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Title:

The interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension

Authors:

Alex Lloyd

Margherita Raccuglia

Simon Hodder

George Havenith

Affiliation:

Environmental Ergonomics Research Centre, Loughborough University, Loughborough, UK

Running Head:

Fatigue in combined hypoxic and thermal stress

Corresponding Author:

George Havenith

Environmental Ergonomics Research Centre,

James France Bldg.,

Loughborough,

Leicestershire

UK

LE11 3TU

Email: G.Havenith@lboro.ac.uk

ABSTRACT

This study investigated causative factors behind the expression of different interaction types during exposure to multi-stressor environments. Neuromuscular fatigue rates and time to exhaustion (TTE) were investigated in active males (n=9) exposed to three climates (5°C,50%-rh/23°C,50%-rh/42°C,70%-rh) at two inspired oxygen fractions (0.209/0.125 F_IO_2; equivalent attitude=4100 m). After 40-mins rest in the environmental conditions, participants performed constant workload (high-intensity) knee extension exercise until exhaustion, with brief assessments of neuromuscular function every 110-s. Independent exposure to cold, heat and hypoxia significantly ($p<0.01$) reduced TTE from thermoneutral-normoxia (reduction of 190, 405, 505-s from 915-s respectively). The TTE decrease was consistent with a faster rate of peripheral fatigue development ($p<0.01$) compared with thermoneutral-normoxia (increase of 1.6, 3.1 and 4.9%.min^{-1} from 4.1%.min^{-1} respectively). Combined exposure to hypoxic-cold resulted in an even greater TTE reduction (-589-seconds), likely due to an increase in the rate of peripheral fatigue development (increased by 7.6%.min^{-1}), but this without significant interaction between stressors ($p>0.198$). In contrast, combined exposure to hypoxic-heat reduced TTE by 609-s, showing a significant antagonistic interaction ($p=0.003$) similarly supported by an increased rate of peripheral fatigue development (increased by 8.3%.min^{-1}). A small decline (<0.4%.min^{-1}) in voluntary muscle activation was only observed in thermoneutral-normoxia. In conclusion, interaction type is influenced by the impact magnitude of the individual stressors' effect on exercise capacity, whereby the greater the stressors impact, the greater the probability that one stressor will be abolished by the other. This indicates humans respond to severe and simultaneous physiological strains based on a 'worst strain takes precedence' principle.

New and Noteworthy: A novel principle of multifactorial integration is proposed; that the type of interaction between physiological stressors is influenced by the impact magnitude of individual stressors' effect on exercise capacity. Mild stressors add up, however the greater the stressors' impact, the greater the trend for one stressor to cancel out the other. This ultimately infers a maximum threshold for performance deterioration, whereby humans respond to severe and simultaneous strains based on a 'worst-strain takes precedence' principle.

Keywords: combined stressors, central motor drive, high altitude, neuromuscular fatigue, thermal stress.

ABBREVIATIONS

ANOVA: Analysis of variance

COLD: Cold-normoxic condition (used in figures and tables only)

CV: Coefficient of variation

F_IO_2: Fraction of inspired oxygen

FNS: Femoral nerve stimulation (twitch interpolation)

HEAT: Heated-normoxic condition (used in figures and tables only)

HR: Heart rate

HYP-COLD: Hypoxic- cold condition (used in figures and tables only)

HYP-NEU: Hypoxic- thermoneutral condition (used in figures and tables only)

HYP-HEAT: Hypoxic- heat condition (used in figures and tables only)

MVC: Maximal voluntary contraction

RFD: Resting twitch rate of force development

RFR: Resting twitch rate of relaxation

NEU: Thermoneutral-normoxic condition (used in figures and tables only)

O_2: Oxygen

PRE-REST: Pre-rest (used in figures and tables only)

POST-REST: Post-rest (used in figures and tables only)

$Q_{tw,pot}$: Resting potentiated twitch force (peripheral fatigue)

$Q_{tw,sup}$: Superimposed twitch force

SpO_2: Oxygen saturation of peripheral blood

T_{core}: Core temperature

T_{env}: Environmental temperature

T_m: Muscle temperature

T_{sk}: Skin temperature

EXH: Exhaustion through task failure or exercise intolerance (used in figures and tables only)

TTE: Time to exhaustion

VA: Voluntary activation

$\%VO_{2max}$: Relative percentage of maximal aerobic capacity

INTRODUCTION

A human's ability to sustain mechanical function - muscular force and power - over time is modulated by numerous environmental factors, including both the oxygen (O_2) availability and the climate (3, 42, 69). While these are widely studied as independent stressors, many real life applications generate hypoxic and thermal stressors in combination e.g. endurance exercise in cold mountainous areas, operating/ piloting unpressurised aircraft or in the use of hypoxic-heat as a training stimulus (19, 31). Research to date suggests many of the key physiological strains associated with thermal (cold and heat) and hypoxic stress are precursors of a given mechanical work being performed at a higher absolute and/or relative aerobic strain i.e. increases in ml $O_2.min^{-1}.W^{-1}$ or $\%VO_{2max}.W^{-1}$ respectively (4, 35, 42). For example, previous studies have reported that during exhaustive or high intensity exercise in the heat (20, 40, 50), the thermoregulatory requirements for skin blood flow, together with progressive dehydration and higher muscle sympathetic nerve activity, may compromise perfusion of (i.e. oxygen transport to) the active muscle (21, 55, 57, 60). This is similar to hypoxia, where a systemic reduction in arterial oxygen content strains the cardiovascular system's ability to meet the required oxygen delivery to active musculature (4, 15). As such, both heat and hypoxic stress exacerbate the rate of peripheral (intramuscular) failure (2, 23), largely due to a net increase in muscle fiber recruitment in order to match the increased anaerobic energy demands of a given mechanical output (4, 23, 63). In the cold, human performance is also limited at the peripheral/intramuscular level (47, 69), partially caused by local vasoconstriction reducing venous washout of metabolic by-products in the active muscle (8). However vasoconstriction of active musculature is likely to be secondary to the progressive reductions in the absolute aerobic-mechanical efficiency caused by shivering (35, 69) and the co-activation of the antagonist muscles (45, 46).

