Articles in PresS. Am J Physiol Regul Integr Comp Physiol (June 7, 2017). doi:10.1152/ajpregu.00055.2017

Title:

Localized and systemic variations in central motor drive at different local skin and muscle temperatures

Authors:

Alex Lloyd

Lewis Picton

Margherita Raccuglia

Simon Hodder

George Havenith

Affiliation:

Environmental Ergonomics Research Centre, Loughborough Design School, Loughborough University, Loughborough, UK

Running Head:

Central motor drive at different muscle temperatures

Corresponding Author:

Alex Lloyd

Environmental Ergonomics Research Centre,

James France Bldg,

Loughborough,

Leicestershire

UK

LE11 3TU Email: A.Lloyd@lboro.ac.uk

ABSTRACT

This study investigated the ability to sustain quadriceps central motor drive while subjected to localized heat and metaboreceptive feedback from the contralateral leg. Eight active males each completed two counter-balanced trials, in which muscle temperature (T_m) of a single-leg (TEMP-LEG) was altered to 29.4 (COOL) or 37.6°C (WARM), while the contralateral leg (CL-LEG) remained thermoneutral; 35.3 and 35.2°C T_m in COOL and WARM respectively. To activate metaboreceptive feedback, participants first performed one 120-s isometric maximal voluntary contraction (MVC) of the knee extensors in the TEMP-LEG, immediately followed by post-exercise muscle ischemia (PEMI) via femoral blood flow occlusion. To assess central motor drive of a remote muscle group immediately following PEMI, another 120-s MVC was subsequently performed in the CL-LEG. Voluntary muscle activation (VA) was assessed using the twitch interpolation method. Perceived mental effort and limb discomfort were also recorded. In a cooled muscle, a significant increase in mean force output and mean VA (force, $p<0.001$; VA, $p<0.05$) as well as a significant decrease in limb discomfort ($p<0.05$) occurred during the sustained MVC in the TEMP-LEG. However, no differences between T_m were observed in mean force output, mean VA or limb discomfort during the sustained MVC in the CL-LEG (Force, $p=0.33$; VA, $p>0.68$, limb discomfort, $p=0.73$). The present findings suggest that elevated local T_{sk} and T_m can increase limb discomfort and decrease central motor drive, but this does not limit systemic motor activation of a thermoneutral muscle group.

PERSPECTIVES AND SIGNIFICANCE

Previous research indicates that as local skin and muscle temperature increase, the capacity to maximally activate skeletal muscle is reduced. The present study aimed to examine the afferent, supracortical and efferent factors that contribute to this phenomenon. It was concluded that changes central motor drive at different local temperatures are unrelated to the co-variance in core temperature, whole-body mean skin temperature, and/ or true mean body temperature. Herein it is proposed that the causative factors are most probably a direct effect of afferent nerve sensitization on conscious motor control, inefficient contractility leading to increased metabolite precipitation, and/or reduced efficiency of sarcolemmal action potential propagation. Further research required to elucidate the contribution of each to the failure in voluntary activation in heated skeletal muscle, with greater consideration given to the interrelationship between supracortical control and multimodal somatosensory feedback from active muscle.

INTRODUCTION

Thermal strain can reduce performance during prolonged physical work (11, 52). In the heat, this is partially attributed to lower maximum cardiac output which compromises oxygen delivery to active muscle, particularly during high-intensity or dehydrated exercise (29, 55, 64). Conversely, in the cold this is attributable to a lower biomechanical efficiency due to antagonist muscle co-activation and a higher joint resistance (11, 48, 78). In both the heat and the cold, the relative aerobic-mechanical efficiency of exercise is therefore reduced. This in turn limits performance through accelerated chemical metabolite accumulation in active muscle (30, 39, 42) due to an increase in the total muscle fiber recruitment required to sustain a given workload (1, 35, 75).

In addition to faster rates of peripheral fatigue development, a down-regulation in voluntary muscle activation (VA) has been observed as body temperature increases (10, 51, 59, 76). This has been attributed to hyperventilation, arterial hypocapnia, and reduced cerebral blood flow instigated by an increase in core temperature (T_{core}). It is thought that this reduction in cerebral blood flow may limit central drive through altered cerebral metabolite and/ or neurotransmitter concentrations (33, 69). However, T_{core} as a variable is highly non-specific, encompassing as much as 90% of all bodily tissues during heat stress (14). Ultimately, this implicates the integration of a range of regional, spatial and specific organ temperatures in the regulation of central motor drive. Indeed, the impact of body temperature on central drive appears to be related to the relative mass of the heated tissue (40, 74), perhaps indicating a proportional response to true mean body temperature rather than T_{core} *per se*.

T_{core} is also often used to reflect the local temperature of the brain or hypothalamus (7, 33, 53). Yet the firing rate of temperature sensitive hypothalamic neurons are highly dependent on extra-hypothalamic, thermal and non-thermal, inputs (8, 9, 71). In line with this, previous observations indicate that both cutaneous-thermal and muscular-ergoreceptive feedback can initiate autonomic thermo-effectors such as sweating and vasodilation (5, 45), as well as behavioral thermoregulation through voluntary alterations in central motor drive (22, 40, 46, 51).

Despite its obvious role as an effector organ, active muscle is also highly innervated with neuro-sensory receptors (49, 73). At least one of these sensory pathways - metaboreceptive feedback via group III and IV afferents – has been suggested to be a critical stimulus for modulations in central drive to active muscle (2–4). Cardiovascular strain arises under heat stress, exacerbating the rate at which muscles fatigue (42, 55, 56). Thus, faster metaboreceptor activation is a likely consequence of exercise in the heat as well (51, 52). Compounding this, as active muscle temperature increases, both heat related and acid-base related increases in intramuscular TRPV1 receptor activation (6, 13, 19, 57), as well as more efficient transduction in peripheral afferent nerve fibers (34, 44, 60), may lead to upregulation in the sensitivity of the muscle to metabolites. As a result exercise with increasing body temperatures may be subject to sensory nerve sensitization, an increase in metaboreceptor activation, and an increased chemical metabolite accumulation caused by increased cardiovascular strain (see paragraph 1). The net result could be increased activation of the thermo-metabolic sensory pathways from the active muscle, intensifying any reductions in VA that occur with heat stress (28, 52).

To investigate the role of muscle temperature (T_m) on VA via metaboreceptive feedback, the present study sought to: a) examine the impact of T_m on perceived limb discomfort in metabolite saturated muscle i.e. fatigued muscle; and b) examine whether elevated or reduced T_m combined with metaboreceptive feedback inhibits motor drive to a remote (i.e. unaffected) muscle group.

To investigate the role of metaboreceptive feedback, post-exercise muscle ischemia (PEMI) in a temperature-manipulated leg was used. PEMI prevents the washout and circulatory distribution of intramuscular metabolites following exhaustive exercise, allowing the metaboreceptive signal to be explored independently of additional factors associated with fiber recruitment (4, 27, 47). Given the need for both a localized change in muscle temperature and minimal cardiovascular strain, the exercise chosen for this study was a 120-s maximal isometric voluntary contraction (MVC) of the knee extensors.

The first hypothesis for the present study was that warm local T_{sk} and T_m would reduce central motor drive in a temperature-manipulated leg. The second hypothesis was that a corresponding decrease in central motor drive of a remote muscle group (i.e. the contralateral- thermoneutral leg) would occur with an

113 increase in both local T_{sk} and T_m in the temperature manipulated leg, due to a combination of: a) thermal
114 sensitization of metaboreceptive feedback in a warmer muscle; and b) higher integrated thermal afferent
115 feedback from an increase in localized T_{sk} and T_m, which leads to an increase in true mean body
116 temperature (total mass normalized whole-body heat content).
117
118 METHODS
119 ***Participants***
120 Eight healthy and physically active male participants were recruited for this study from Loughborough
121 University's Gymnasium (mean ± SD: age, 22 ± 3 yrs.; stature, 189 ± 1 cm; body mass, 93.4 ± 18.9 kg).
122 All participants were regularly performing moderate to vigorous physical activity (4.5 ± 1.4 exercises/
123 week) as confirmed using self-assessment prior to the start of the familiarization session. Participants were
124 right leg dominant with no history of cardiovascular, neuromuscular or metabolic debility. At least 24-
125 hours prior to undertaking any trials, participants were requested to refrain from strenuous exercise, as well
126 as caffeine and alcohol consumption. Participants were given an information sheet detailing the
127 experimental protocol before completing both a health screening questionnaire and written informed
128 consent. The experimental protocol was approved by the Loughborough University Ethical Advisory
129 Committee, and all procedures were conducted by trained experimenters. The experiments were conducted
130 in spring (UK) with the likely levels of heat acclimation low.
131
132 ***Experimental design and overview***
133 Each participant visited the laboratory on three separate occasions to complete one preliminary, and two
134 experimental trials. All trials were separated by a minimum of 2 days recovery. The experimental trial
135 order was counter-balanced. All contractions during the experimental protocols were performed with
136 femoral nerve stimulation using the twitch interpolation technique to ascertain changes in VA (50). Figure
137 1, Panel A outlines the general procedure for the main experimental sessions.
138
139 **[INSERT FIGURE 1 HERE]**
140

On arrival at the laboratory, participant's baseline neuromuscular performance was assessed using three brief MVCs on each leg, in a random order, and each interspersed with 30-s rest. Participants were then prepared and instrumented with the temperature recording equipment and vastus lateralis T_m was assessed in both legs. Following this, the right leg was temperature manipulated in a water bath (hereafter referred to as the TEMP-LEG). The two experimental trials differed by water temperature (T_{water}) and consisted of either: a) 25 minutes immersion in 8°C T_{water} (COOL) changing vastus lateralis T_m to 29.4 ± 4.0ºC at 2-cm depth; or b) 15 minutes immersion in 44°C T_{water} (WARM) changing vastus lateralis T_m to 37.6 ± 0.4ºC at 2-cm depth. During immersion, the thermoneutral contralateral (left) leg was suspended out of the water by a support frame (hereafter referred to as the CL-LEG).

After single leg water-immersion, T_m was immediately reassessed in both legs. No T_m changes occurred in the CL-LEG. To check for influences on VA that could not be attributed to a change in TEMP-LEG T_m, participants completed another three brief MVCs (each interspersed with 30-s rest) in their CL-LEG only (Figure 1).

To complete the trial, participants conducted a sustained 120-s MVC immediately followed by PEMI in their TEMP-LEG. With PEMI maintained in the TEMP-LEG, participants immediately sustained a 120-s MVC in their CL-LEG. This allowed the self-regulated distribution of VA to be assessed in a local temperature-manipulated muscle, shortly followed by an assessment of the systemic influence of T_m on VA, as assessed in a remote and unaffected muscle group.

Experimental rationale

The immersion times were chosen based on the COOL and WARM conditions used by Lloyd et al. (2015*b*), shown to induce minimal (≤ 0.1°C) variations in rectal temperature. Given the previously observed linearity between VA and T_m in the range of 22 to 38.5°C (40), it was assumed that including a condition that corresponds with resting T_m (e.g. ~34°C), would provide no additional value to the present study. Similarly, a brief contraction immediately following immersion was not deemed necessary in the TEMP-LEG for the following reasons: a) the force and VA relation during brief isometric contractions has already been characterized at different T_m (40); b) any influential factors other than T_m or local T_{skin} should

influence the CL-LEG and TEMP-LEG proportionally; c) any acute changes in force and VA would be apparent at the initiation of the sustained contraction in the TEMP-LEG; and d) the requirement for a localized change in T_m imposes strict time restraints.

It is important to note that due to changes in contractile efficiency during isometric exercise at different T_m (10, 20, 40, 70, 76), a submaximal isometric contraction would not have provided a viable method for inducing similar metabolite concentrations in the TEMP-LEG. The chosen self-regulated 120-s MVC circumvents this issue by allowing participants to alter recruitment patterns to maintain the maximum tolerable level of metabolite saturation independent of T_m.

Preliminary session

Prior to the main experiment, participants attended a preliminary session to complete training in the procedure and an initial assessment of neuromuscular function in both left and right legs. After a series of contractions to potentiate the quadriceps, participants were then asked to perform repeated MVCs with femoral nerve stimulation in each leg. The contractions were repeated until participants reached a coefficient of variation below 5% for three successive MVCs in each leg. During this session, the positioning of all stimulation electrodes were ascertained then marked with indelible ink for the following experimental sessions (see 'neuromuscular assessment' for details). The current necessary for supramaximal femoral nerve stimulation of each leg was also ascertained using progressive increases in current until a plateau in the mechanical response of the muscle was observed (2, 40, 59). After adding 25% to the stimulator current to ensure supramaximal depolarization of the femoral nerve, the same output was then used for the main experimental trials: 186 ± 26 and 184 ± 27 mA for TEMP-LEG and CL-LEG respectively. During the familiarization sessions, the maximum force output was 1067 ± 186 and 973 ± 252 N in the TEMP-LEG and CL-LEG, respectively. Resting potentiated twitch force (using doublet stimulation; details below) was 413 ± 71 and 409 ± 65 N in the TEMP-LEG and CL-LEG respectively.

