not CON (+0.143 [-0.531;+0.817], p=0.655). The associated latency of response was also shorter in HYP than CON ($p=0.014$, $\eta^2=0.385$). While the interaction effect was large ($\eta^2=0.179$) it did not reach significance ($p=0.116$). However, post hoc analyzes showed that the latency of response was shorter in HYP than CON pre-acclimation (-12.342 [-19.754;-4.930], p=0.003), but not post-acclimation (-1.895 [-11.545;+7.754], p=0.678).

**Peripheral neural drive transmission**

M-wave (Table 2) amplitudes were lower in HYP than CON both at rest ($p=0.006$, $\eta^2=0.550$) and during 10% MVC ($p<0.001$, $\eta^2=0.862$) for SOL, but the difference did not reach significance for the GM at rest ($p=0.054$, $\eta^2=0.322$) or during 10% MVC

361 ($p=0.184$, $\eta^2=0.187$). However, none of the M-waves at rest ($p\geq0.444$, $\eta^2\leq0.060$ for
362 both muscles) or during 10% MVC ($p\geq0.251$, $\eta^2\leq0.143$ for both muscles) were
363 affected by acclimation.
364
365 M-waves recorded using intramuscular EMG (Fig. 2) confirmed that amplitude was
366 lower in HYP than CON (rest: $p=0.067$, $\eta^2=0.359$; 10%-MVC: $p=0.038$, $\eta^2=0.434$),
367 but was not affected by acclimation (rest: $p=0.832$, $\eta^2=0.006$; 10%-MVC: $p=0.708$,
368 $\eta^2=0.018$). The interaction between thermal state and acclimation was also not
369 significant for any of the M-waves (all $p\geq0.061$, $\eta^2\leq0.337$).
370
371 **Spinal modulation**
372 H-reflex amplitudes (Table 2) were consistently lower in HYP than CON for SOL at
373 rest ($p<0.001$, $\eta^2=0.899$) and during 10% MVC ($p<0.001$, $\eta^2=0.920$), as well as for
374 GM at rest ($p<0.001$, $\eta^2=0.634$) and during 10% MVC ($p=0.002$, $\eta^2=0.557$).
375 However, none of the H-reflexes at rest ($p\geq0.326$, $\eta^2\leq0.074$ for both muscles) or
376 during 10% MVC ($p\geq0.347$, $\eta^2\leq0.074$ for both muscles) were affected by acclimation.
377 There was no interaction effect between thermal state and acclimation on the H-reflex
378 amplitude of the SOL (rest: $p=0.184$, $\eta^2=0.142$; 10%-MVC: $p=0.488$, $\eta^2=0.045$) or the
379 GM (rest: $p=0.533$, $\eta^2=0.031$; 10% MVC: $p=0.940$, $\eta^2=0.000$).
380
381 **Nerve conduction velocity**
382 As presented in Table 3, the latency durations for all evoked potentials were
383 significantly shorter in HYP than CON (all $p\leq0.027$, $\eta^2\geq0.484$). There was no effect
384 of acclimation (all $p\geq0.297$, $\eta^2\leq0.083$) or interaction effects (all $p\geq0.116$, $\eta^2\leq0.179$)
385 on any latencies.

386

387 **Sustained maximal voluntary isometric contraction**

388 Brief maximal torque was lower in HYP than CON ($p<0.001$, $\eta^2=0.700$) and
389 increased from pre- to post-acclimation ($p=0.004$, $\eta^2=0.484$), without an interaction
390 effect ($p=0.588$, $\eta^2=0.023$). Mix model analyzes showed that the percentage of
391 maximal force sustained during the 120 s MVC decreased during the contraction
392 ($p<0.001$), was higher in CON than HYP ($p<0.001$), and higher post- compared with
393 pre-acclimation ($p<0.001$). This was accompanied by an interaction effect time (i.e.
394 fatigue) x condition ($p<0.001$) and time x condition x acclimation ($p<0.001$). As
395 displayed in Figure 3, the greater rate of fatigue in HYP than CON observed pre-
396 acclimation was attenuated post-acclimation due to a significant effect of acclimation
397 on fatigue in HYP ($p<0.001$), but not in CON ($p=0.791$).