While peripheral adaptations may partly explain environmental influences on exercise, both conscious and autonomic-inhibitory neural factors (i.e. central fatigue) (5, 17, 33, 64) have been recognized for their role in hot, cold and hypoxic performance decrements (9, 22, 41). For example, suboptimal voluntary muscle activation (VA) independent of peripheral fatigue, has been reported under extreme heat stress (39, 65, 67) and severe systemic hypoxemia (22, 37). Such acute reductions in VA have primarily been attributed to changes in cerebral temperature (44) and cerebral oxygenation (43) respectively. However, the identification of the involvement of numerous 'limiting' factors at the point

of exhaustion has also highlighted the importance of psycho- and neurophysiological interactions during exercise regulation, including cognitive-behavioral management of thermal and muscular metabolic homeostasis (5, 13, 32). In this regard, the afferent neural networks stemming from metabo-, mechano-, thermo- and baroreceptors are likely crucial in integrating the cardiovascular and mechanical (peripheral) adaptations under central control (38, 62).

Human performance during exposure to multifactorial environments is notably complex to investigate, and literature examining inter-stressor interactions is sparse (66). Real world exposures to high-altitude often consist of combinations of hypobaric hypoxia, solar radiation, winter clothing and cold ambient temperatures (30, 31, 66, 68). In these complex situations, the effect of one stressor on performance may be subject to change, simply due to the presence of another independent stressor. Such differential influences can occur in three basic forms; additive, antagonistic and synergistic (31). Each term defines a fundamental concept of inter-parameter interactions, which are most effectively represented using example parameters 'A' and 'B'. Starting from an additive interaction, which mathematically can either be the sum of absolute impacts:

$$\text{Impact of 'A' and 'B' combined (\% reduction)} = \text{Impact of 'A'(\% reduction)} + \text{Impact of 'B'(\% reduction)}$$

Or the product of the relative impacts, expressed as:

$$\text{Impact of 'A' and 'B' combined (\% reduction)} = \left[1-\left(1-\frac{\text{Impact of 'A'(\% reduction)}}{100}\right)\cdot\left(1-\frac{\text{Impact of 'B'(\% reduction)}}{100}\right)\right]\cdot 100$$

The other options are that the combined effect is significantly larger (synergistic) than the sum of the individual effects of each parameter:

$$\text{Impact of 'A' and 'B' combined (\% reduction)} > \text{Impact of 'A'(\% reduction)} + \text{Impact of 'B'(\% reduction)}$$

Or smaller (antagonistic) than the sum of the individual effects of each parameter:

Impact of 'A' and 'B' combined (% reduction) <
Impact of 'A'(% reduction) + Impact of 'B'(% reduction)

An extreme expression of the antagonistic interaction might also include the complete abolition of one or both stressors impact, and thereby the mathematical equivalent of the 'worst strain takes precedence' up to a full 'strain nullification' respectively. Of the few studies that have investigated combined temperature and hypoxia, Lloyd et al. (31) reported that forearm flexor fatigue increases additively when hypoxia and mild cold are combined during repeated dynamic contractions. Likewise, Van Cutsem et al. (68) and Aldous et al. (1) recently observed additive performance decrements when combining hypoxia and a warm environment during 30 min self-paced cycling and an intermittent soccer performance test respectively. In contrast however, Girard and Racinais (19) observed an antagonistic interaction during a fixed intensity cycling (66% VO_{2max}) in combined moderate hypoxia and mild heat stress. At present, the reasons for these varying observations are unclear.

As well as the natural occurrence of thermal and hypoxic stressors in combination (31, 66, 68), understanding interactions is fundamental to experimentally modelling how multiple physiological strains integrate in their influence on – or regulation of - exercise intensity. Based on this, the present study was primarily formulated to understand the causative factors behind different interaction expressions, and thereby how interaction types can quantitatively define multifactorial integration during exhaustive exercise. To achieve this, rates of peripheral and central fatigue development and time to exhaustion (TTE) were examined across a variety of single and multi-stressor environments. By examining both cold and heat stress combined with hypoxia (hypoxic-cold and hypoxic-heat respectively), this study aimed to investigate both the influence of the individual stressor's mechanism of impact (i.e. 'nature') as well as the individual stressor's magnitude of influence (i.e. 'impact severity') on the interaction types expressed during combined exposure to simultaneous stressors.

Based on previous research, it was hypothesized that 1) short duration (40-mins) exposure to cold, heat and hypoxia would each increase fatigue development and reduce TTE compared to thermoneutral normoxia (3, 42, 69); 2) the effect of short duration exposure to cold and hypoxia combined would be

additive on TTE, central and peripheral fatigue development (31); 3) the effect of short duration exposure to heat and hypoxia would be **antagonistic** on TTE, central and peripheral fatigue development (19); and 4) TTE and fatigue would be principally mediated by peripheral factors in hypoxic-cold (31), while the contribution of central factors to TTE and fatigue would rise **synergistically** in combined hypoxic-heat (37, 41).