Muscle temperature manipulation

The procedure to manipulate T_m (single leg water-immersion) has been discussed in detail previously (40). Briefly, participants sat in a water-immersion bath with their TEMP-LEG immersed, and the CL-LEG held

out of the water. To restrict the temperature changes to the TEMP-LEG, participants sat suspended in a sling to keep as much of their trunk and non-exercising leg out of the water as possible, while still fully immersing the TEMP-LEG up to the iliac crest. Seated immersion with the leg horizontal and water level just covering the leg was used to minimize hydrostatic pressure to the lower limb. T_{water} was maintained using active circulation, and continuously measured using a Grant Squirrel SQ2010 data logger and calibrated thermistor (Grant Instruments Ltd., Cambridge, UK). Following an initial start-up dose of 20 mg/l, the water chlorine level was maintained within 3-5 mg/l.

Participants were supplied with two sixteen inch floor-standing, variable intensity (4 speed) electric fans. To promote behavioral thermoregulation and to minimize the change in T_{core} across conditions, participants were permitted adjust the fan intensity, as well as add or upper body remove clothing, as required.

Body temperature measurement

T_{sk} was continuously recorded (1/min) using six wireless thermistors (Maxim, San Jose, USA), secured to the skin using Transpore tape (3M, Loughborough, UK). The thermistors were placed over the forehead, upper left chest, left bicep, stomach, CL-LEG thigh, and CL-LEG calf. Mean T_{sk} was calculated using equal weighting from each of the six measurement sites. The towel dried thigh T_{sk} in the TEMP-LEG was recorded using an infra-red sensor (FLUKE 566, Fluke Corporation, USA) immediately after water-immersion.

T_{core} was measured rectally, recorded via a Grant Squirrel SQ2010 data logger (Grant Instruments Ltd., Cambridge, UK) with a sample rate of 4/min. The rectal thermistor was inserted to a depth of 10-cm beyond the anal sphincter. During each experimental trial, heart rate (HR) was monitored using a Polar monitor (Polar Electro Oy, Kempele, Finland) at a sample rate of 12/min.

T_m was assessed in the vastus lateralis muscle of both legs, at 1- 2- and 3-cm using a solid needle thermocouple (MKA08050A275T; Ellab, Copenhagen, Denmark). The probe was inserted to a depth of 3-cm, and allowed to stabilize for 3-s. The needle was then withdrawn to 2- and 1-cm, with each depth

recorded upon temperature stabilization (21). T_m was recorded a total of three times in each leg, at three time points: 1) pre-water-immersion; 2) post-water-immersion; and 3) on completion of the exercise protocol (Figure 1). The three insertions were located in an equilateral triangle, with each insertion separated by ~5mm. In the subsequent trial, insertions were delivered ~1cm proximal of the previous insertion site. All needle thermocouples were sterilized before use using a vacuum autoclave (Little Sister SES 225B, Eschmann, UK). All procedures were conducted by trained personnel and in accordance with a strict sterility protocol. The insertion sites were towel dried and re-sterilized using iodine before each insertion.

Neuromuscular assessment

The procedures and equipment used for neuromuscular assessment have been detailed previously (40). Briefly, participants changed into a swimsuit and were secured into a bespoke knee extension dynamometer using a waist and chest belt system. The dynamometer was adjusted for each individual's femoral and tibial lengths, as well as their popliteal to patella width, whereas the hip and knee joint angles were set to 90 and 100° respectively. An adjustable, non-compliant harness was applied around the ankle malleolus to secure the leg to the force transducer (2000N, Model 615, Tedea- Huntleigh, Vishay Precision Group, California, USA). A thin (1-cm) layer of padding was also applied between the tibia and the harness to prevent bruising. Knee-extension force output was visually displayed (line trace and numerical value) to all participants during all contractions (DataLog software, Biometrics Ltd, UK). This was achieved using a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK) and PC interface.

To calculate the mechanical properties of the muscle (i.e. peripheral fatigue) (2, 40, 59) as well as VA (i.e. central command, central motor drive, central fatigue), the twitch interpolation technique was used (26, 50). To this end, two superimposed twitches ($Q_{tw,sup}$) were evoked over the force plateau of each brief (3-s) MVC (Figure 1, Panel B). In the case of the sustained 120-s MVCs, a single $Q_{tw,sup}$ was evoked at initial peak force, then again every 10-s during the MVC (totaling 13 $Q_{tw,sup}$). All MVCs (sustained and brief) were followed by two resting potentiated twitches ($Q_{tw,pot}$), 1-second and 3-seconds after full muscle relaxation respectively (40) (Figure 1, Panel B).

To circumvent the thermal influence associated with the use of singlet twitches (26, 40, 42), the present twitches were evoked by two 0.2-ms rectangular pulses spaced 10-ms apart (i.e. doublet twitch) using a high voltage simulation of the femoral nerve (max voltage 400 V; Digitimer DS7AH, Hertfordshire, UK) (23). All stimulations were delivered manually by the same experimenter. The stimulator anode was placed in the femoral triangle and the cathode over the greater trochanter (40). To ensure potentiation prior to the brief (3-s) MVCs, each assessment was conducted 20-s after a series of incremental practice contractions (2-s at 50, 50, 50, 75 and 90% MVC). All participants received moderate encouragement during all MVCs.

Analysis of the neuromuscular variables

VA was calculated using the following two equations. VA_1 is considered to be a more conservative estimate of central drive than VA_2, while VA_2 provides an estimation of changes in voluntary activation over time during a sustained isometric contraction. For a detailed discussion see ref: (40).

$$VA_1 = (1- Q_{tw,sup}/Q_{tw,pot}) \times 100 \quad (1)$$

$$VA_2 = MVC/ (MVC+ Q_{tw,sup}) \quad (2)$$

For completeness, both calculations were used in the calculation of VA during the brief contractions also. In addition, $Q_{tw,pot}$ was used as an index of the mechanical (contractile) status of the muscle (2, 40, 59). The mean rate of force development (RFD) and mean rate of force relaxation (RFR) were also calculated for all $Q_{tw,pot}$ (2). For all contractions, for each set of two $Q_{tw,pot}$, mean RFR, and mean RFD were averaged.

For all brief MVCs pre- and post-water-immersion, only the MVC with highest peak force was assessed; the two remaining contractions were discarded from the analysis. Likewise, to analyze the maximum attainable VA for the pre- and post-water-immersion brief MVCs, the $Q_{tw,sup}$ delivered closest in time to peak force was also used. In contrast, for all 120-s MVCs mean VA_1 was calculated using the mean $Q_{tw,sup}$ (thirteen twitches total) and mean pre- and post- $Q_{tw,pot}$ (four twitches total), resulting in a conservative index of average central motor drive to the quadriceps femoris (40).

286

287 ***Post-exercise muscle ischemia***

288 PEMI was used in order to restrict femoral arterial and venous blood flow following the 120-s MVC in the
289 TEMP-LEG; thereby trapping quadriceps intramuscular metabolites. Using PEMI prevents direct
290 circulatory metabolite effects on the CL-LEG while maintaining metaboreceptive feedback from the
291 TEMP-LEG. This was achieved by rapid inflation (180mmHg) of a vascular cuff (SC12L, Hokanson,
292 Bellevue, WA, USA) applied to the TEMP-LEG upper thigh. Occlusion pressure is inversely related to the
293 vascular cuff width; thus 180mmHg was selected, as this is approximately one SD above the mean arterial
294 occlusion pressure for a wide cuff (13.5cm) (43). The cuff was positioned to avoid any interference with
295 the femoral nerve stimulation equipment. Rapid inflation was initiated 10-s prior to the completion of the
296 120-s MVC in the TEMP-LEG (Figure 1).

297

298 ***Perceptual variables***

299 Participants were asked to retrospectively rate (on completion of both sustained MVCs) their mental effort
300 and limb discomfort for each sustained MVC using a modified Borg's CR-10 scale (12, 32). To this end,
301 participants were retrospectively asked: A) 'what was your internal sense of mental effort, independent of
302 all discomforts?' and B) 'what was your sense of active muscle discomfort?' for both the TEMP-LEG and
303 CL-LEG during the sustained 120-s MVCs.

304

305 ***Statistical analysis***

306 To examine the main effect of T_m on neuromuscular function at time points pre- (TEMP-LEG and CL-
307 LEG) and post-water- immersion (CL-LEG only), a one-way (COOL vs WARM) repeated measures
308 ANOVA was used. The tested outcome variables for each brief (3-s) MVC were peak force, 1-s mean
309 force (over the MVC force plateau, including both $Q_{tw,sup}$), peak VA_1, peak VA_2, $Q_{tw,pot}$, $Q_{tw,sup}$, mean RFD
310 and mean RFR. In circumstances where Mauchly's Test of Sphericity was significant, the Greenhouse-
311 Geisser correction was applied.

312

313 For each 120-s sustained MVC in each leg, the main effect of force output and VA_2 were assessed over
314 time (i.e. for each $Q_{tw,sup}$) using a two-way-repeated measures ANOVA (T_m x Time; 2 x 13). Subsequently,

peak force, mean force (whole 120-s), mean force (every 30-s), mean VA_1, mean VA_2, VA_2 (at each time point), as well as post-exercise $Q_{tw,pot}$, mean RFR and mean RFD were then analyzed using a one-way repeated measures ANOVA. A one-way ANOVA was also used to test the thermal variables (T_{core}, mean T_{sk}, and T_m in both legs at all measured depths), HR, mental effort (for each leg) and limb discomfort (for each leg) at the pertinent time points. Parametric testing has been shown to be robust against violations of normal distribution (68); as such non-normally distributed data, which included the perceptual data only, were also analyzed using one-way repeated measures ANOVA. All statistical tests were assessed to a 95% confidence level ($p < 0.05$). All data are displayed as mean ± SD.

RESULTS

Temperature responses

Table 1 shows T_{core}, mean T_{sk}, and T_m at all depths (1-, 2- and 3-cm) and in both legs (TEMP-LEG & CL-LEG). In response to TEMP-LEG water-immersion, none of T_{core}, non- immersed mean T_{sk} or T_m of the CL-LEG at any depth were affected by condition. Immediately (~10-s) post-water-immersion, a spot measurement of thigh T_{sk} in the TEMP-LEG was 13.8 ± 1.1 and 36.5 ± 1.2 °C in the COOL and the WARM conditions, respectively ($p < 0.001$). Thus, TEMP-LEG temperature was independently manipulated, with T_m significantly ($p = 0.001$) higher in WARM compared to COOL conditions, across all measured depths. By completion of the exercise protocol in the WARM condition, T_{core} had become significantly ($p = 0.02$) elevated (0.34°C), while mean T_{sk} was significantly ($p = 0.04$) reduced (~2°C), likely due to increased convective (fan) and evaporative (sweating) cooling during muscle heating. Mean T_{sk} did not change during COOL, and while end T_{core} was lower (~0.1°C) during COOL, the effect was not significant ($p = 0.06$). T_m in the CL-LEG remained unaffected by condition immediately post-exercise, while T_m in the TEMP-LEG remained significantly ($p < 0.001$) higher (~4.5°C) in the WARM compared to the COOL conditions, at all measured depths immediately post-exercise.