398

399 Voluntary activation calculated every 30 s during showed a similar pattern with an
400 effect of time (i.e. decreased during the contraction, $p=0.001$, $\eta^2=0.836$), an effect of
401 thermal state (i.e. CON > HYP, $p=0.017$, $\eta^2=0.363$) and an effect of acclimation (i.e.
402 post > pre, $p=0.012$, $\eta^2=0.395$). Post hoc analyzes revealed that VA was lower in
403 HYP than CON pre-acclimation (-7.995 [-12.048;-3.941]%, $p=0.001$), but not post-
404 acclimation (-3.259 [-11.994;5.475]%, $p=0.435$). In addition, despite the absence of a
405 significant interaction effect between fatigue x condition x acclimation ($p=0.405$,
406 $\eta^2=0.307$), VA was lower in HYP than CON from 60 s onward pre-acclimation (60 s:
407 $p=0.014$; 90 s: $p=0.002$; 120 s: $p=0.046$), whereas VA was not different between HYP
408 and CON post-acclimation (all $p>0.298$) (Fig. 3).

409

410 **DISCUSSION**

411 This study used heat acclimation as a model to investigate the pathway(s) responsible
412 for the impairment in neural drive transmission in hyperthermic humans. Despite the
413 occurrence of classic and significant heat acclimation responses in core temperature,
414 sweat rate and heart rate, we observed that the amplitudes of electrically evoked
415 potentials (i.e. a total of 3360 M-wave and H-reflex were analyzed) remained
416 depressed in HYP relative to CON. The second aim of this study was to determine
417 whether heat acclimation can attenuate the influence of hyperthermia on neural drive
418 generation and executive function. The current data showed that heat acclimation
419 allowed both to better sustain voluntary muscle activation during a sustained
420 contraction, and enhance special planning and working memory in hyperthermic
421 humans. These observations occurred without any changes in control conditions and
422 suggest supraspinal adaptations to heat acclimation.
423
424 **Classic acclimation responses**
425 The current data show that repeated passive heat exposure induces adaptations
426 consistent with heat acclimation, such as a decrease in core temperature at rest, an
427 increase in sweat rate, along with a decrease in heart rate (24). Core temperature
428 during the HYP test did not differ from pre- to post-acclimation due to the ~39ºC
429 clamp used, but the time to reach this temperature was longer post-acclimation. Our
430 data also confirm that acute passive heat exposure alters perceptual and emotional
431 responses as evidenced by significant increases in thermal sensation and discomfort,
432 along with an increase in the negative/positive affects ratio (11). However, we did not
433 observe any effects of heat acclimation on these subjective responses. The absence of
434 adaptation is partly in contrast with previous reports that heat acclimation can
435 improve thermal comfort (14, 19). However, these previous observations were not

436 obtained in controlled hyperthermic conditions at given core temperature's. The
437 current data suggest that acclimation *per se* may not significantly improve the
438 perception, comfort and affective responses to a given hyperthermic state (i.e. 39ºC
439 body core temperature).
440
441 **Peripheral nervous system function and nerve conduction velocity**
442 Similar to previous reports, passive hyperthermia significantly decreased M-wave
443 amplitude in the SOL, whilst the decrease was not significant in other muscles (25-
444 27). The decrease in SOL M-wave was confirmed both at rest and using a controlled
445 contraction to maintain a constant level of background activity (i.e. 10% MVC).
446 Currently, there is no direct explanation for the larger sensitivity of the M-wave in
447 SOL than other muscles. However, this could be partly related to the slower nerve
448 conduction velocity in predominantly slow twitch muscles, which is linked to the
449 mechanical (2) and discharge (6) properties of the muscle.
450 Of note, when comparing HYP and CON, it has to be acknowledged that the decrease
451 in M-wave amplitude might be related to a higher cutaneous blood flow, which has
452 been suggested to attenuate surface EMG (4, 30). However, as shown in Fig. 2, the
453 current data indicate that a decrease in M-wave amplitude can also be observed using
454 intra-muscular EMG. This shows that the decrease in M-wave is at least partly related
455 to neural factors and not solely to the methodological artefact of skin temperature.
456
457 Two different physiological mechanisms have been proposed in the literature to
458 explain the decrement in M-wave with hyperthermia. The traditional view is that an
459 increase in temperature reduces the opening time of voltage-gated sodium channels,
460 leading to a decrease in the amplitude, duration and area of the axon potential (34).