MATERIALS AND METHODS

Ethical approval

This study was approved by Loughborough University Ethical Advisory Committee and was conducted in accordance with the World Medical Associations Declaration of Helsinki. Participants were provided with a detailed document explaining the risks and requirements of experimental protocol, prior to providing written informed consent. All participants conducted a health screening questionnaire prior to the start of the experiment.

Subjects

Nine healthy, moderately trained male volunteers participated in the study (mean ± SD, height: 181 ± 0.08 cm, weight: 78.8 ± 17.5 kg, age: 22.1 ± 2.1 yrs., activity level: 4.8 ± 1.2 exercise bouts.wk^{-1}, resting heart rate during 5-mins supine rest: 64 ± 4 b.min^{-1}). All were right leg dominant with no previous history of cardiovascular, neurological and muscle debility. Participants were requested to preserve their normal exercise routines, but abstain from exercise, caffeine and alcohol 24 hrs. prior to each experimental session. The experiments were conducted in autumn (UK), presumably indicating little or no heat acclimation. Participants were not acclimated to hypoxia prior to participation in the experiment.

General Study Overview

Following initial familiarization, participants conducted six experimental sessions at three levels of environmental temperature (T_{env}) and two levels of fraction of inspired oxygen (F_IO_2) in an environmental chamber (T.I.S.S. Peak Performance, Series 2009 Climate Chambers). Specifically, the conditions included:

HYP- COLD	5°C T_{env} & 50% rh in 0.125 F_IO_2
COLD	5°C T_{env} & 50% rh in 0.209 F_IO_2
HYP- NEU	23°C T_{env} & 50% rh in 0.125 F_IO_2
NEU	23°C T_{env} & 50% rh in 0.209 F_IO_2
HYP- HEAT	42°C T_{env} & 70% rh in 0.125 F_IO_2
HEAT	42°C T_{env} & 70% rh in 0.209 F_IO_2

As a general overview of the experimental protocol, upon entering into the test conditions, participants first performed an isometric assessment of neuromuscular function (PRE-REST). Following 40-mins period of seated rest (REST), participants then performed a post-rest isometric assessment of neuromuscular function (POST-REST). Subsequently, participants performed repeated bouts of dynamic knee extension (DYN) at a fixed load (50.3 ± 11.1 W; 60 extensions.min^{-1}; 80- 140° of knee extension) until exhaustion (EXH). Every 110-s, dynamic exercise was interspersed with an isometric neuromuscular test to calculate central and peripheral fatigue (ISO). Participants then continued dynamic exercise until exhaustion in all conditions, completing a final isometric neuromuscular function test at task failure (EXH). A complete schematic of the experimental protocol is provided in Fig. 1.

[Insert Figure 1 here]

Familiarization sessions

Participants conducted at least two and up to four (dependent on the time necessary to ascertain an appropriate workload; see below) preliminary sessions to familiarize with the experimental procedures and requirements of the experiments. During these sessions, participants were accustomed to performing brief maximal isometric voluntary contractions (MVC) with femoral nerve stimulation (FNS; see procedural details below). In all sessions, this was followed by complete run through of the experimental procedure, minus any rest periods (Fig. 1). To identify an appropriate workload for the main experimental sessions, initial power was prescribed at a mental effort of two (light; Borg's CR-10 scale). Following this the change in mental effort was used to adjust power output, aiming to evoke exhaustion in thermoneutral-normoxia (maximal on Borg's CR-10 scale) within 15 to 20 min of the

start of the exercise. On subsequent familiarization visits, the procedure was repeated, applying a fixed (constant) workload based on each individual's performance in their previous familiarization session. Participants then progressed to the main experimental sessions upon satisfactory completion of a full trial at a fixed workload that was observed to evoke exhaustion after no longer than 20 min. The final knee extension workload (different for each individual) was 50.3 ± 11.1 W. During familiarization, initial (fresh) MVC force was 940 ± 156 N with an average co-efficient of variation (CV) in 3 successive trials of 3.81 ± 1.4%. Initial (fresh) resting potentiated twitch force was 384 ± 53 N with an average CV in 3 successive trials of 3.03 ± 1.7%.

Main experimental sessions

The main experimental procedure was the same across all conditions. Participants wore shorts and socks for all conditions. Participants were instrumented with the temperature recording and neuromuscular testing equipment, and muscle temperature (T_m) was assessed prior to entering the environmental chamber (see procedural details at section: temperature recording and oximetry). Following this, participants sat in a custom-built knee extension dynamometer (inside the environmental chamber) and following potentiation (2-s plateau at 50, 50, 50, 75 and 90% MVC) of the quadriceps they performed a pre-rest assessment of neuromuscular function (MVC of 2-s plateau duration with FNS, 3 times, with 30-s rest). Winter clothing was provided for pre-rest assessments in cold conditions. Following this neuromuscular assessment, participants were then seated for 40-min rest in the environmental chamber. During the rest periods, participants were instructed to maintain an upright posture, with their arms relaxed by their side. After seated rest, T_m was reassessed inside the environmental chamber. Participants were then re-secured into the knee extension dynamometer (details below), and following potentiation they performed a post-rest assessment of neuromuscular function (MVC of 2-s plateau duration with FNS, 3 times, with 30-s rest). Following a final 90-s rest, participants carried out dynamic knee extension (active concentric, passive eccentric) at a fixed (constant) intensity (50.3 ± 11.1 W; 60 extensions.min^{-1}; 80- 140° of knee extension) until exhaustion. Each workload was specific to each individual and was selected based on their performance in the familiarization trial. After every 110-secconds of dynamic knee extension exercise, the dynamometer was locked in position (knee joint angle = 100°), following which participants performed a single MVC (2-s plateau duration with FNS) to quantify central and peripheral fatigue development (16).