Heart rate responses

During single-leg water immersion, HR was significantly ($p = 0.004$) higher in the WARM (93.8 ± 15.3 $b.min^{-1}$) compared to the COOL (80.5 ±18.7 $b.min^{-1}$) conditions. Likewise, during the 120-s MVC in the TEMP-LEG, a trend ($p = 0.07$) for higher HR in WARM was observed (WARM = 136.1 ± 28.3, COOL =

344 131.7 ± 24.3 b.min^{-1}); however during the 120-s MVC in the CL-LEG there were no significant
345 differences in HR (WARM = 143.2 ± 26.8, COOL = 139.5 ± 28.6 b.min^{-1}).
346
347 **[INSERT TABLE 1 HERE]**
348
349 ***Neuromuscular function before water-immersion***
350 Table 2 shows the neuromuscular characteristics before and after water-immersion. Prior to immersion,
351 there were no significant differences between conditions in peak force, mean force (1-s plateau), $VA_{1,}$
352 VA_2, $Q_{tw,pot}$, $Q_{tw,sup}$ or mean RFD during the brief (3-s) MVC, in either TEMP-LEG or CL-LEG. In the
353 TEMP-LEG, a significant ($p = 0.04$) difference in mean RFR was observed between conditions (faster in
354 WARM by 0.3 N.ms^{-1}). This was not observed for mean RFR in the CL-LEG.
355
356 ***Neuromuscular function after water-immersion***
357 After the water-immersion protocol, there were no significant differences for all neuromuscular outcome
358 variables between conditions in the CL-LEG, during the brief MVCs. This indicates that water-immersion
359 of the TEMP-LEG had no effect on the brief MVC characteristics of CL-LEG, immediately post-
360 immersion (Table 2).
361
362 **[INSERT TABLE 2 HERE]**
363
364 ***Sustained central motor drive at different muscle temperatures***
365 Figure 2 shows both mean force output (Panel A) as well as a more conservative estimate of mean VA
366 (VA_1; Panel B) for each 120-s MVC, in both legs, in COOL and WARM conditions. Figure 3 shows a
367 more detailed (over time) representation of MVC force output (Panel A and B) as well as the
368 corresponding change in VA_2 (Panel C and D) in each leg and between conditions.
369
370 **[INSERT FIGURE 2 HERE]**
371
372 **[INSERT FIGURE 3 HERE]**

373

374 At the start of the sustained (120-s) contractions, both peak force in absolute and relative (% pre) terms
375 were not significantly different between conditions, during either contraction. This was reflected in starting
376 (peak) VA_2. Together this indicates there were no central or peripheral alterations in maximal force
377 production at the start of the 120-s MVC, in either the TEMP-LEG or CL-LEG (Figure 3). However over
378 time, both TEMP-LEG force output (condition main effect over time $p = 0.02$) and TEMP-LEG VA_2
379 (condition main effect over time $p = 0.01$) were significantly reduced in the WARM compared to the
380 COOL condition (Figure 3: A and C). This amounted to a significant reduction ($p < 0.001$) in mean force
381 output across the whole contraction in WARM (Figure 2: A), as well as a significant ($p = 0.04$) reduction
382 in the most conservative estimate of mean VA (i.e. VA_1) in WARM (Figure 2: B). While the sense of
383 mental effort was near maximal in both conditions, limb discomfort was significantly ($p < 0.05$) higher in
384 WARM; however, it should be noted that four of eight participants rated limb discomfort as maximal (i.e.
385 10) in both WARM and COOL conditions (Table 3).
386
387 **[INSERT TABLE 3 HERE]**
388
389 Following the sustained MVC and subsequent PEMI in the TEMP-LEG, CL-LEG force output and VA_2
390 were unaffected by the change in condition (Figure 3: B, D). This was reflected in mean MVC force, mean
391 VA_1 as well as the sensation of limb discomfort in the CL-LEG (Figure 2: A, B and Table 3, respectively).
392
393 ***Post-exercise twitch characteristics***
394 There were no significant differences between conditions in post-exercise $Q_{tw,pot}$, $Q_{tw,sup}$ or mean RFD, in
395 either the TEMP-LEG or CL-LEG. However in the CL-LEG, a significant ($p = 0.04$) difference of 0.21
396 $N.ms^{-1}$ was observed in post-exercise mean RFR between conditions. This was not observed for post-
397 exercise mean RFR in the TEMP-LEG.
398
399 DISCUSSION
400 This study investigated whether thermo-metabolic feedback from a warm local skin and muscle
401 temperature would have a larger impact on central motor drive to a remote and thermoneutral muscle

group, compared to thermo-metabolic feedback from a cold local skin and muscle temperature. In order to sustain metaboreceptive feedback from the temperature-manipulated and exercising muscle, as well as to exclude a systemic effect of circulating metabolites on the remote muscle group, post-exercise venous occlusion was used. It was hypothesized that an increase in local skin and muscle temperature would augment metaboreceptive feedback, thereby reducing VA in both a temperature-manipulated leg and the contralateral (thermoneutral) leg.

In the temperature-manipulated leg, the present study showed that during active central motor drive of a muscle characterized by high levels of metaboreceptive feedback, both force output and VA were significantly reduced in a warm limb compared to a cooled limb (Figure 2; Figure 3: A, C). Extending previous findings (40), the present study showed that at the same perceived mental effort, peripheral limb discomfort is significantly higher with increasing muscle temperature (Table 3). However, contrary to our hypothesis, the present study also indicated that any influence of increased local skin and muscle temperature on leg thermo-metabolic feedback does not appear to inhibit voluntary muscle activation of a remote muscle group, as represented by an equal force output and central motor drive in the thermoneutral, contralateral leg (Figure 3).

Research context

Aerobic-mechanical efficiency is considered a critical determinant of self-selected pacing strategy during exercise in extreme environments (55, 56); however humans are not provided with specific receptors for sensing oxygen consumption (24). On the contrary, peripheral fatigue development rates - a direct consequence of reductions in aerobic-mechanical efficiency - can be centrally assimilated through two modalities (42): a) progressive deactivation of mechanoreceptive muscle afferents for a given central motor drive (58); and b) a progressive activation of metaboreceptive muscle afferents (3, 57). In turn, this composite of ergoreceptive activity likely provides the necessary inputs on which humans can modulate VA (or ‘pace’), without exposing specific organs to excessive or intolerable homeostatic disturbances (3, 4, 27). Despite the importance of mechano- and metabo-receptive sensory modalities, the impact of thermal factors on ergoreceptive feedback during fatiguing exercise are not well understood (28, 46, 52).

The influence of heat on metaboreceptor activation and afferent signal transmission

As T_m increases, a faster transduction velocity and higher discharge frequency in metaboreceptive afferent fibers occurs (34, 36, 44, 49). Based on evidence from small muscle groups in humans, cooling delays, while heating increases, muscle sympathetic nerve activity during sustained isometric contractions; the effect of which has been attributed to altered mechano- and/or metabo-receptive sensitivity at different T_m (60, 61). TRPV1 receptors located at the terminal end of III-IV muscle afferents have also been implicated in evoking noxious sensations in response to both thermal factors e.g. heat (63) and non-thermal metabolites produced during fatiguing exercise e.g. lactate (37, 57). As such, decreases in the temperature threshold and/or increases in the thermal sensitivity of TRPV1 may occur in the presence of low pH (13, 19, 63). A net result could be increased noxious sensations in combined heated and lactate saturated muscle (31, 57, 77). Such a thermal sensitization of metaboreceptive afferents is partially indicated in the present study. The findings show that during a prolonged high-intensity and fatiguing contraction, warmer muscle results in both a higher perceived limb discomfort and a lower VA. However, a parallel impact on the contralateral leg was not observed (Figure 3). This suggests that the change in limb discomfort from the temperature-manipulated leg did not influence systemic (whole-body) modulations of VA. This may indicate limited impact of thermal factors on the sensitivity of metaboreceptive afferents during whole-body exercise performance.

Chemical metabolite accumulation under local and systemic thermal strain

The present increase in limb discomfort and decrease in VA in the temperature-manipulated leg may also arise as a direct response to an increase in metabolite production and accumulation rate in heated muscle. In this regard, the reduction in VA may be caused by the muscle Q_{10} effect on tetanic fusion and the consequent reduction in the efficiency of the sustained contractions in warm muscle (10, 40, 70, 76). Such an effect can explain why the corresponding effect was not observed in a remote muscle group i.e. the contralateral leg, where no thermal influence on metabolite production rate was present. If so, a proportional change in VA due to changes in peripheral fatigue rate may still have important implications for whole-body dynamic exercise, where metabo-receptive feedback in active muscle is accelerated by cardiovascular (heat) and biomechanical (cold) strain (41, 42).

460 ***Local improvements in muscle recruitment***

461 The reduction in VA in the temperature-manipulated but not in the contralateral leg may also result from a
462 decrease in sarcolemmal action potential propagation amplitude and/or the reduction in efficiency of
463 peripheral transmission of neural drive, as T_m increases (18, 54, 65). In this regard, an increase in VA at
464 lower T_m could be attributed to longer depolarization time in the peripheral nerve and sarcolemma (54, 65),
465 thereby more effective recruitment of inactive muscle fibers. However, this does not explain why
466 discomfort is increased in some participants, nor why the effect is not exhibited during a brief MVC (40)
467 or at the start of the sustained MVC (Figure 3). Importantly, a combination of factors – direct and indirect
468 influences on afferent feedback as well as sarcolemmal transmission - should not be excluded from the
469 present conclusions.

470

471 ***No influence of whole-body heat content, cerebral or core temperature***

472 Since during both brief and sustained contractions the contralateral leg was unaffected by condition, the
473 change in temperature-manipulated leg VA can only be attributed to local T_m and T_{sk}. This opposes
474 changes in T_{core}, cerebral temperature, true mean body temperature and/or mean T_{sk}, which would have
475 resulted in similar observations in both legs. This finding helps to further elucidate the observations by
476 Lloyd et al. (40), where the impact of T_{core} and T_{sk} could not be fully excluded. From the present study, it
477 can be unequivocally concluded that the changes in motor drive here, and in Lloyd et al. (40) are
478 independent of T_{core}, mean T_{sk} or true mean body temperature (body mass normalized whole-body heat
479 content) and the measurement modality of these variables e.g. rectal vs esophageal temperature
480 assessment.

481

482 ***Local skin temperature***

483 Feedback from localized T_{sk} cannot be ruled out as a potential explanation for the present findings. Recent
484 research indicates a strong link between human behavior, voluntary movement and the activation of
485 cutaneous-thermal group III [Aδ] and IV [C] fibers (66, 67). Given the skin is more densely innervated
486 with thermoreceptors than muscle (31, 49), a reasonable conclusion may be that local T_{sk} is responsible for
487 the alterations in central drive to the active muscle in this study. However, if so, it remains unclear why

this did not influence both legs proportionally, given that the cutaneous thermoreceptive feedback remains active during the sustained contractions in both legs.

Conscious awareness and post-exercise muscle ischemia

The differential roles of autonomic and conscious pathways to reduction in VA under thermal strain are not well understood. As such, it is not possible to exclude a supracortical influence of T_m on VA in the present study (15–17, 62). Indeed, the conscious assimilation of metabo- and thermo-receptive afferents may be influenced by whether a fatigued muscle group is under voluntary control. In the present study, participants would have been consciously aware that reducing VA would not alleviate the increased sensory discomfort of fatigue. In contrast, this is not the case during PEMI and contralateral leg exercise; in which any attenuation of contralateral leg drive will not relieve discomfort experienced in a temperature-manipulated leg.

Final considerations

The absence of a thermoneutral trial may be considered a potential limitation of the present study. However, a linearity between local VA and T_m for the range investigated presently has already been shown (40). Moreover, the present study aimed at understanding whether a local thermal stimulus had any capacity to impose changes in limb discomfort and systemic VA, for which a thermoneutral trial was not required. Had an influence on VA been observed in the contralateral leg, further research may have been warranted to compare dynamic exercise in a neutral (e.g. 33-36°C T_m) or hot environment (e.g. 40-42 °C T_m); although it is important to note, that substantial methodological difficulties are associated with investigating very high T_m in large muscle masses, independently of large changes in T_{core} (31).

Another consideration is that during the later stages of the sustained MVC in the temperature-manipulated leg, muscle force output was lower in the warm muscle (21%MVC) compared to the cool muscle (29%MVC). Consequently, this may have resulted in a different intramuscular pressure and metabolite flushing in the seconds prior to the inflation of the occlusion cuff (10-second before relaxation). On the contrary, research studies have widely reported that muscle is kept fully ischemic at isometric contractions

forces above 10% MVC (25, 72). It should also be recognized that the insertion of a solid needle thermocouple may have impacted the participants' ability to perform a maximal voluntary contraction.

Conclusions

The present study examined the interaction between thermal and metaboreceptive feedback from muscle, to the distribution of central motor drive to a remote and thermoneutral body part. It was shown that increased cutaneous and quadriceps muscle temperature combined with metaboreceptive feedback in a single leg has little or no effect on voluntary activation of a remote muscle group during a 120-s isometric contraction. The foremost implications of these findings are: a) the effects of local skin and muscle temperature change on central motor drive and limb discomfort are localized to actively driven warm muscle groups only; b) if metaboreceptive feedback is enhanced due to afferent nerve warm-sensitization, it is unlikely to systemically or autonomically inhibit motor drive of other (thermoneutral) muscles; and c) the previously observed changes in central motor drive at different local skin and muscle temperatures (40) appear to be unrelated to the change in either core, whole-body mean skin temperature, or true mean body temperature. Further research is necessary to understand the individual and combined impacts of local mechano-, baro-, thermo- and metabo-receptive feedback on exercise performance during thermal strain (38).

534 REFERENCES

535 1. **Amann M**, **Calbet JAL**. Convective oxygen transport and fatigue. *J Appl Physiol* 104: 861–70, 2007.