461 This view is partly based on the negative correlation between skin temperature and
462 the amplitude, duration, area and latency of a compound action potential (5). Such a
463 mechanism can be considered a side effect of the rule of Vant-Hoff, stating that there
464 is a 2 to 4-fold increase in chemical reaction rate for each 10ºC increase in
465 temperature. Given that the components of the equation of Arrhenius characterizing
466 the temperature dependence of the chemical reaction rate are chemical constants, this
467 effect is likely independent of acclimation status. Our data demonstrating that heat
468 acclimation does not modify the M-wave latency and amplitude support this view.
469
470 **Synaptic failure**
471 The acute decrease in H-reflex amplitude in HYP has been discussed in previous
472 studies (25, 26). Briefly, *in vitro* models suggest that both M-wave and H-reflex
473 decrement in HYP could be related to a synaptic failure in the transmission of an
474 action potential from the pre- to the post-synaptic element (26). *In vitro*, stimulation
475 of a pre-synaptic element always produced one or more post-synaptic quantal events
476 for each nerve impulse at 22°C (16). However, as temperature increased, the
477 amplitude of the response declined and failures became evident until transmission
478 completely failed when nerve temperature reached 35°C (16). However, these
479 observations were noted in Drosophila with a range of temperatures lower than those
480 recorded *in vivo* in hyperthermic humans, which failed to report synaptic alterations
481 (23, 25). Importantly, it has been shown that thermal preconditioning could protect
482 from this synaptic failure, perhaps via an increase in HSP (15, 17). For example, the
483 effect of thermal preconditioning was mimicked by incubating the slice preparation
484 into a solution containing HSP72 (17), and an increase in HSP70 was sufficient for
485 synaptic thermoprotection in insects (33), with neurons able to take-up the HSP from

the extracellular fluid (38). Despite an increase in circulating eHSP following heat acclimation in the current study (Table 1), none of the 10 different types of M-waves or H-reflexes recorded were protected (Table 2). This suggest that the decrease in neural drive transmission in humans might not be linked to synaptic failure. Indeed, human neuromuscular junctions have a very high safety factor, with far more acetylcholine released per stimulus than necessary to induce a muscle fibre depolarization (35). Moreover, even if M-wave and H-reflex amplitude were acutely reduced in HYP, they would never totally disappear, suggesting a more gradual effect of temperature than synaptic failure. Taken together, these data suggest that the primary pathway for neural drive transmission alterations with hyperthermia in humans relates to the increase in axonal conduction velocity rather than synaptic failure.

**Central motor drive**

The current study used a passive hyperthermia model to avoid any confounding effect of exercise-induced fatigue (acutely) or training (chronically). This model has previously shown that hyperthermia can acutely reduce voluntary activation (22, 26, 36), partly in relation with a decrease in supraspinal neural drive generation (23, 26, 37). However, it remained unknown if heat acclimation could partly restore neural drive in hyperthermic individuals. To date, the only report on regarding this query suggested that heat acclimation did not affect central fatigue (7). However, this previous study was conducted with a different muscle group (knee extensors), a localised heating procedure (lower limb heating) and a sub-optimal acclimation procedure (every second day). As displayed in Fig. 3, the current data show that hyperthermia-induced alterations during a sustained MVC can be at least partly

recovered following heat acclimation. Of note, maximal torque remained lower in HYP than CON, even post-acclimation (29), but the ability to sustain this torque was recovered by acclimation. The improvement was associated with a recovery of the loss of voluntary activation (Fig. 3). Importantly, this recovery was not accompanied by any spinal or peripheral nervous system adjustments, suggesting a supraspinal adaptation.