110-s periods were selected to reserve 10-s for the assessment of central and peripheral fatigue during every 2-mintues of exercise. The locking of the knee joint angle was undertaken by a practiced experimenter. The average time from muscle relaxation after dynamic exercise to the start of MVC (i.e. the locking time) was 2.9 ± 0.8-s. Dynamic exercise resumed exactly 10-s after relaxation from the previous bout. It should be noted that while a single MVC may be considered a less reliable measure of central and peripheral fatigue, it has the significant benefit of minimizing recovery time, thereby preventing the common underestimation of central and peripheral fatigue following dynamic exercise and at exhaustion (48).

Exhaustion was defined as either a) volitional cessation of exercise (i.e. exercise intolerance) or b) a failure to maintain the required rate (e.g. 60 extensions.min^{-1}) or range (e.g. 80- 140° of knee extension) during three concentric knee extensor contractions in succession (i.e. task failure) (15).

Application of the dynamic workload

In this study a custom-built knee extension dynamometer was used. The equipment was designed based on the work of Andersen et al (7) and Fulco et al (16). The initial seat and frame was taken from commercial knee extension equipment (GymanoElite Pro, UK). To apply a concentric contraction only load to the knee extensors, the weights system was replaced with an electromagnetically-braked flywheel (Angio V2, Lode, Groningen, Netherlands). The flywheel was powered using a non-compliant adjustable crank arm and chain driven gearbox. A locking mechanism was constructed to allow rapid changes between an isometric MVC (with FNS) and dynamic exercise (15, 16, 48). A visual-analogue scale was used to display current knee extension angle to the participants. The present range of movement (e.g. 80- 140° of knee extension) was selected based on piloting. This was determined by an absence of antagonist stretching at extended ranges (>150°), with the aim of minimizing monosynaptic co-activation of biceps femoris. The required rate (e.g. 60 extensions.min^{-1}) was controlled using an audible metronome set to 2Hz (a 'beep' for extension and flexion respectively). The device was calibrated using dead weights of known mass (force) and goniometry (range). During this experiment, the mean force transducer signal during each dynamic exercise bout was strongly correlated (r = 0.82) with the calculated power output (see below) across all participants.

The CV in mean force transducer output during dynamic exercise across conditions was also small (3.86%), indicating equal and proportional workloads were successfully applied to the knee extensors.

Dynamometer set up

For all experiments, subjects were seated with a hip and knee joint angle of 90 and 100° respectively. The dynamometer was adjusted for each individual's femoral and tibial lengths, as well as their popliteal to patella width. The right leg was secured to a force transducer (Tedea- Huntleigh, Model 615, Vishay Precision Group, California, USA) using an adjustable, non-compliant harness around ankle malleolus. A layer of padding was applied to the ankle to protect against harness bruising/ rubbing during the dynamic component of the exercise protocol. Participants were secured using a waist belt system. Force data was visually displayed on a PC (DataLog software, Biometrics Ltd, UK) via a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK). Baseline noise was less than 0.5N once ambient and force transducer temperature had stabilized. No discernable (over and above baseline noise) differences were observed in force transducer sensitivity at different T_{env}.

Knee extension power output calculations

All power outputs were calculated from the kinetic energy measured on the flywheel, plus the power required to move the lower leg, foot, force transducer and crank arm through -20° to +40° (perpendicular to the floor) at an angular velocity of 2.0944 $rad.s^{-1}$ (i.e. movement through a range of 80- 140° knee extension every 0.5 secs). Combined lower leg and foot weight were calculated as total body weight *0.0592 (12).

Since gravity alters the torque requirement at each circular section, a torque decay curve was calculated for each 10° segment moved. In this case, when the crank arm was perpendicular to the floor the additional torque requirement equals zero; whereas this is equal to 100% when parallel to the floor. A correction was then applied to the torque required at each 10° segment to move the lower leg, foot, force transducer and crank arm through the range used in this study, before calculating the total power in watts (W) from flywheel power, angular velocity ($rad.s^{-1}$) and total corrected torque ($N.m^{-1}$).

Isometric neuromuscular assessments

Peripheral and central fatigue were calculated using the twitch interpolation technique during an MVC (36). To this end, two superimposed twitches ($Q_{tw,sup}$) were evoked over the force plateau of each MVC, each followed by two resting potentiated twitches ($Q_{tw,pot}$) 1-second after muscle relaxation. VA (i.e. central fatigue) was calculated using the following equation (32, 41): VA = MVC/ (MVC+ $Q_{tw,sup}$). $Q_{tw,pot}$ was used an index of the mechanical (contractile) properties of the muscle (i.e. peripheral fatigue) (4, 32, 53). The mean rate of force development (RFD) and mean rate of force relaxation (RFR) were calculated for all $Q_{tw,pot}$ (4).

In analysis, the values of the three MVC's pre- and post- rest were averaged and each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled 1-ms prior to delivery of each $Q_{tw,sup}$.