536 2. **Amann M**, **Romer LM**, **Pegelow DF**, **Jacques AJ**, **Hess CJ**, **Dempsey JA**. Effects of arterial oxygen
537 content on peripheral locomotor muscle fatigue. *J Appl Physiol* 101: 119–27, 2006.

538 3. **Amann M**, **Sidhu SK**, **Weavil JC**, **Mangum TS**, **Venturelli M**. Autonomic responses to exercise:
539 Group III/IV muscle afferents and fatigue. *Auton Neurosci* 188: 19–23, 2015.

540 4. **Amann M**, **Venturelli M**, **Ives SJ**, **McDaniel J**, **Layec G**, **Rossman MJ**, **Richardson RS**. Peripheral
541 fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal
542 output. *J Appl Physiol* 115: 355–64, 2013.

543 5. **Amano T**, **Ichinose M**, **Inoue Y**, **Nishiyasu T**, **Koga S**, **Kondo N**. Modulation of muscle
544 metaboreceptor activation upon sweating and cutaneous vascular responses to rising core temperature in
545 humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* (2015). doi: 10.1152/ajpregu.00005.2015.

546 6. **Benham CD**, **Gunthorpe MJ**, **Davis JB**. TRPV channels as temperature sensors. *Cell Calcium* 33:
547 479–487, 2003.

548 7. **Boulant JA**. Hypothalamic Neurons Regulating Body Temperature. *Compr Physiol* : 105–126, 2011.

549 8. **Boulant JA**, **Bignall KE**. Hypothalamic neuronal responses to peripheral and deep-body temperatures.
550 *Am J Physiol* 225: 1371–1374, 1973.

551 9. **Boulant JA**, **Hardy JD**. The effect of spinal and skin temperatures on the firing rate and
552 thermosensitivity of preoptic neurones. *J Physiol* 240: 639–660, 1974.

553 10. **Cahill F**, **Kalmar JM**, **Pretorius T**, **Gardiner PF**, **Giesbrecht GG**. Whole-body hypothermia has
554 central and peripheral influences on elbow flexor performance. *Exp Physiol* 96: 528–38, 2011.

555 11. **Castellani JW**, **Tipton MJ**. Cold Stress Effects on Exposure Tolerance and Exercise Performance.
556 *Compr Physiol* 6: 443–469, 2015.

557 12. **Christian RJ**, **Bishop DJ**, **Billaut F**, **Girard O**. The role of sense of effort on self-selected cycling
558 power output. *Front Physiol* 5: 115, 2014.

559 13. **Clapham DE**. TRP channels as cellular sensors. *Nature* 426: 517–524, 2003.

560 14. **Colin J**, **Timbal J**, **Houdas Y**, **Boutelier C**, **Guieu JD**. Computation from rectal of mean body
561 temperature and skin temperatures. *J Appl Physiol* 31: 484–489, 1971.

562 15. **Craig AD**. Interoception: The sense of the physiological condition of the body. *Curr Opin Neurobiol*
563 13: 500–505, 2003.

564 16. **Craig ADB**. Significance of the insula for the evolution of human awareness of feelings from the body.
565 *Ann N Y Acad Sci* 1225: 72–82, 2011.

566 17. **Critchley HD**. Neural mechanisms of autonomic, affective, and cognitive integration. *J Comp Neurol*
567 493: 154–166, 2005.

568 18. **Dewhurst S**, **Riches PE**, **Nimmo MA**, **De Vito G**. Temperature dependence of soleus H-reflex and M
569 wave in young and older women. *Eur J Appl Physiol* 94: 491–499, 2005.

570 19. **Dhaka A**, **Uzzell V**, **Dubin AE**, **Mathur J**, **Petrus M**, **Bandell M**, **Patapoutian A**. TRPV1 Is
571 Activated by Both Acidic and Basic pH. *J Neurosci* 29: 153–158, 2009.

572 20. **Edwards RH**, **Harris RC**, **Hultman E**, **Kaijser L**, **Koh D**. Effect of temperature on muscle energy
573 metabolism and endurance during successive isometric contractions, sustained to fatigue, of the
574 quadriceps muscle in man. *J Physiol* 220: 335–352, 1972.

575 21. **Faulkner SH**, **Ferguson RA**, **Gerrett N**, **Hupperets M**, **Hodder SG**, **Havenith G**. Reducing muscle
576 temperature drop after warm-up improves sprint cycling performance. *Med Sci Sport Exerc* 45: 359–65,
577 2013.

578 22. **Flouris AD**, **Schlader ZJ**. Human behavioral thermoregulation during exercise in the heat. *Scand J*
579 *Med Sci Sports* 25: 52–64, 2015.

580 23. **Folland JP**, **Williams AG**. Methodological issues with the interpolated twitch technique. *J*
581 *Electromyogr Kinesiol* 17: 317–27, 2007.

582 24. **Forster H V.**, **Haouzi P**, **Dempsey JA**. Control of breathing during exercise. *Compr Physiol* 2: 743–

777, 2012.

25. **Gaffney F a**, **Sjøgaard G**, **Saltin B**. Cardiovascular and metabolic responses to static contraction in man. *Acta Physiol Scand* 138: 249–258, 1990.

26. **Gandevia SC**. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725–89, 2001.

27. **Gandevia SC**, **Allen GM**, **Butler JE**, **Taylor JL**. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex [Online]. *J Physiol* 490: 529–536, 1996. http://jp.physoc.org/content/490/Pt_2/529.short [17 Dec. 2014].

28. **Girard O**, **Brocherie F**, **Bishop DJ**. Sprint performance under heat stress: A review. *Scand J Med Sci Sports* 25: 79–89, 2015.

29. **González-Alonso J**, **Crandall CG**, **Johnson JM**. The cardiovascular challenge of exercising in the heat. *J Physiol* 586: 45–53, 2008.

30. **Grassi B**, **Rossiter HB**, **Zoladz JA**. Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? *Exerc Sport Sci Rev* 43: 75–83, 2015.

31. **Graven-Nielsen T**, **Arendt-Nielsen L**, **Mense S**. Thermosensitivity of muscle: high-intensity thermal stimulation of muscle tissue induces muscle pain in humans. *J Physiol* 540: 647–656, 2002.

32. **Hamilton AL**, **Killian J**, **Summers E**, **Jones NL**. Quantification of intensity muscular work by normal of sensations subjects during. *J Appl Physiol* 81: 1156–1161, 1996.

33. **Hasegawa H**, **Piacentini MF**, **Sarre S**, **Michotte Y**, **Ishiwata T**, **Meeusen R**. Influence of brain catecholamines on the development of fatigue in exercising rats in the heat. *J Physiol* 586: 141–149, 2008.

34. **Hertel HC**, **Howaldt B**, **Mense S**. Responses of group IV and group III muscle afferents to thermal stimuli. *Brain Res* 113: 201–205, 1976.

35. **Krustrup P**, **Söderlund K**, **Relu MU**, **Ferguson RA**, **Bangsbo J**. Heterogeneous recruitment of quadriceps muscle portions and fibre types during moderate intensity knee-extensor exercise: effect of thigh occlusion. *Scand J Med Sci Sports* 19: 576–84, 2009.

36. **Kumazawa T**, **Mizumura K**. Thin-fibre receptors responding to mechanical, chemical, and thermal stimulation in the skeletal muscle of the dog. *J Physiol* 273: 179–194, 1977.

37. **Light AR**, **Hughen RW**, **Zhang J**, **Rainier J**, **Liu Z**, **Lee J**. Dorsal root ganglion neurons innervating skeletal muscle respond to physiological combinations of protons, ATP, and lactate mediated by ASIC, P2X, and TRPV1. *J Neurophysiol* 100: 1184–1201, 2008.

38. **Lloyd A**, **Havenith G**. Interactions in human performance: an individual and combined stressors approach. *Temperature* 120: 567–579, 2016.

39. **Lloyd A**, **Hodder S**, **Havenith G**. The interactive effect of cooling and hypoxia on forearm fatigue development. *Eur J Appl Physiol* 115: 2007–2018, 2015.

40. **Lloyd A**, **Hodder S**, **Havenith G**. The interaction between peripheral and central fatigue at different muscle temperatures during sustained isometric contractions. *Am J Physiol Regul Integr Comp Physiol* 309: R410–R420, 2015.

41. **Lloyd A**, **Hodder SG**, **Raccuglia M**, **Havenith G**. The Interaction Between Temperature And Hypoxia On The Rate Of Neuromuscular Fatigue Development. *Med Sci Sport Exerc* 47: 610, 2015.

42. **Lloyd A**, **Raccuglia M**, **Hodder S**, **Havenith G**. Interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension. *J Appl Physiol* 120: 567–579, 2016.

43. **Loenneke JP**, **Fahs CA**, **Rossow LM**, **Sherk VD**, **Thiebaud RS**, **Abe T**, **Bemben DA**, **Bemben MG**. Effects of cuff width on arterial occlusion: Implications for blood flow restricted exercise. *Eur J Appl Physiol* 112: 2903–2912, 2012.

44. **Ludin HP**, **Beyeler F**. Temperature dependence of normal sensory nerve action potentials. *J Neurol* 216: 173–180, 1977.

45. **Mallette MM**, **Hodges GJ**, **McGarr GW**, **Gabriel DA**, **Cheung SS**. Investigating the roles of core and local temperature on forearm skin blood flow. *Microvasc Res* 106: 88–95, 2016.

46. **Martin PG**, **Marino FE**, **Rattey J**, **Kay D**, **Cannon J**. Reduced voluntary activation of human skeletal muscle during shortening and lengthening contractions in whole body hyperthermia. *Exp Physiol* 90:

225–36, 2005.

47. **Martin PG**, **Smith JL**, **Butler JE**, **Gandevia SC**, **Taylor JL**. Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans. *J Neurosci* 26: 4796–4802, 2006.

48. **Mcardle D**, **Magel JR**, **Lesmes GR**, **Pechar GS**. Metabolic and cardiovascular adjustment to work in air and water at 18, 25 and 33° C. *J Appl Physiol* 40: 85–90, 1976.

49. **Mense S**, **Meyer H**. Different types of slowly conducting afferent units in cat skeletal muscle and tendon. *J Physiol* 363: 403–17, 1985.

50. **Merton PA**. Voluntary Strength and Fatigue. *J Physiol* 1: 553–564, 1954.

51. **Nybo L**, **Nielsen B**. Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol* 91: 1055–1060, 2001.

52. **Nybo L**, **Rasmussen P**, **Sawka MN**. Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. *Compr Physiol* 4: 657–689, 2014.

53. **Nybo L**, **Secher NH**, **Nielsen B**. Inadequate heat release from the human brain during prolonged exercise with hyperthermia. *J Physiol* 545: 697–704, 2002.

54. **Périard JD**, **Christian RJ**, **Knez WL**, **Racinais S**. Voluntary muscle and motor cortical activation during progressive exercise and passively induced hyperthermia. *Exp Physiol* 99: 136–48, 2014.

55. **Périard JD**, **Racinais S**. Performance and Pacing during Cycle Exercise in Hyperthermic and Hypoxic Conditions. *Med Sci Sports Exerc* : 845–853, 2015.

56. **Périard JD**, **Racinais S**. Self-paced exercise in hot and cool conditions is associated with the maintenance of %V̇o2peak within a narrow range. *J Appl Physiol* 118: 1258–1265, 2015.

57. **Pollak KA**, **Swenson JD**, **Vanhaitsma TA**, **Hughen RW**, **Jo D**, **White AT**, **Light KC**, **Schweinhardt P**, **Amann M**, **Light AR**. Exogenously applied muscle metabolites synergistically evoke sensations of muscle fatigue and pain in human subjects. *Exp Physiol* 99: 368–80, 2014.

58. **Proske U**, **Gandevia SC**. The Proprioceptive Senses: Their Roles in Signaling Body Shape, Body Position and Movement, and Muscle Force. *Physiol Rev* 92: 1651–1697, 2012.

59. **Racinais S**, **Gaoua N**, **Grantham J**. Hyperthermia impairs short-term memory and peripheral motor drive transmission. *J Physiol* 586: 4751–4762, 2008.

60. **Ray CA**, **Gracey KH**. Augmentation of exercise-induced muscle sympathetic nerve activity during muscle heating. *J Appl Physiol* 82: 1719–1733, 1997.

61. **Ray CA**, **Hume KM**, **Gracey KH**, **Mahoney ET**. Muscle cooling delays activation of the muscle metaboreflex in humans exercise humans. *Am J Physiol Heart Circ Physiol* 273: H2436–H2441, 1997.

62. **Robertson C**, **Marino F**. A role for the prefrontal cortex in exercise tolerance and termination. *J. Appl. Physiol.* (2015). doi: 10.1152/japplphysiol.00363.2015.