**Cognitive responses**

HYP increased thermal sensation and thermal discomfort, and decreased positive affects whereas negative affects were increased (Table 1). The unpleasantness of these responses (20) has been likened to a cognitive load decreasing available resources and thus limiting cognitive function (11). Whilst we observed a significant impairment in executive function in HYP pre-acclimation, it was offset post-acclimation due to a selective improvement in HYP, but not CON (Fig. 1). This was obtained using planning tasks and add to previous reports that acclimation can also protect attention tasks (31) and psychomotor performance (39). Importantly, this protective effect occurred in a clamped hyperthermic setting, without any improvement in thermal sensation, thermal discomfort, and positive or negative affects (Table 1), highlighting a dissociation between thermal perception and its consequences.

Of note, a training effect cannot be ruled-out when performing sequential measures pre- and post-acclimation. However, the pre-tests were also preceded by a familiarization session a week before the test, plus the acclimation period allowed for 11-days of wash-out. In addition, the current data showed that accuracy improved in HYP, but not in CON, suggesting a specific adaptation to hyperthermia rather than an overall improvement during the test.

Based on recent data showing that passive hyperthermia increased the rate of false alarms during a sustained attention task (12) and led to faster but false responses during a complex planning task (10), it has been suggested that hyperthermia increases impulsivity (11). The current data confirm that the lower accuracy in the planning task pre-acclimation was associated with a faster response rate. Moreover, our results show that the latency of response was shorter in HYP than CON pre-acclimation, but not post-acclimation. Taken together, these results suggest a decrease in impulsivity allowing for better accuracy, despite a similar rectal temperature and thermal perception.

## Conclusion

There is currently contention in the literature as to the pathway by which hyperthermia affects neural drive transmission. The current results show that heat acclimation has no protective effect on the decrement of electrically evoked potential amplitude (i.e. M-wave and H-reflex). This suggests that the decrement in neural drive transmission in hyperthermic humans is not driven by synaptic failure, but may relate to an increase in nerve conduction velocity, that is independent of acclimation status. Conversely, the current data show that heat acclimation allowed to better sustain voluntary neural drive during a prolonged contraction, and protect executive function, during hyperthermia. Hence, heat acclimation appears to induce beneficial central, but not peripheral nervous system adaptations.

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**Competing interests**

The authors declare no competing interests, financial or otherwise.

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680 **Figure 1:** Accuracy and latency of responses during a cognitive task. Pre acclimation,
681 hyperthermia (○) decreased accuracy and latency as compared to control (● ,
682 $p<0.05$). Acclimation increased accuracy and latency in HYP (△, $p<0.05$) toward
683 control values (▲, $p>0.05$). Values represent mean and SD of 14 participants.
684
685 **Figure 2.** Electrical stimulations (a) were applied to the α-motoneuron (b,d) and Ia-
686 afferents (c,e) on relaxed (b,c) and contracted (d,e) muscles. As displayed in panel (f)
687 (average of 6 H-reflexes in the soleus for one representative participant), evoked
688 potentials occurred earlier and with a lower amplitude in hyperthermic participants.
689 Repeated-measures ANOVA showed no changes from pre- to post-acclimation in any
690 electrically evoked M-wave or H-reflex, measured from soleus or gastrocnemius, via
691 surface or intramuscular fine wire electromyography. A total of 3360 evoked
692 potentials were analyzed from 14 participants.
693
694 **Figure 3.** Relative torque sustained during a 120 s maximal voluntary isometric
695 contraction in CON (i.e. normothermic state) and HYP (i.e. hyperthermic state). The
696 larger decrement in torque in HYP than CON observed pre-acclimation (left panel)
697 was minimized post-acclimation (right panel) (mixed-model analyses, $p<0.001$).
698 Voluntary activation was lower in HYP (open circle) than CON (closed disk) pre-
699 acclimation only (repeated-measures ANOVA, * $p<0.05$). Values represent mean and
700 SD of 14 participants.
701

**Table 1:** Environmental conditions, thermoregulatory, physiological and perceptual responses during the tests in CON (i.e. normothermic state) and HYP (i.e. hyperthermic state), pre- and post-acclimation.