The femoral nerve was stimulated by two 0.2-ms rectangular pulses spaced 10-ms apart (i.e. doublet twitch), delivered using a high voltage simulator (max voltage 400 V; Digitimer DS7AH, Hertfordshire, UK) (14). The stimulator anode was placed in the femoral triangle and the cathode over the greater trochanter (11, 32, 41, 48). During familiarization the precise electrode placement was ascertained then marked with indelible ink. During familiarization the current necessary for supramaximal nerve depolarization was also calculated (126 ± 19 mA), using progressive increases until a plateau in the mechanical response of the muscle (i.e. $Q_{tw,pot}$) was observed (32, 53). Potentiation prior to both pre- and post-rest neuromuscular assessments (e.g. MVC of 2-s plateau duration with FNS, 3 times, with 30-s rest) was ensured using a series of five incremental practice contractions (2-s plateau at 50, 50, 50, 75 and 90% MVC). Each neuromuscular test was conducted 15-s after potentiation. Subjects were encouraged moderately during all MVC's and all twitches were delivered manually by the same experimenter.

Temperature, heart rate and pulse oximetry

Rectal temperature (T_{core}), skin temperature (T_{sk}), heart rate (HR) and oxygen saturation of peripheral blood (SpO_2) were logged every 1 min from pre-exposure until exhaustion. Rectal temperature was measured 10-cm beyond the anal sphincter using a flexible thermistor and squirrel data logger (Series

2010, Grant International, UK). Skin temperature was measured on the forehead, shoulder, chest, right thigh, left thigh, right calf and left calf using wireless thermistors and in built memory (Ibutton, UK). Pulse oximetry was measured using a Nonin Pulse Oximeter (Nonin, US) attached to the ear lobe.

T_m was measured at 1, 2 and 3-cm depth using a solid needle thermocouple (Ellab, Denmark) inserted into vastus lateralis of both the exercising and non-exercising leg. Depth was corrected for adipose tissue, calculated using skin calipers over the insertion site. A strict sterility procedure was administered for all assessments. Data was collected prior to entering the environmental chamber (PRE-REST), after a 40-min seated rest period (POST-REST) and immediately following exhaustion (EXH) (Fig. 1). For simplicity, as well as to present the average gradient across the vastus lateralis muscle, T_m is displayed as a three depth mean (1, 2 and 3-cm). Mean T_{sk} was calculated using equal weighting from each of the seven measurement sites.

Perceptual Ratings

Immediately after every MVC intervention participants were asked to retrospectively rate their subjective sense of a) mental effort; b) leg muscle fatigue; and c) leg muscle pain for the previous bout of dynamic exercise. All participants were clearly instructed that sense of mental effort was the internal sense of effort expended, independent of all peripheral discomforts (10). All questions were answered by giving a rating on a modified Borg CR-10 scale (10). The specific questions were visibly printed above the scale and stated: a) *'what was your sense of leg effort i.e. how hard did you have to drive your leg'*; b) *'what was your sense of fatigue in your exercising muscles'*; and c) *'what was your sense of pain in your exercising muscles'*.

Statistical Analysis

To examine the main effect of F_IO_2 (e.g. normoxia and hypoxia), and T_{env} (e.g. cold, neutral and heat) on all dependent variables, a two-way (3 x 2; T_{env} x F_IO_2) repeated measures analysis of variance (ANOVA) was used. Two-way ANOVA's were conducted at time point's pre-rest, post-rest and exhaustion, as well on the rate of change (%.min^{-1}) between time points post-rest and exhaustion (i.e. between the start and end of the dynamic exercise). Given a main effect at any given time point (i.e. two-way ANOVA) will typically yield a significant interaction with time, for straightforwardness, the

effect of time was not deemed necessary to assess using a three-way ANOVA. Significance was tested at a 95% confidence level ($p < 0.05$). The Greenhouse- Geisser correction was applied when Mauchly's test of Sphericity was significant. When a significant F ratio was observed for T_{env}, then pairwise comparisons (Bonferroni corrected) were conducted to assess the independent variance of cold or heat from neutral T_{env}. When a significant stressor interaction (T_{env} x F_IO_2) was observed in conjunction with significant main effects of heat, cold as well as F_IO_2, an additional two-way (2 x 2; HEAT or COLD vs NEU x F_IO_2) repeated measures ANOVA was conducted to examine the interaction type expressed during hypoxic-heat and hypoxic-cold (31).

A different number of MVC's were performed by each participant across conditions (repeated every 110-s across varying exercise times; Fig. 1). It is therefore not appropriate to compare dependent variables across each MVC collection point. Consequently, non-linear regression analysis (dependent variable vs time) was used to define the temporal changes in central (VA) and peripheral fatigue ($Q_{tw,pot}$). Regression analyses were conducted on the individual data points (as opposed to group mean data) between start and end of the dynamic exercise, allowing overall mean curves, using the same time base, for each condition to be determined. Data are displayed as mean ± SD.

RESULTS

Pre-rest measures

Prior to the seated rest, there was no main effect of T_{env} or F_IO_2 on T_{core}, T_{sk}, quadriceps T_m (both legs), SpO_2, HR, VA, $Q_{tw,sup}$, $Q_{tw,pot}$, mean RFD or mean RFR. Average MVC force across the three pre-rest contractions was significantly lower during heat ($p = 0.020$) and cold ($p = 0.003$) compared with neutral conditions (-5 and -7%, respectively). While there were no significant pre-rest modulations in VA, VA did significantly ($p < 0.001$) correlate with changes in MVC force ($R^2 = 0.52$ for condition normalized VA vs MVC). Moreover, the reductions in average MVC force were independent ($p = 0.246$) of any corresponding pre-experimental muscle fatigue ($R^2 = 0.01$ for condition normalized $Q_{tw,pot}$ vs MVC).