63. **Romanovsky AA**. Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. *Am J Physiol Regul Integr Comp Physiol* 292: R37–R46, 2007.

64. **Rowell LB**. Human Cardiovascular Adjustments Exercise and Thermal Stress. *Physiol Rev* 54: 75–159, 1974.

65. **Rutkove SB**. Effects of temperature on neuromuscular electrophysiology. *Muscle and Nerve* 24: 867–882, 2001.

66. **Schlader Z**. The relative overlooking of human behavioral temperature regulation: An issue worth resolving. *Temperature* 1: 20–21, 2014.

67. **Schlader Z**, **Perry BG**, **Jusoh MR**, **Hodges LD**, **Stannard SR**, **Mündel T**. Human temperature regulation when given the opportunity to behave. *Eur J Appl Physiol* 113: 1291–1301, 2013.

68. **Schmider E**, **Ziegler M**, **Danay E**, **Beyer L**, **Bühner M**. Is It Really Robust?: Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. *Methodology* 6: 147–151, 2010.

69. **Secher NH**, **Seifert T**, **Van Lieshout JJ**. Cerebral blood flow and metabolism during exercise: implications for fatigue. *J Appl Physiol* 104: 306–14, 2008.

70. **Segal SS**, **Faulkner JA**, **White TP**. Skeletal muscle fatigue in vitro is temperature dependent. *J Appl Physiol* 61: 660–665, 1986.

71. **Silva NL**, **Boulant J a**. Effects of testosterone, estradiol, and temperature on neurons in preoptic tissue slices. *Am J Physiol - Regul Integr Comp Physiol* 19: R625–R632, 1986.

72. **Sjogaard G**, **Savard G**, **Juel C**. Muscle blood-flow during isometric activity and its relation to muscle fatigue. *Eur J Appl Physiol Occup Physiol* 57: 327–335, 1988.

73. **Stacey MJ**. Free nerve endings in skeletal muscle of the cat. *J Anat* 105: 231–254, 1969.

74. **Stolwijk JA**. A mathematical model of physiological temperature regulation in man. National Aeronautics and Space Administration.

75. **Taylor AD**, **Bronks R**, **Smith P**, **Humphries B**. Myoelectric evidence of peripheral muscle fatigue during exercise in severe hypoxia: some references to m. vastus lateralis myosin heavy chain composition. *Eur J Appl Physiol* 75: 151–9, 1997.

76. **Todd G**, **Butler JE**, **Taylor JL**, **Gandevia SC**. Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* 563: 621–31, 2005.

77. **VanHaitsma TA**, **Light AR**, **Light KC**, **Hughen RW**, **Yenchik S**, **White AT**. Fatigue sensation and gene expression in trained cyclists following a 40 km time trial in the heat. *Eur. J. Appl. Physiol.* (2015). doi: 10.1007/s00421-015-3311-9.

78. **Wakabayashi H**, **Oksa J**, **Tipton MJ**. Exercise performance in acute and chronic cold exposure. *J Phys Fit Sport Med* 4: 177–185, 2015.

FIGURE & TABLE LEGENDS

Table 1: Temperature recordings before (PRE-WI) and after water-immersion (POST-WI) and immediately post-exercise (POST-EX). TEMP-LEG indicates the muscle temperature-manipulated leg; CL-LEG indicates the contralateral-thermoneutral leg. The two experimental conditions are COOL: 25 minutes single-leg immersion in 8°C water, and WARM: 15 minutes single-leg immersion in 44°C water. Core temperature is measured rectally. Mean T_{sk} was calculated using equal weighting from each of the six measurement sites. Muscle temperature is displayed at each measured depth (1, 2 and 3-cm). All data are presented as mean ± SD (n = 8). *significant difference between WARM and COOL, $p < 0.05$. $^{\#}$trend for difference between WARM and COOL, $p < 0.1$.

Table 2: Neuromuscular function during a brief (3-s) maximal voluntary contraction (MVC). Data is displayed for the assessments before water-immersion (PRE-WI), in both the temperature-manipulated (TEMP-LEG) and the contralateral, thermoneutral leg (CL-LEG), as well as after water-immersion (POST-WI), in the CL-LEG only (see Figure 1). The two experimental conditions are COOL: 25 minutes single-leg immersion in 8°C water, and WARM: 15 minutes single-leg immersion in 44°C water. VA_1, voluntary activation calculated using equation 1; VA_2, voluntary activation calculated using equation 2; $Q_{tw,sup}$, superimposed twitch force; $Q_{tw,pot}$, resting potentiated twitch force; mean RFD, resting twitch mean rate of force development; mean RFR, resting twitch mean rate of relaxation. All PRE-WI and POST-WI values are in relation to the MVC with highest peak force. Each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC. All data are presented as mean ± SD (n = 8). *significant difference between WARM and COOL, $p < 0.05$.

Table 3: Subjective ratings of mental effort and limb discomfort immediately post-exercise. TEMP-LEG indicates the muscle temperature-manipulated leg; CL-LEG indicates the contralateral-thermoneutral leg. The two experimental conditions are COOL: 25 minutes single-leg immersion in 8°C water, and WARM: a 15 minutes single-leg immersion in 44°C water. All perceptions were assessed using modified Borg's

729 CR-10 scale. All data are presented as mean ± SD (n = 8). *significant difference between WARM and
730 COOL, $p < 0.05$.

731 Figure 1: The general methods. Panel A illustrates schematic of the general procedure. White boxes
732 indicate the schematic overview of the experimental protocol. Grey boxes indicate the outcome measures.
733 Dark grey provides a visual reference for muscle contraction and supramaximal twitches. Panel B shows
734 an example force trace during a brief (3-s) maximal voluntary contraction in one participant. Vertical lines
735 indicate the doublet simulation of the femoral nerve. The two corresponding superimposed twitches
736 evoked during each contraction are indicated using the abbreviation $Q_{tw,sup}$. The two resting potentiated
737 twitches are indicated by the abbreviation $Q_{tw,pot}$. T_{re}, rectal temperature; T_m, muscle temperature; T_{sk}, skin
738 temperature; MVC, maximal isometric voluntary contraction force of knee extensors; HR, heart rate;
739 TEMP-LEG, the muscle temperature-manipulated leg; CL-LEG, the contralateral-thermoneutral leg.

740

741 Figure 2: The effect of temperature condition on mean contraction force and mean voluntary activation
742 percentage (equation 1) during a 120-s sustained maximal isometric voluntary knee extension. Panel A
743 shows the contraction force in the both the TEMP-LEG and the contralateral, thermoneutral leg (CL-LEG).
744 Panel B shows the voluntary muscle activation in the TEMP-LEG and the CL-LEG. For the 120-s MVCs,
745 mean VA_1 was calculated using the mean $Q_{tw,sup}$ (thirteen twitches total) and mean pre- and post- $Q_{tw,pot}$
746 (four twitches total). All data are presented as mean ± SD (n = 8). The p-value for each repeated measured
747 one-way ANOVA (t-test) is displayed above the corresponding set of bars. *significant difference between
748 WARM and COOL, $p < 0.05$.

749

750 Figure 3: The effect of muscle temperature (T_m) on contraction force (as a percentage of pre-water-
751 immersion values) and voluntary activation percentage (equation 2 as a percentage of pre-water-immersion
752 values) during a 120-s sustained maximal isometric voluntary contraction. Panel A shows the contraction
753 force in the temperature-manipulated leg (TEMP-LEG). Panel B shows the contraction force in the
754 contralateral, thermoneutral leg (CL-LEG). Panel C shows the voluntary muscle activation in the TEMP-
755 LEG. Panel D shows the voluntary muscle activation in the CL-LEG. Grey lines represent the COOL

condition, while black lines represent WARM. All data are presented as mean ± SD (n = 8). In all panels, the p-value for each repeated measured one-way ANOVA is displayed for each 30-s mean of the contraction. In addition, panels C and D are analyzed for each superimposed twitch (13 total). *significant difference between WARM and COOL, $p < 0.05$. $^{\#}$trend for difference between WARM and COOL, $p < 0.1$.

762 **Table 1:**

Variable	Time Point	COOL	WARM
Core temperature (°C)	PRE-WI	37.43 ± 0.25	37.41 ± 0.31
	POST-WI	37.49 ± 0.16	37.62 ± 0.30
	POST-EX	37.32 ± 0.21	37.68 ± 0.20*
TEMP-LEG 1 - cm muscle temperature (°C)	PRE-WI	33.5 ± 2.3	33.5 ± 2.0
	POST-WI	26.0 ± 4.7	37.2 ± 1.1*
	POST-EX	30.1 ± 2.9	36.2 ± 2.1*
CL-LEG 1 - cm muscle temperature (°C)	PRE-WI	33.7 ± 2.2	33.8 ± 2.3
	POST-WI	33.5 ± 2.2	33.7 ± 1.4
	POST-EX	34.9 ± 2.0	34.7 ± 2.1
TEMP-LEG 2 - cm muscle temperature (°C)	PRE-WI	35.1 ± 1.2	35.0 ± 0.9
	POST-WI	29.4 ± 4.0	37.6 ± 0.4*
	POST-EX	32.9 ± 2.2	37.5 ± 0.5*
CL-LEG 2 - cm muscle temperature (°C)	PRE-WI	35.1 ± 1.1	35.3 ± 0.9
	POST-WI	35.3 ± 0.7	35.2 ± 1.0
	POST-EX	36.6 ± 0.7	36.7 ± 0.8
TEMP-LEG 3 - cm muscle temperature (°C)	PRE-WI	35.8 ± 1.0	35.5 ± 0.7
	POST-WI	31.4 ± 2.9	37.7 ± 0.4*
	POST-EX	34.0 ± 1.5	37.8 ± 0.4*
CL-LEG 3 - cm muscle temperature (°C)	PRE-WI	35.8 ± 0.7	35.9 ± 0.6
	POST-WI	35.7 ± 0.5	35.7 ± 0.7
	POST-EX	36.8 ± 0.4	37.2 ± 0.5#
Mean skin temperature (°C)	PRE-WI	33.2 ± 0.8	32.5 ± 1.7
	POST-WI	33.0 ± 0.8	32.2 ± 1.2
	POST-EX	33.2 ± 0.4	30.5 ± 2.5*

763

764

765

766 **Table 2:**

Variable	Time Point	COOL	WARM
Peak MVC Force (N)	PRE-WI CL-LEG	983 ± 268	977 ± 268
	PRE-WI TEMP-LEG	1086 ± 226	1029 ± 232
	POST-WI CL-LEG	1039 ± 287	1052 ± 238
1-s Mean MVC Force (N)	PRE-WI CL-LEG	791 ± 272	791 ± 264
	PRE-WI TEMP-LEG	917 ± 206	892 ± 258
	POST-WI CL-LEG	890 ± 279	908 ± 202
Peak VA_1 (%)	PRE-WI CL-LEG	68.7 ± 16.1	63.8 ± 22.0
	PRE-WI TEMP-LEG	73.0 ± 12.5	73.1 ± 13.3
	POST-WI CL-LEG	73.9 ± 11.9	74.5 ± 10.4
Peak VA_2 (%)	PRE-WI CL-LEG	85.4 ± 9.7	83.8 ± 10.6
	PRE-WI TEMP-LEG	89.1 ± 5.4	88.8 ± 6.5
	POST-WI CL-LEG	88.0 ± 8.4	89.8 ± 5.5
Mean $Q_{tw,sup}$ (N)	PRE-WI CL-LEG	134 ± 89	144 ± 99
	PRE-WI TEMP-LEG	115 ± 53	108 ± 57
	POST-WI CL-LEG	114 ± 62	108 ± 66
Mean $Q_{tw,pot}$ (N)	PRE-WI CL-LEG	415 ± 118	400 ± 93
	PRE-WI TEMP-LEG	424 ± 88	399 ± 71
	POST-WI CL-LEG	431 ± 118	403 ± 94
Mean RFD ($N.ms^{-1}$)	PRE-WI CL-LEG	5.66 ± 2.07	5.12 ± 1.59
	PRE-WI TEMP-LEG	5.91 ± 1.57	5.73 ± 1.29
	POST-WI CL-LEG	5.66 ± 2.02	6.02 ± 2.65
Mean RFR ($N.ms^{-1}$)	PRE-WI CL-LEG	1.68 ± 0.75	1.76 ± 0.60
	PRE-WI TEMP-LEG	1.72 ± 0.52	1.42 ± 0.52*
	POST-WI CL-LEG	1.66 ± 0.75	1.53 ± 0.53

767

768

769

770

771

772

Table 3:

Variable	Time Point	COOL	WARM
Mental Effort (CR-10)	TEMP-LEG	10.0 ± 0.0	9.9 ± 0.4
	CL-LEG	9.9 ± 0.4	10.0 ± 0.0
Sense of Limb Discomfort (CR-10)	TEMP-LEG	7.4 ± 3.0	8.6 ± 2.3*
	CL-LEG	7.9 ± 2.8	7.9 ± 3.1

Figure 1:

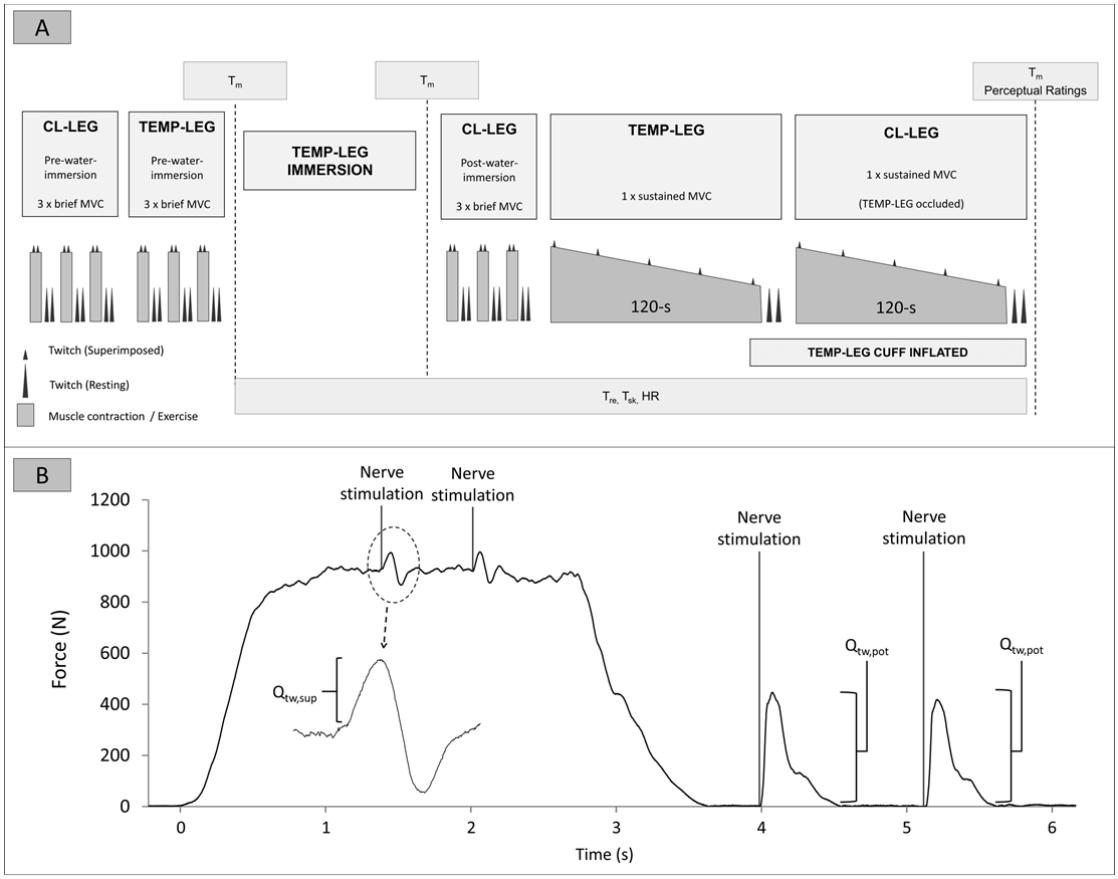


Figure 2:

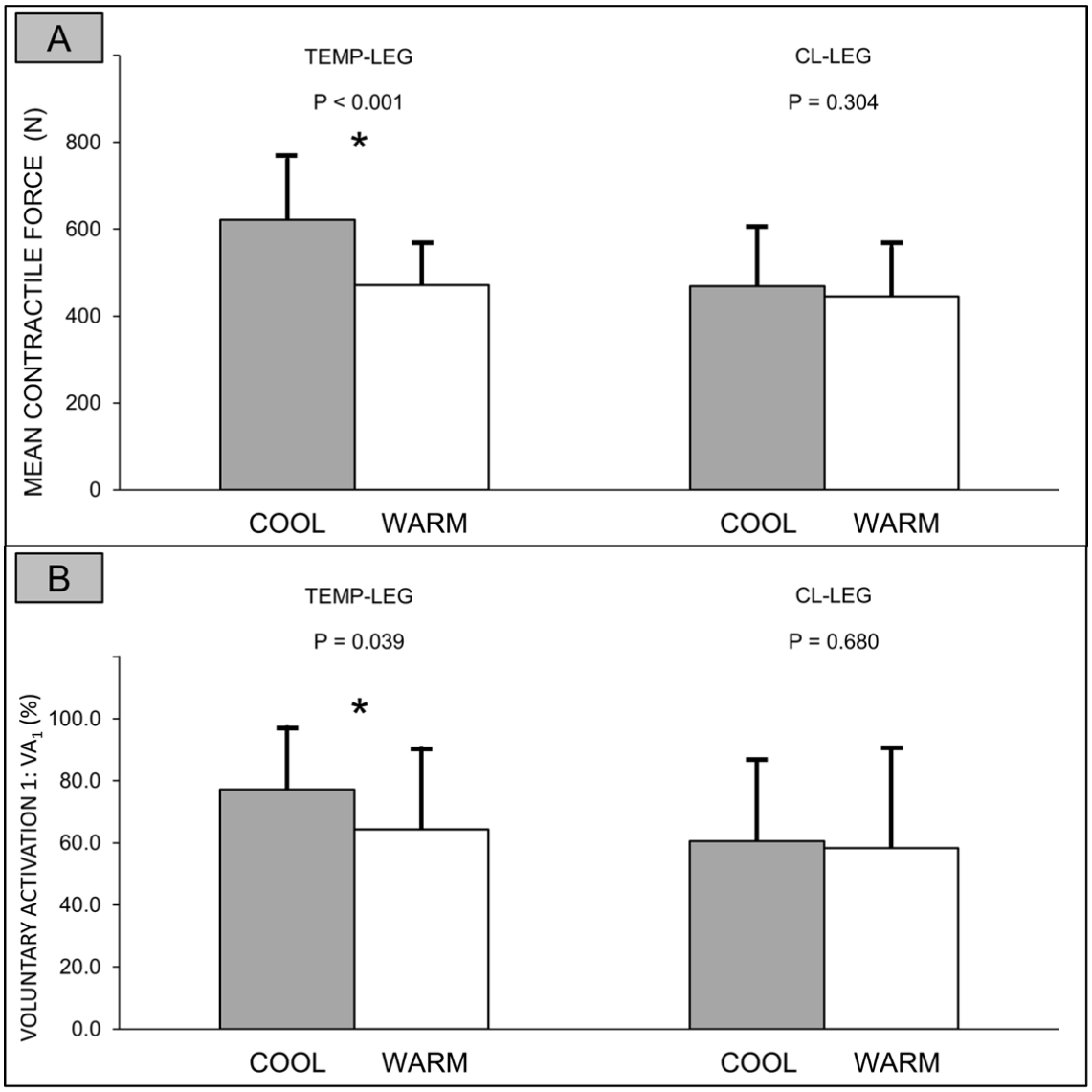
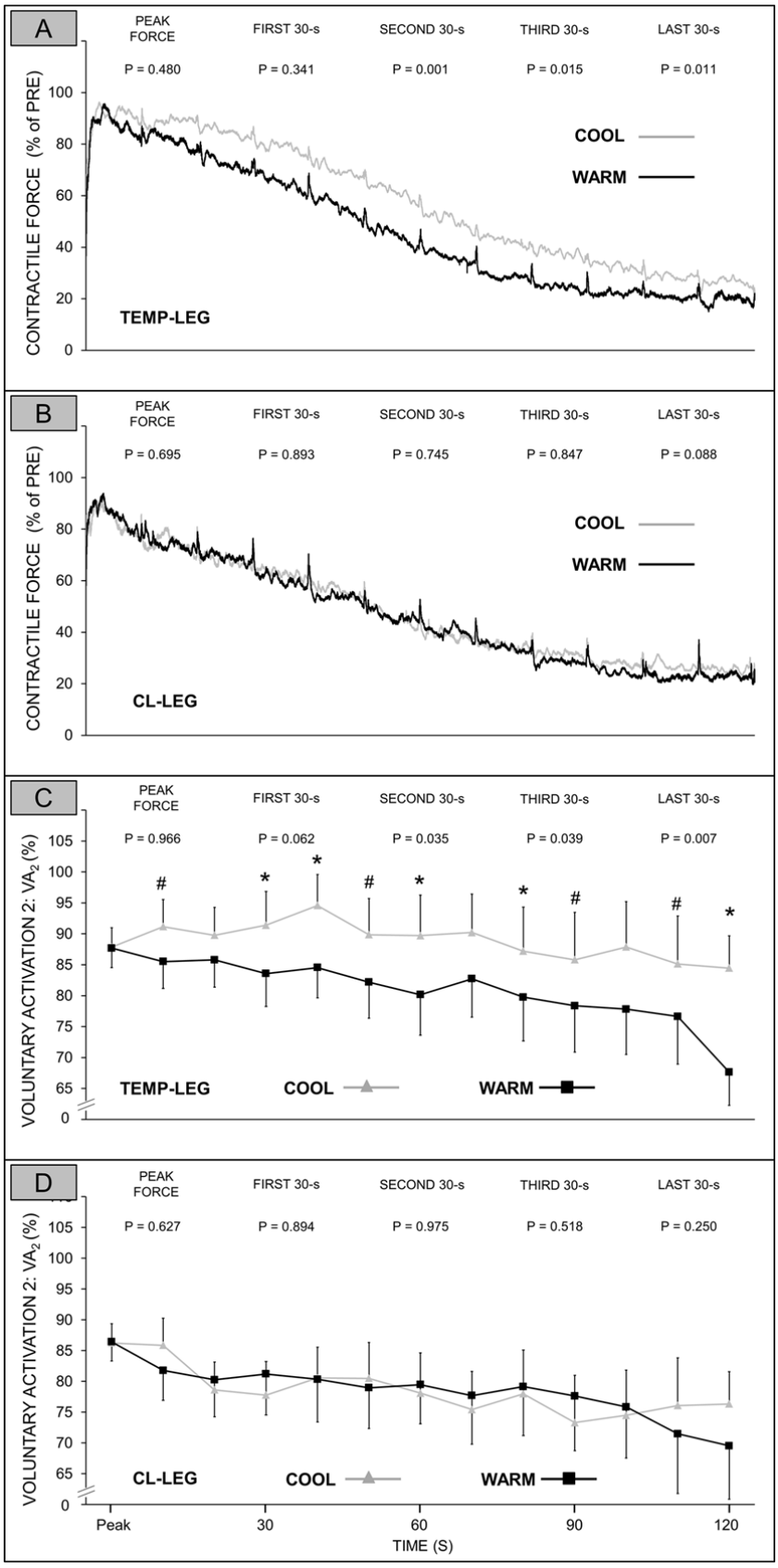

A
TEMP-LEG
P < 0.001
*
CL-LEG
P = 0.304
MEAN CONTRACTILE FORCE (N)
800
600
400
200
0
COOL
WARM
COOL
WARM
B
TEMP-LEG
P = 0.039
*
CL-LEG
P = 0.680
VOLUNTARY ACTIVATION 1: VA1 (%)
100.0
80.0
60.0
40.0
20.0
0.0
COOL
WARM
COOL
WARM

Figure 3:


A
PEAK FORCE
FIRST 30-s
SECOND 30-s
THIRD 30-s
LAST 30-s
P = 0.480
P = 0.341
P = 0.001
P = 0.015
P = 0.011
CONTRACTILE FORCE (% of PRE)
COOL
WARM
TEMP-LEG
B
P = 0.695
P = 0.893
P = 0.745
P = 0.847
P = 0.088
CL-LEG
C
P = 0.966
P = 0.062
P = 0.035
P = 0.039
P = 0.007
VOLUNTARY ACTIVATION 2: VA2 (%)
D
P = 0.627
P = 0.894
P = 0.975
P = 0.518
P = 0.250
Peak
30
60
90
120
TIME (S)

A

T_m

T_m

T_m
Perceptual Ratings

CL-LEG

Pre-water-immersion

3 x brief MVC

TEMP-LEG

Pre-water-immersion

3 x brief MVC

TEMP-LEG IMMERSION

CL-LEG

Post-water-immersion

3 x brief MVC

TEMP-LEG

1 x sustained MVC

120-s

CL-LEG

1 x sustained MVC

(TEMP-LEG occluded)