| | CON | | | HYP | |
|---|---|---|---|---|---|
| | Pre | Post | | Pre | Post |
| **Environmental conditions** | | | | | |
| Temperature (ºC) | 24.1 ±0.2 | 24.0 ±0.2 | < | 45.8 ±1.3 | 46.0 ±0.8 |
| Relative Humidity (%) | 37.1 ±2.7 | 37.5 ±3.3 | < | 49.4 ±4.1 | 49.2 ±3.7 |
| **Thermoregulatory responses** | | | | | |
| Core temperature (ºC) | 36.6 ±0.4 | 36.4 ±0.4* | < | 39.2 ±0.3 | 39.1 ±0.3 |
| Skin temperature (ºC) | 30.8 ±0.5 | 30.7 ±0.5 | < | 38.9 ±0.7 | 38.6 ±0.7 |
| Sweat rate (L/h) | 0.1 ±0.3 | 0.1 ±0.1 | < | 1.3 ±0.4 | 2.0 ±0.7* |
| Heart rate (bpm) | 74 ±8 | 78 ±13 | < | 126 ±9 | 116 ±9* |
| eHSP72 (pg/ml) | 70.6 ±36.2 | 75.4 ±43.1 | < | 78.4 ±42.3 | 90.6 ±45.5* |
| **Perceptual responses** | | | | | |
| Thermal sensation (/7) | 3.0 ±0.6 | 3.0 ±0.9 | < | 6.3 ±0.7 | 5.8 ±0.7 |
| Thermal discomfort (/7) | 3.1 ±0.6 | 3.1 ±1.0 | < | 6.1 ±0.8 | 5.8 ±0.7 |
| **Affects** | | | | | |
| Positive (a.u.) | 24.8 ±6.5 | 23.7 ±6.0 | > | 21.2 ±6.9 | 20.3 ±7.9 |
| Negative (a.u.) | 11.7 ±1.8 | 10.9 ±1.1 | < | 16.1 ±4.9 | 15.4 ±4.1 |
| Negative/Positive ratio | 0.5 ±0.2 | 0.5 ±0.2 | < | 0.9 ±0.5 | 0.9 ±0.4 |

Values in mean ±SD. < Significant differences between HYP and CON ($p<0.05$). *Significant differences between Post and Pre ($p\leq0.05$). For eHSP72, the CON value was measured before HYP exposure on the same day.

**Table 2:** Amplitude of electrically evoked potentials during the tests in CON (i.e. normothermic state) and HYP (i.e. hyperthermic state), pre- and post-acclimation.

| | CON | | | HYP | |
|---|---|---|---|---|---|
| | Pre | Post | | Pre | Post |
| **M-wave amplitude (mV)** | | | | | |
| Soleus - Rest | 7.5 ±2.9 | 6.6 ±2.4 | > | 4.5 ±2.0 | 5.0 ±2.2 |
| Soleus - 10% MVC | 8.7 ±2.9 | 7.1 ±3.0 | > | 5.2 ±3.2 | 5.1 ±3.1 |
| Gastroc Med – Rest | 7.4 ±3.8 | 6.0 ±2.6 | | 5.0 ±3.2 | 5.6 ±2.8 |
| Gastroc Med – 10%MVC | 7.8 ±3.6 | 6.6 ±3.1 | | 5.7 ±4.2 | 6.0 ±4.0 |
| Intramuscular – Rest | 2.9 ±2.1 | 3.1 ±1.4 | | 1.8 ±1.3 | 1.8 ±1.0 |
| Intramuscular – 10%MVC | 3.1 ±1.9 | 3.3 ±1.3 | > | 1.8 ±1.2 | 1.9 ±1.1 |
| **H-reflex amplitude (mV)** | | | | | |
| Soleus - Rest | 6.0 ±2.6 | 5.3 ±1.8 | > | 2.8 ±1.4 | 2.8 ±1.4 |
| Soleus - 10% MVC | 5.6 ±2.0 | 5.4 ±1.7 | > | 2.5 ±1.1 | 2.6 ±1.3 |
| Gastroc Med – Rest | 2.7 ±1.5 | 2.8 ±1.2 | > | 1.5 ±0.7 | 1.9 ±1.2 |
| Gastroc Med – 10%MVC | 2.6 ±1.0 | 2.7 ±1.1 | > | 1.6 ±0.5 | 1.8 ±1.2 |