[Insert Table 1 here]

Temperature, heart rate and pulse oximetry

413 Table 2 shows the temperature recordings and pulse oximetry before and after the rest period, as well
414 as at exhaustion. T_{core} was unaffected by condition ($p > 0.2$) except at the post-exercise time point
415 where T_{core} was 0.25°C higher ($p = 0.017$) in the heated conditions compared to neutral. Conversely,
416 after the rest period, right leg quadriceps T_m and mean T_{sk} decreased ($p < 0.002$) by 3.8 ± 1.8°C and 5.4
417 ± 0.6°C in cold conditions, increased ($p < 0.001$) by 2.2 ± 1.4°C and 5.1 ± 1.1°C in heated conditions,
418 and decreased by 0.8 ± 1.2°C and 0.3 ± 0.5°C in neutral conditions. Rest in hypoxia did not affect
419 exercising quadriceps T_m ($p = 0.234$); however mean T_{sk} was marginally increased ($p < 0.001$) by 0.4 ±
420 0.5°C in hypoxic conditions. The dynamic exercise protocol increased exercising T_m in all conditions;
421 however exercising T_m remained significantly different ($p < 0.001$) in the same order across
422 environmental temperatures at exhaustion.
423
424 Across the whole exposure, mean SpO_2 was significantly ($p < 0.001$) reduced to 85 ± 4% in hypoxia
425 compared to 99 ± 1% in normoxia. SpO_2 remained significantly ($p < 0.001$) lower in hypoxia at
426 exhaustion. HR was significantly increased after the rest period in heated ($p = 0.002$) and hypoxic ($p =$
427 0.013) conditions.
428
429 There was no significant interaction ($p > 0.1$) between F_IO_2 and T_{env} on any dependent variable
430 immediately after the rest period. At exhaustion however, there was a trend ($p = 0.084$) for synergistic
431 increases in T_{sk}, and a trend ($p = 0.062$) for antagonistic decreases in SpO_2, when hypoxia and cold
432 were combined. In addition, hypoxia significantly ($p = 0.039$) antagonized non-exercising T_m in heated
433 conditions compared to neutral conditions; however this is most likely explained by the higher non-
434 exercising T_m at the start of the exercise in the hypoxic-thermoneutral condition (Table 2).
435 **[Insert Table 2 here]**
436
437 **Post-rest neuromuscular measures**
438 After the rest period, there was no main effect of F_IO_2 on MVC force, VA, $Q_{tw,sup}$, $Q_{tw,pot}$, mean RFD,
439 mean RFR, perceived mental effort, perceived leg fatigue or perceived leg pain. Additionally there was
440 no main effect of T_{env} on mean RFD, mean RFR, perceived mental effort, perceived leg fatigue or
441 perceived leg pain. However MVC force and VA were significantly reduced during heated exposures

compared to neutral conditions ($p = 0.011$ and 0.006 respectively), suggesting participants displayed a small degree of post-rest central fatigue in the heat (– 4.0% VA).While the main effect of T_{env} on $Q_{tw,pot}$ was also significant ($p = 0.005$), neither heat or cold were different from neutral ($p > 0.132$), thus the main effect was due to pairwise differences between heat and cold only ($p = 0.012$).

Time to exhaustion

Fig. 2, Panel A shows the absolute TTE in seconds across all conditions, as well as the relative reductions in TTE (percentage) caused by each individual stressor at each level of the other stressor (Fig. 2 Table Insert). In response to dynamic exercise, independent exposure to hypoxia and to cold reduced TTE by 505-s ($p = 0.002$) and 190-s ($p = 0.006$) respectively, from 915-s in control (thermoneutral-normoxic) conditions. During independent exposure to the heated condition, TTE was significantly ($p < 0.001$) reduced by 405-s (Fig. 2, Panel A).

[Insert Figure 2 here]

During combined hypoxic-cold, exercise time was reduced further (-589-s) compared to thermoneutral normoxia (-60 ± 14%); however there was no significant interaction between stressors ($p = 0.198$; Fig. 2, Panel A). While the reduction in TTE in absolute terms did not visually appear additive of the two stressors (suggesting antagonism) (Fig. 2, Panel A), the relative percentage reductions in TTE caused by cold and hypoxia were similar irrespective whether this was during combined or individual stressor exposure (Fig. 2, Table Insert). This suggests an additive relative effect (percentage reduction) when cold and hypoxia are combined. Conversely, combined hypoxic-heat reduced exercise time by 609-s compared to thermoneutral normoxia (-63 ± 13%), with a significant antagonistic interaction between stressors ($p = 0.003$). The relative influences of hypoxia and heat each were different in the presence of the other stressor (Fig. 2, Table Insert) supporting a significant antagonism between heat and hypoxia on TTE when combined. The interaction types expressed at group level varied slightly between participants during both hypoxic-cold (6 additive, 2 antagonistic, 1 synergistic) and hypoxic-heat (2 additive, 7 antagonistic).

In nearly all cases, volitional exercise intolerance occurred simultaneously with failure to maintain the required knee extension range (e.g. 80 to 140°) for three concentric knee extensor contractions in succession.

Temporal change in central and peripheral fatigue

Fig. 2, Panel B (peripheral fatigue/ $Q_{tw,pot}$) and Table 3 (all other dependent variables) show rate of change in neuromuscular and perceptual variables between the start and end of the dynamic exercise (i.e. between post-rest and exhaustion). The rate of increase in peripheral fatigue ($Q_{tw,pot}$) was faster during independent exposure to cold ($p = 0.004$), heat ($p = 0.006$) and hypoxia ($p < 0.001$) compared to thermoneutral-normoxia (increases of 1.6 ± 2.3, 3.1 ± 2.3 and 4.9 ± 2.7 $\%.min^{-1}$ for cold, heat and hypoxia respectively). Moreover, the combined effects of hypoxia and cold as well as that of hypoxia and heat on peripheral fatigue rate were additive (increases of 7.6 ± 3.2 and 8.3 ± 4.4 $\%.min^{-1}$) with no significant interaction ($p = 0.525$).