120-s

TEMP-LEG CUFF INFLATED

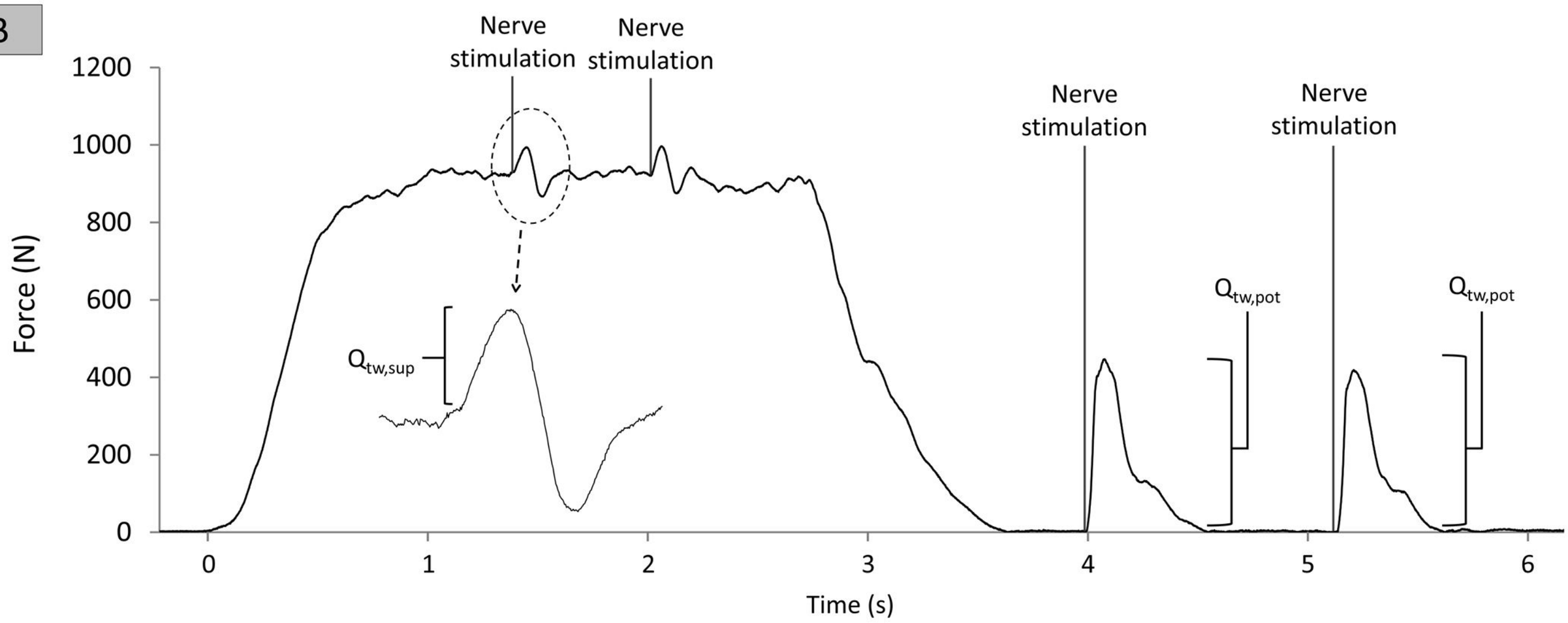
Twitch (Superimposed)

Twitch (Resting)

Muscle contraction / Exercise

T_{re}, T_{sk}, HR

B



A

TEMP-LEG

P < 0.001

*

CL-LEG

P = 0.304

MEAN CONTRACTILE FORCE (N)

800

600

400

200

0

COOL WARM

COOL WARM

B

TEMP-LEG

P = 0.039

*

CL-LEG

P = 0.680

VOLUNTARY ACTIVATION 1: VA_1 (%)