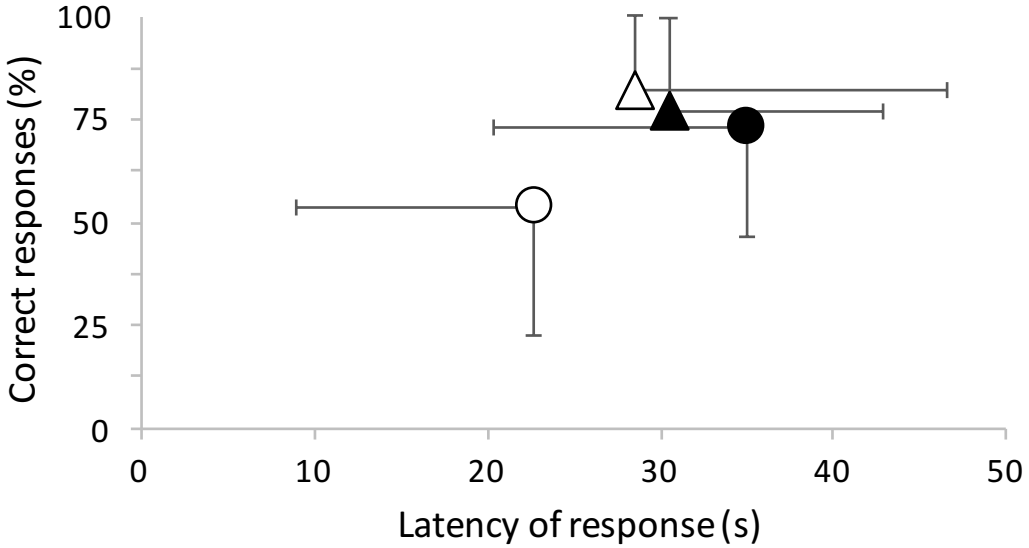
Values in mean ±SD. > significant differences between HYP and CON ($p<0.05$).

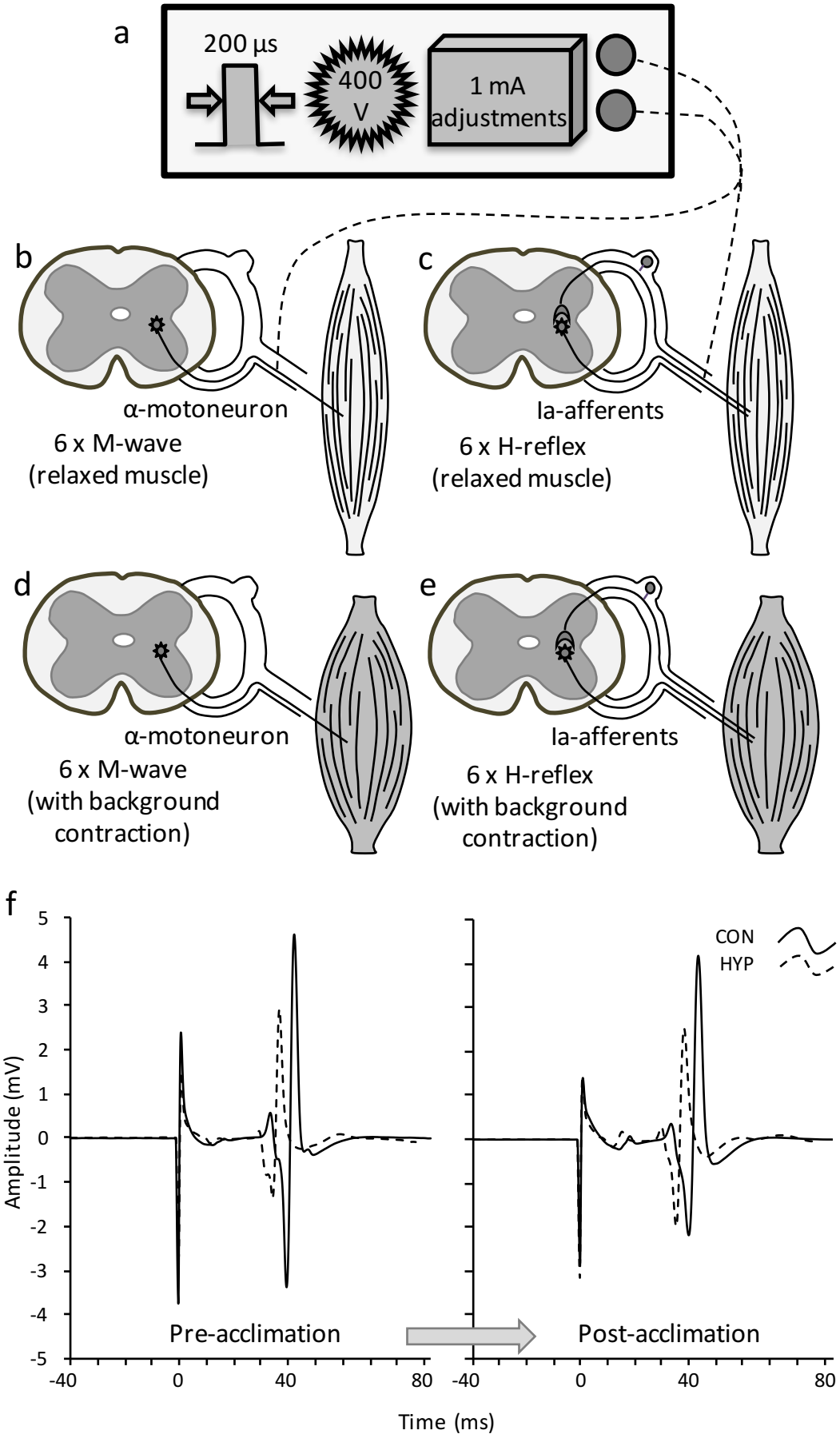
713 **Table 3:** Latency of electrically evoked potentials during the tests in CON (i.e.
714 normothermic state) and HYP (i.e. hyperthermic state), pre- and post-acclimation.