[Insert Table 3 here]

Interestingly, volitional (central) fatigue (VA) was largely unaffected at exhaustion (Table 1). Moreover, while the rates of change in VA were significantly greater in cold ($p = 0.004$) and heat ($p = 0.006$), these were actually indicative of minor increases in VA (i.e. decreases in central fatigue). Also the rate of change in volitional fatigue was not affected by hypoxia ($p > 0.37$). When VA did decline (thermoneutral conditions only), the variance was less than $0.4\%.min^{-1}$ (Table 3).

Central and peripheral contributions to exhaustion

Fig. 3, Panels A, B, C and D illustrate the decline in $Q_{tw,pot}$ (increase in peripheral fatigue) over time and across conditions. Based on post-hoc observation, $Q_{tw,pot}$ was fitted with a 2-order polynomial function ($\Delta Q_{tw,pot}$ (%) = $0.0011x^2 - 0.6617x - 0.3017$, $R^2 = 0.89$, where X is percentage TTE). Fig. 4, Panels A, B, C and D illustrate the change in VA (central fatigue) over time and across conditions. VA was fitted with a linear function (VA = $-0.0065x + 93.383$, $R^2 = 0.00$, where X is percentage TTE).

MVC force (an index of integrated fatigue) and perceptual exercise ratings (i.e. effort, fatigue and pain) each correlated with peripheral fatigue ($R^2 > 0.79$, $p < 0.001$) (Fig. 3, Panel E, F G and H), thus also

following a 2-order polynomial function when expressed against time. Moreover, across conditions and over time, the perception of mental effort and quadriceps fatigue tracked an almost identical function to each other (perceived mental effort = $0.0003x^2 + 0.1252x + 0.1411$, $R^2 = 0.91$; perceived muscle fatigue = $0.0004x^2 + 0.128x + 0.1349$, $R^2 = 0.91$, where X is percentage TTE), resulting in similar values at the point of exhaustion. On the contrary, muscle pain was on average 1 point lower on the Borg CR-10 scale at any given time point (perceived muscle pain = $-0.0001x^2 + 0.0967x + 0.0024$, $R^2 = 0.86$, where X is percentage TTE).

VA was largely unchanged over time and did not correlate in a meaningful way ($R^2 < .02$) with the decline in MVC force ($p = 0.013$), $Q_{tw,pot}$ ($p = 0.407$), or the increase in mental effort, perceived limb fatigue and perceived limb pain ($p > 0.39$) (Fig. 4, Panel E, F G and H).

[Insert Fig. 3 here]

[Insert Fig. 4 here]

DISCUSSION

The main focus of this study was to examine the effect of hypoxia and thermal stress (heat, neutral and cold environments) individually and combined, on the development of central and peripheral fatigue, as well as subsequent times to exhaustion. The results confirm the first hypothesis, that independent exposure to cold, heat and hypoxia each significantly reduced time to exhaustion with the effect increasing in that order (Fig. 2, Panel A); a finding related to changes in the rate of peripheral, not central, fatigue development (Fig. 2, Panel B; Fig. 3, Panel D). Since changes in peripheral fatigue occurred despite minimal increases in T_{core}, the present data appear to support that thermoregulatory strain, and thereby muscle fatigue in the heat, is largely influenced the observed narrowing of the skin to core temperature gradient during high intensity exercise (42, 50, 57, 60) (Table 2), though a direct effect of the raised skin and muscle temperature itself cannot be excluded.

In part confirmation of the second hypothesis, during combined exposure to hypoxia and cold, the reductions in time to exhaustion were **additive** of the relative effects of hypoxia and cold independently i.e. the fraction (percentage) decreases attributed to hypoxia and cold respectively were similar during both combined and single stressor exposure (Fig. 2, Panel A). This differs from the

findings of Lloyd et al. (31), where an absolute additive effect on fatigue was observed. The additive reductions in times to exhaustion during hypoxic-cold (Fig. 2, Panel A) were also consistent with additive rates of peripheral fatigue development (Fig. 2, Panel B; Fig. 3, Panel D) and an additive progression in the perception of mental effort and leg muscle fatigue (Table 3; Fig. 3 Panels F and G respectively). In contrast, combining moderate hypoxia with severe heat stress resulted in a significant **antagonistic** interaction on both the absolute and relative reductions in time to exhaustion (Fig. 2) i.e. the combined effect being significantly less than the sum of the individual effects. This confirms the third hypothesis. Taking all observations together, the results suggest humans respond to severe and simultaneous physiological strains based on a 'worst strain takes precedence' principle (details below).

Interestingly, the rate of increase in peripheral fatigue was strongly correlated with the decline in isometric maximal voluntary force (a measure of integrated fatigue) and not accompanied by substantial changes in voluntary activation (central fatigue) over time or across conditions. The results suggest peripheral fatigue is likely the main driver behind faster neuromuscular fatigue (Fig. 3, Panel E) and shorter times to exhaustion (Fig. 2) in the combined stressors tested presently, thus confirming the additive hypothesis of hypoxic-cold, but opposing the hypoxic-heat hypothesis for central fatigue.