100.0

80.0

60.0

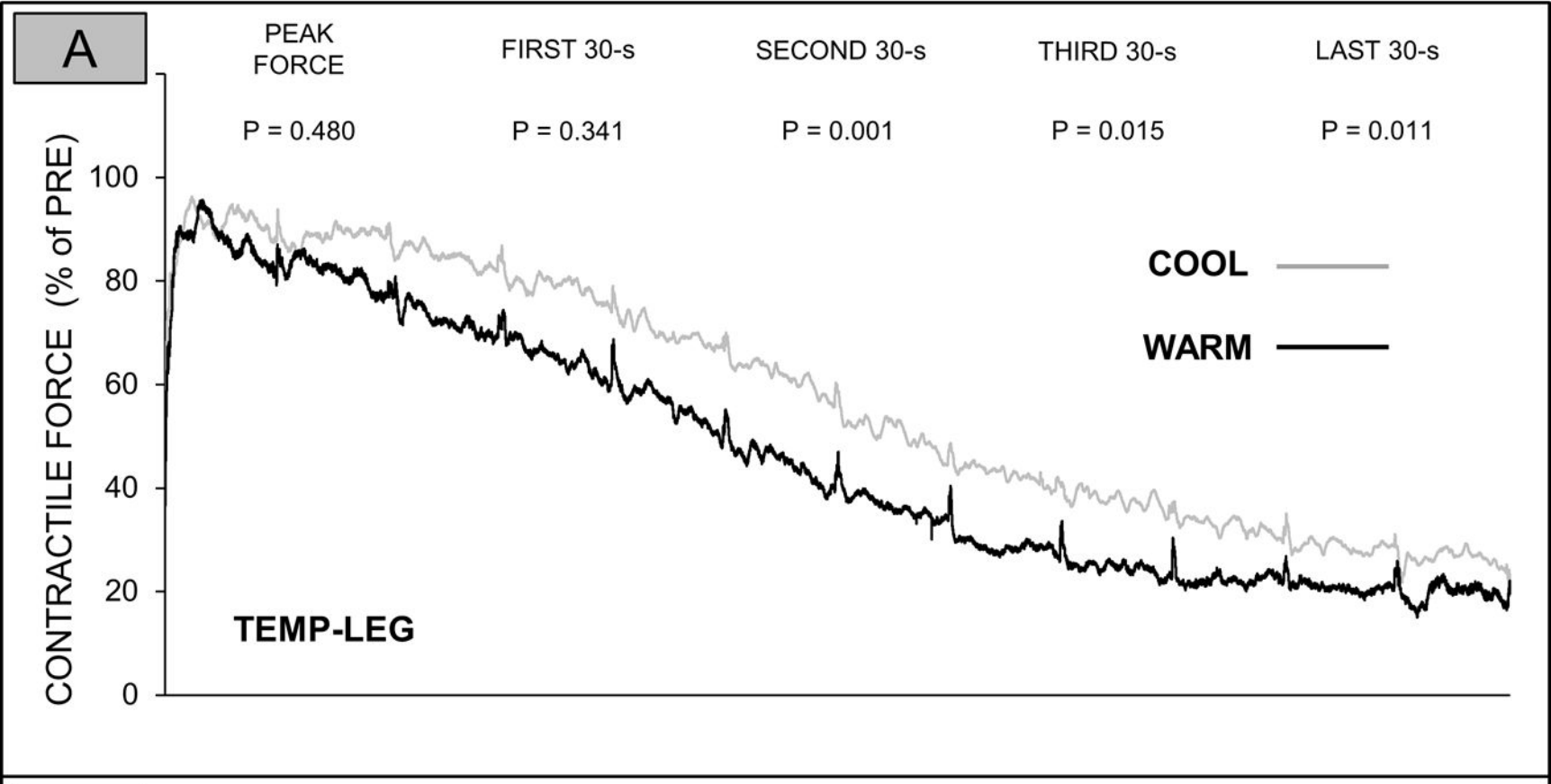
40.0

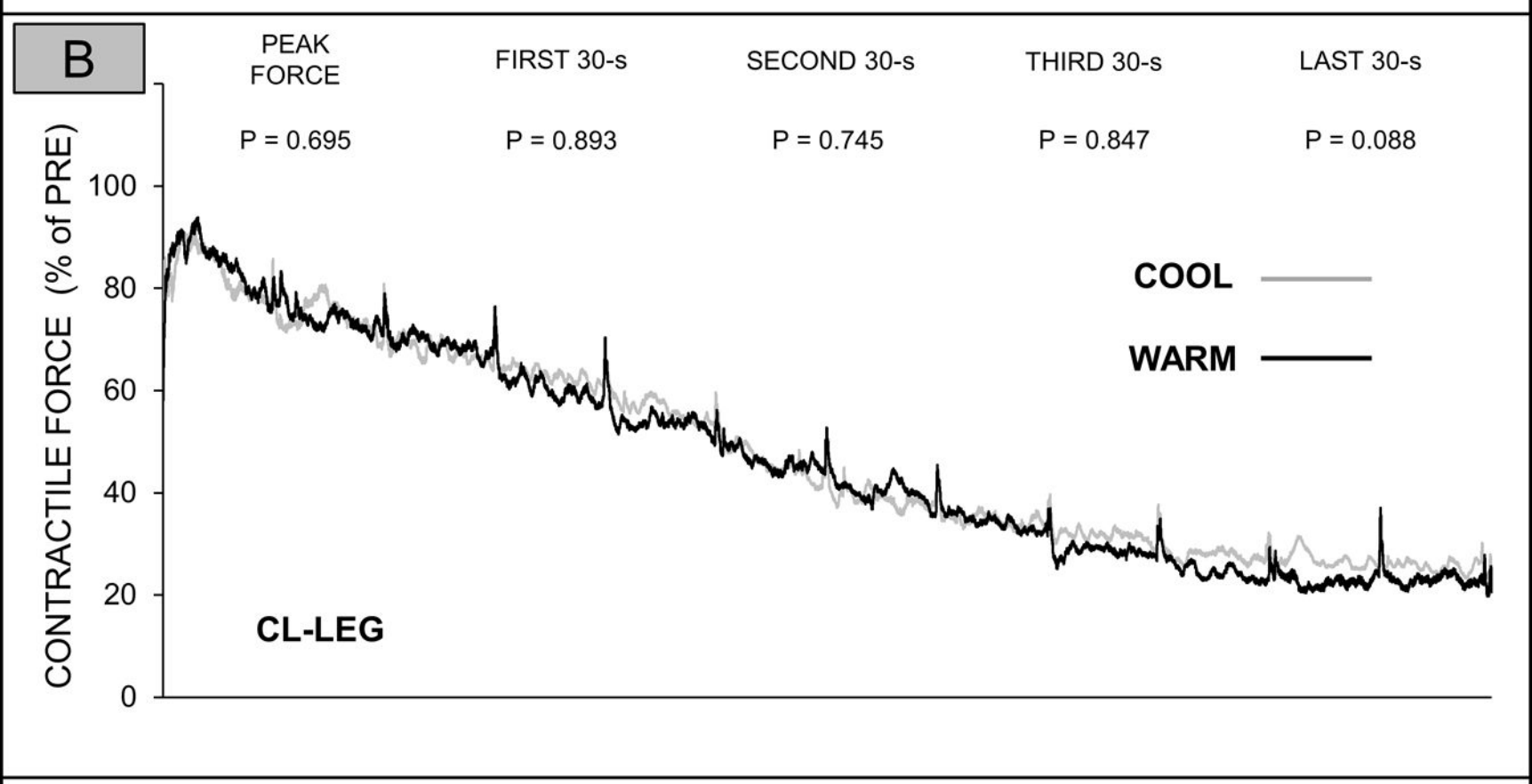
20.0

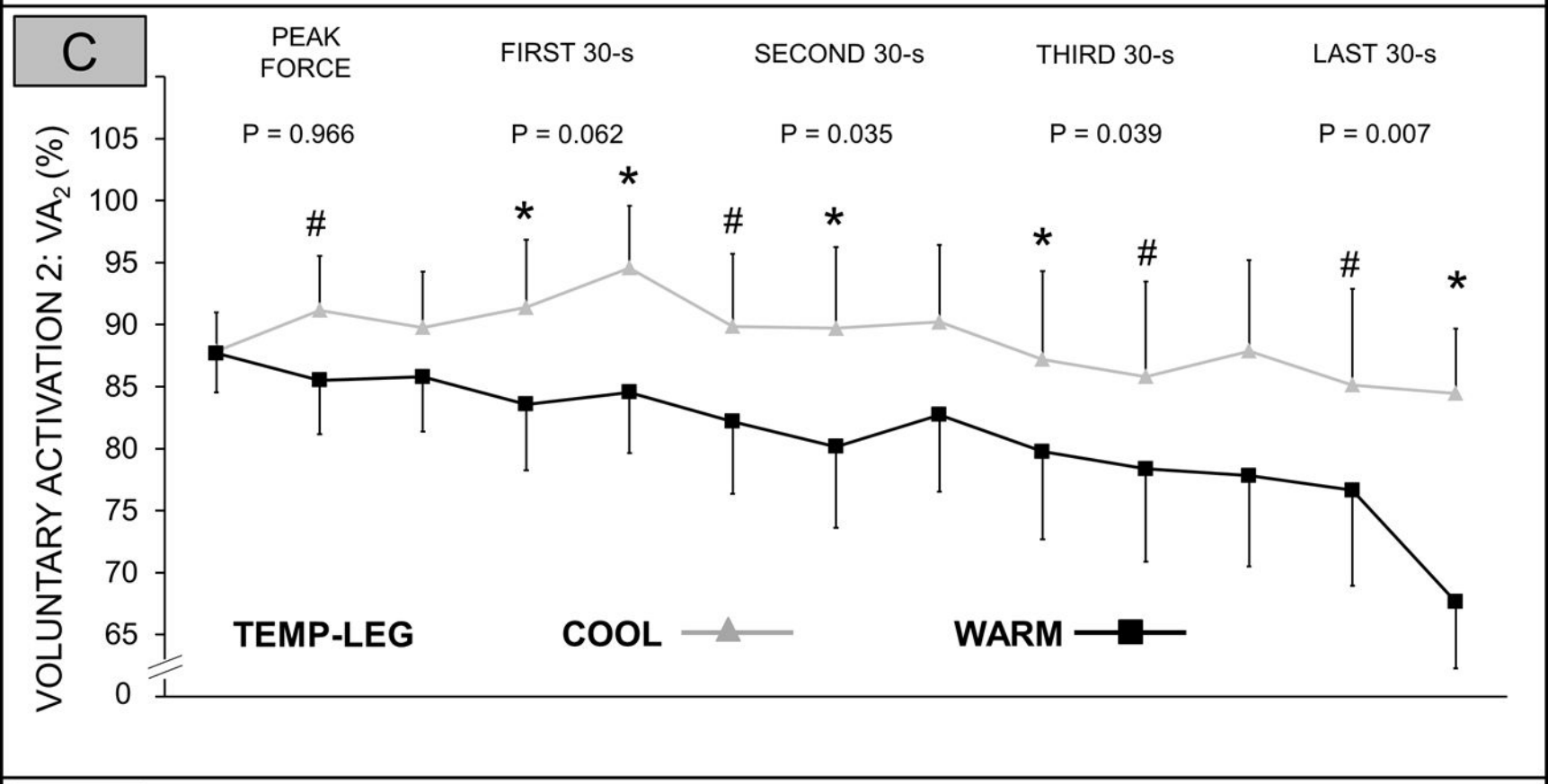
0.0

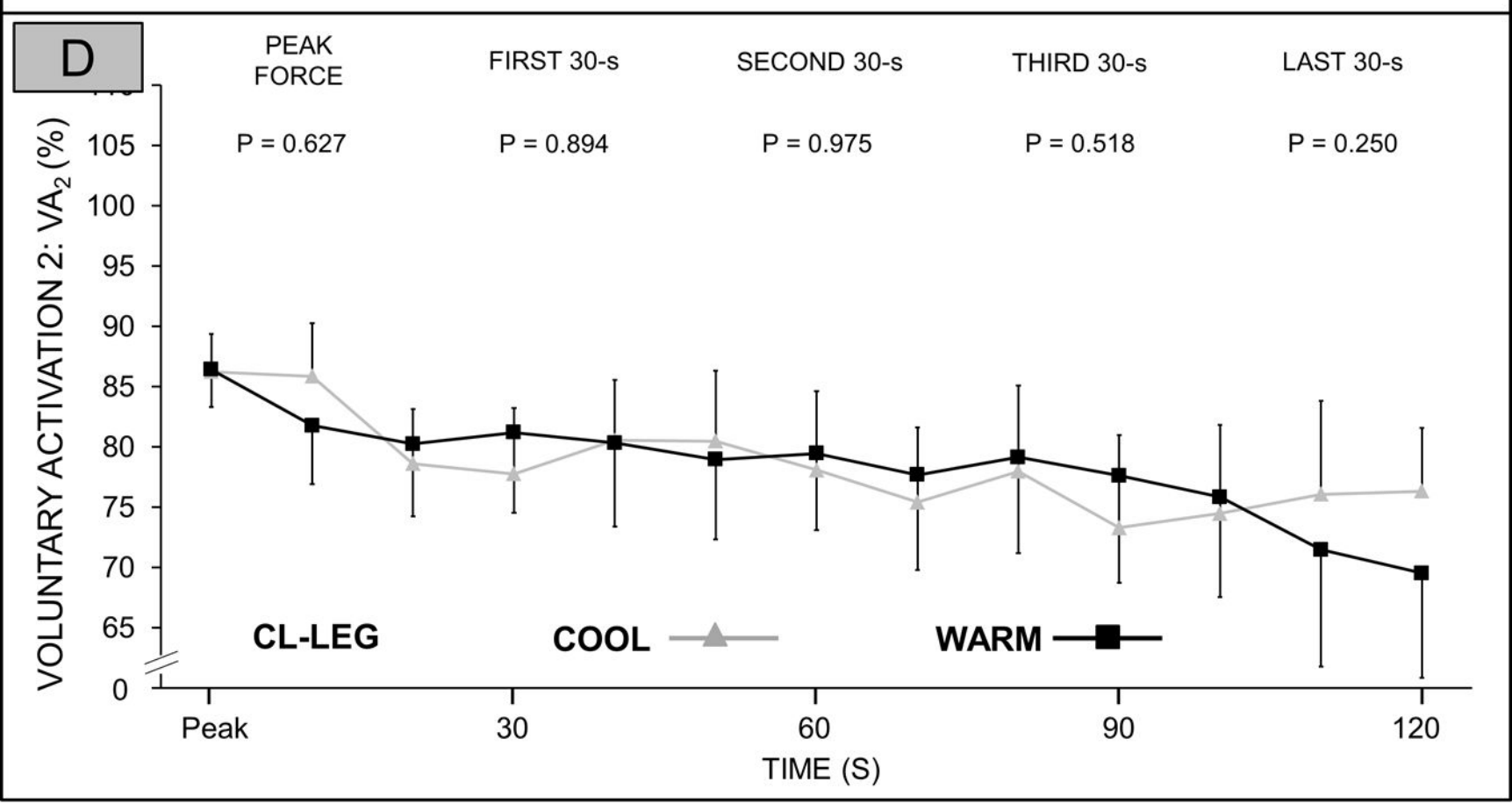
COOL WARM

COOL WARM


A
PEAK FORCE
FIRST 30-s
SECOND 30-s
THIRD 30-s
LAST 30-s
P = 0.480
P = 0.341
P = 0.001
P = 0.015
P = 0.011
CONTRACTILE FORCE (% of PRE)
100
80
60
40
20
0
COOL
WARM
TEMP-LEG


B
PEAK FORCE
FIRST 30-s
SECOND 30-s
THIRD 30-s
LAST 30-s
P = 0.695
P = 0.893
P = 0.745
P = 0.847
P = 0.088
CONTRACTILE FORCE (% of PRE)
100
80
60
40
20
0
COOL
WARM
CL-LEG


C
PEAK FORCE
FIRST 30-s
SECOND 30-s
THIRD 30-s
LAST 30-s
P = 0.966
P = 0.062
P = 0.035
P = 0.039
P = 0.007
VOLUNTARY ACTIVATION 2: VA2 (%)
105
100
95
90
85
80
75
70
65
0
#
*
*
#
*
*
#
#
*
TEMP-LEG
COOL
WARM


D
PEAK FORCE
FIRST 30-s
SECOND 30-s
THIRD 30-s
LAST 30-s
P = 0.627
P = 0.894
P = 0.975
P = 0.518
P = 0.250
VOLUNTARY ACTIVATION 2: VA2 (%)
105
100
95
90
85
80
75
70
65
0
CL-LEG
COOL
WARM
Peak
30
60
90
120
TIME (S)

Variable	Time Point	COOL	WARM
Core temperature (°C)	PRE-WI	37.43 ± 0.25	37.41 ± 0.31
	POST-WI	37.49 ± 0.16	37.62 ± 0.30
	POST-EX	37.32 ± 0.21	37.68 ± 0.20*
TEMP-LEG 1 - cm muscle temperature (°C)	PRE-WI	33.5 ± 2.3	33.5 ± 2.0
	POST-WI	26.0 ± 4.7	37.2 ± 1.1*
	POST-EX	30.1 ± 2.9	36.2 ± 2.1*
CL-LEG 1 - cm muscle temperature (°C)	PRE-WI	33.7 ± 2.2	33.8 ± 2.3
	POST-WI	33.5 ± 2.2	33.7 ± 1.4
	POST-EX	34.9 ± 2.0	34.7 ± 2.1
TEMP-LEG 2 - cm muscle temperature (°C)	PRE-WI	35.1 ± 1.2	35.0 ± 0.9
	POST-WI	29.4 ± 4.0	37.6 ± 0.4*
	POST-EX	32.9 ± 2.2	37.5 ± 0.5*
CL-LEG 2 - cm muscle temperature (°C)	PRE-WI	35.1 ± 1.1	35.3 ± 0.9
	POST-WI	35.3 ± 0.7	35.2 ± 1.0
	POST-EX	36.6 ± 0.7	36.7 ± 0.8
TEMP-LEG 3 - cm muscle temperature (°C)	PRE-WI	35.8 ± 1.0	35.5 ± 0.7
	POST-WI	31.4 ± 2.9	37.7 ± 0.4*
	POST-EX	34.0 ± 1.5	37.8 ± 0.4*
CL-LEG 3 - cm muscle temperature (°C)	PRE-WI	35.8 ± 0.7	35.9 ± 0.6
	POST-WI	35.7 ± 0.5	35.7 ± 0.7
	POST-EX	36.8 ± 0.4	37.2 ± 0.5#
Mean skin temperature (°C)	PRE-WI	33.2 ± 0.8	32.5 ± 1.7
	POST-WI	33.0 ± 0.8	32.2 ± 1.2
	POST-EX	33.2 ± 0.4	30.5 ± 2.5*

Variable	Time Point	COOL	WARM
Peak MVC Force (N)	PRE-WI CL-LEG	983 ± 268	977 ± 268
	PRE-WI TEMP-LEG	1086 ± 226	1029 ± 232
	POST-WI CL-LEG	1039 ± 287	1052 ± 238
1-s Mean MVC Force (N)	PRE-WI CL-LEG	791 ± 272	791 ± 264
	PRE-WI TEMP-LEG	917 ± 206	892 ± 258
	POST-WI CL-LEG	890 ± 279	908 ± 202
Peak VA_1 (%)	PRE-WI CL-LEG	68.7 ± 16.1	63.8 ± 22.0
	PRE-WI TEMP-LEG	73.0 ± 12.5	73.1 ± 13.3
	POST-WI CL-LEG	73.9 ± 11.9	74.5 ± 10.4
Peak VA_2 (%)	PRE-WI CL-LEG	85.4 ± 9.7	83.8 ± 10.6
	PRE-WI TEMP-LEG	89.1 ± 5.4	88.8 ± 6.5
	POST-WI CL-LEG	88.0 ± 8.4	89.8 ± 5.5
Mean $Q_{tw,sup}$ (N)	PRE-WI CL-LEG	134 ± 89	144 ± 99
	PRE-WI TEMP-LEG	115 ± 53	108 ± 57
	POST-WI CL-LEG	114 ± 62	108 ± 66
Mean $Q_{tw,pot}$ (N)	PRE-WI CL-LEG	415 ± 118	400 ± 93
	PRE-WI TEMP-LEG	424 ± 88	399 ± 71
	POST-WI CL-LEG	431 ± 118	403 ± 94
Mean RFD ($N.ms^{-1}$)	PRE-WI CL-LEG	5.66 ± 2.07	5.12 ± 1.59
	PRE-WI TEMP-LEG	5.91 ± 1.57	5.73 ± 1.29
	POST-WI CL-LEG	5.66 ± 2.02	6.02 ± 2.65
Mean RFR ($N.ms^{-1}$)	PRE-WI CL-LEG	1.68 ± 0.75	1.76 ± 0.60
	PRE-WI TEMP-LEG	1.72 ± 0.52	1.42 ± 0.52*
	POST-WI CL-LEG	1.66 ± 0.75	1.53 ± 0.53

Variable	Time Point	COOL	WARM
Mental Effort (CR-10)	TEMP-LEG	10.0 ± 0.0	9.9 ± 0.4
	CL-LEG	9.9 ± 0.4	10.0 ± 0.0
Sense of Limb Discomfort (CR-10)	TEMP-LEG	7.4 ± 3.0	8.6 ± 2.3*
	CL-LEG	7.9 ± 2.8	7.9 ± 3.1