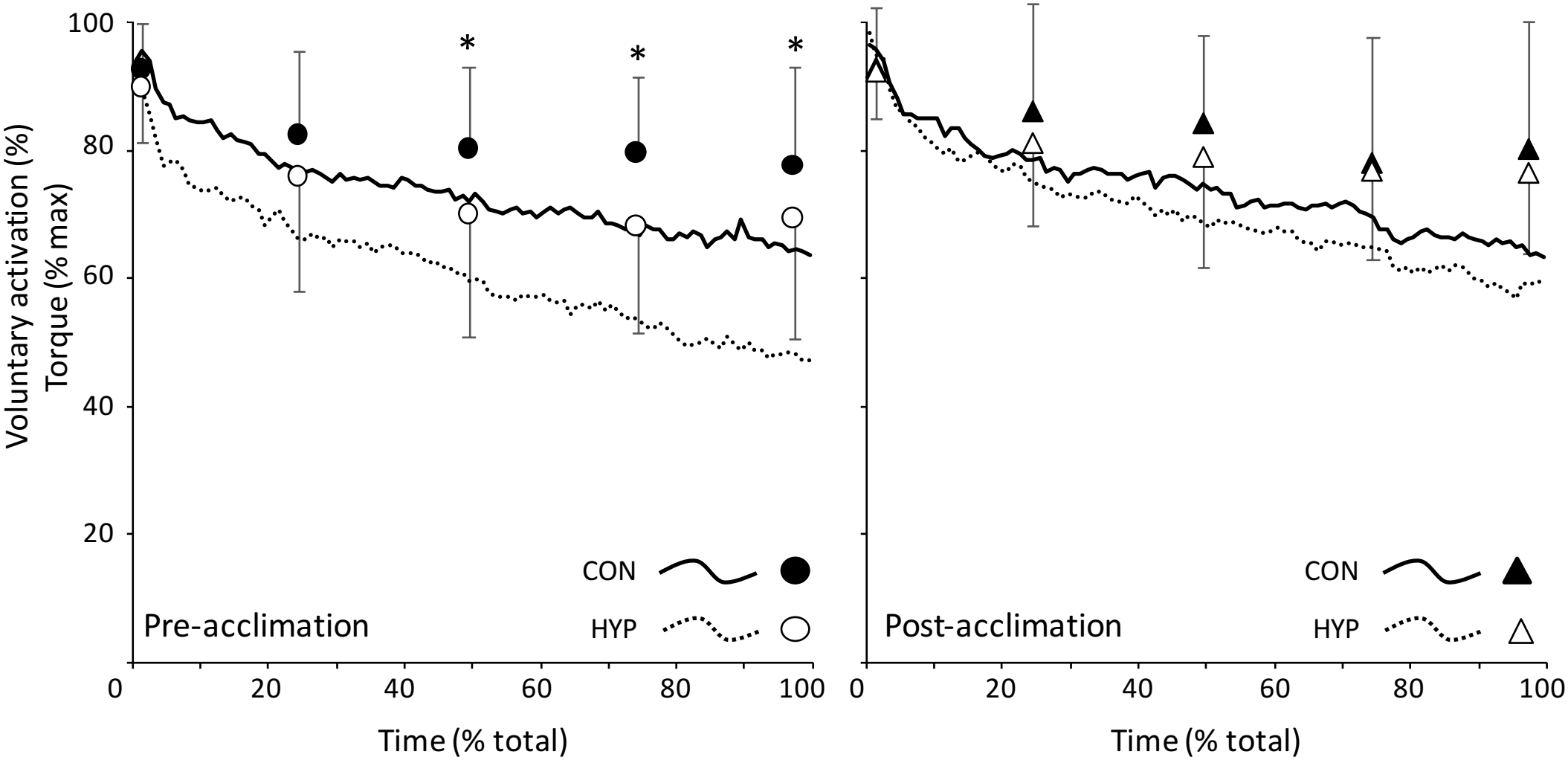
| | CON | | | HYP | |
|---|---|---|---|---|---|
| | Pre | Post | | Pre | Post |
| **Latency M-wave (ms)** | | | | | |
| Soleus - Rest | 15.8 ±2.3 | 16.0 ±2.7 | > | 12.6 ±1.5 | 12.5 ±1.8 |
| Soleus - 10% MVC | 15.0 ±2.2 | 14.8 ±2.4 | > | 11.9 ±1.7 | 12.2 ±2.5 |
| Gastroc. Med. – Rest | 11.3 ±1.6 | 11.7 ±2.6 | > | 9.8 ±2.0 | 9.7 ±2.6 |
| Gastroc. Med. – 10%MVC | 12.0 ±2.2 | 12.6 ±3.4 | > | 10.2 ±3.0 | 10.2 ±2.7 |
| **Latency H-reflex (ms)** | | | | | |
| Soleus - Rest | 41.6 ±3.0 | 41.9 ±3.3 | > | 36.5 ±2.7 | 36.4 ±2.5 |
| Soleus - 10% MVC | 41.1 ±3.1 | 41.4 ±3.2 | > | 36.0 ±2.5 | 35.8 ±2.8 |
| Gastroc. Med. – Rest | 37.5 ±2.4 | 37.5 ±2.6 | > | 33.8 ±2.2 | 34.2 ±2.3 |
| Gastroc. Med. – 10%MVC | 37.3 ±2.4 | 37.3 ±2.4 | > | 33.4 ±2.1 | 34.6 ±3.2 |

715 Values in mean ±SD. > significant differences between HYP and CON ($p<0.05$).

716


100
75
50
25
0
Correct responses (%)
0
10
20
30
40
50
Latency of response (s)


a
200 µs
400 V
1 mA adjustments
b
α-motoneuron
6 x M-wave
(relaxed muscle)
c
Ia-afferents
6 x H-reflex
(relaxed muscle)
d
α-motoneuron
6 x M-wave
(with background contraction)
e
Ia-afferents
6 x H-reflex
(with background contraction)
f
CON
HYP
Amplitude (mV)
5
4
3
2
1
0
-1
-2
-3
-4
-5
-40
0
40
80
Pre-acclimation
Post-acclimation
Time (ms)


Voluntary activation (%)
Torque (% max)
100
80
60
40
20
0
20
40
60
80
100
Time (% total)
*
*
*
CON
HYP
Pre-acclimation
Post-acclimation