Single stressor exposure to cold, heat and hypoxia

Rate changes in peripheral (intermuscular/ mechanical) fatigue development (Fig. 2, Panel B; Fig. 3, Panel D) are in line with previous reports using prolonged single-joint exercise under cold and hypoxic stress (15, 28, 45, 67) as well as during high intensity exercise under heat stress (20, 40, 50). In hypoxia, an increase in peripheral fatigue development is commonly attributed to faster intra-muscle-fiber metabolite production (e.g. inorganic phosphate, reactive oxygen species and hydrogen ion) (2, 24–26) due to a given mechanical work being performed at a higher relative aerobic strain (i.e. $\%VO_{2max}.W^{-1}$), with greater type II muscle fiber recruitment required to compensate inefficient oxygen availability (4, 28, 63). In the cold, an increase in muscle fatigue can be attributed to reductions in active muscle perfusion (71), lowering both net oxygen delivery, and reducing muscle metabolite washout (8). However in the cold, such factors are likely secondary to the reductions in aerobic-mechanical efficiency (i.e. increase in ml $O_2.min^{-1}.W^{-1}$) (35) due to increases in antagonist co-activation and increasing relative agonist muscle work (45, 46), which ultimately increases fiber

recruitment and thereby muscle (peripheral) fatigue (4, 23, 63, 69). While such mechanisms are likely responsible for the present findings, it should be noted that due to methodological constraints, changes in fiber recruitment and/or in co-activation were not directly addressed in this study (i.e. using EMG).

In the heat, dynamic exercise of isolated muscle groups has received less focus in the research compared to whole-body, multi-joint exercises. This is perhaps based on evidence suggesting that any reduction in central blood-volume (i.e. higher skin blood flow) is fully compensated for by increases in HR and redistribution of cardiac output away from non-essential vascular beds (e.g. renal, splanchnic, non-exercising muscle), leaving active muscle blood flow uncompromised during prolonged exercise of an isolated muscle group (21, 42, 59). In contrast however, the present results report a 57% faster rate of peripheral fatigue development – and shorter TTE also - during high intensity, single-joint exercise in the heat compared to thermoneutral conditions (Fig. 2, Panel B; Fig. 3 Panel A, B, C, D); a finding in the absence of meaningful changes in both T_{core} (Table 2) and VA (central fatigue) (Table 1; Fig 4).

It has been suggested that thermoregulatory strain (higher skin blood flow) is associated with the observed narrowing of the skin to core temperature gradient (42, 57, 60), as acutely observed in the present study (42, 50, 57, 60) (Table 2). Thus an increase in muscle fatigue may have occurred because higher HR and/or blood redistribution away from viscera was not able to entirely compensate the requirement for both high skin and muscle blood flow. In this regard, it is pertinent to consider the present exercise intensity and current test population, for whom the exercise workload was both high-intensity and unsustainable in all environments, thus requiring high levels of muscle oxygen delivery (Fig. 2, Panel A). By comparison, the maintained muscle blood flow observed by Savard et al. (59) employed an exercise workload that was sustainable for over 75-min. The increase in peripheral fatigue in the heat may also be related to the Q^{10} effect of T_m on contractile efficiency (i.e. twitch fusion or oxygen uptake kinetics) (23, 27, 54, 61). At present however, the significance of the Q^{10} effect remains equivocal, especially during dynamic exercise, owing to the number of studies reporting an unchanged absolute oxygen consumption during localized and whole-body heat strain (29, 42).

Combined exposure to thermal stress and hypoxia

In this study, when thermal and hypoxic stressors were combined, TTE was substantially reduced, compared with independent exposure to each stressor. Interestingly however, the stressor interaction type differed between hypoxic-cold (relative addition) and hypoxic-heat (antagonistic) exposures (Fig. 2, Panel A). During combined hypoxic-cold, TTE reduced by a magnitude equal to the product of the relative performance effects of each stressor individually. This is in partial support of previous studies examining hypoxic-cold exposure during exercise of isolated muscle groups (31). However the present results also differ in that the mean absolute reduction in performance (i.e. TTE) were not additive as seen with previous additive interactions (31, 68). Supporting the findings of Lloyd et al. (31) however, the present data do show that the influence of hypoxic-cold is mediated predominantly by peripheral (intramuscular/ mechanical) factors, in the absence of alterations in central motor drive or fiber recruitment (Fig. 3, 4). Thus, the present findings extend previous observations made in the smaller forearm muscles, to demonstrate a relative additive effect in larger muscle groups at higher intensities, as well as indicating that a faster development of peripheral fatigue (Fig. 2, Panel B) is a major precursor to exhaustion in hypoxic-cold (Fig. 2, Panel A).

In contrast to hypoxic-cold, during combined hypoxia and heat, a significant antagonistic interaction was observed on TTE. Thus, the relative effect magnitudes were reduced when hypoxia and heat were in the presence of the other stressor (Fig. 2, Table Insert). Opposing the hypothesis, there was no substantial influence of VA (central fatigue) on the decline in force with time (Table 1, Fig 4). Therefore as with hypoxic-cold, the findings appeared to be primarily attributable to significant increases in peripheral fatigue rate (Fig. 2, Panel B; Fig 3, Panel D).

The influence of individual stressor mechanisms and impact on the interaction between stressors

Lloyd et al. (31) suggested that when stressors with dissimilar mechanistic characteristics are combined, an additive effect may be observed (e.g. cold and hypoxia); while combining stressors that work through similar mechanisms, may result in an interactive effect (e.g. heat and hypoxia). This suggestion was formulated on the basis that if two mechanisms work through similar physiological pathways, there is a greater possibility for one stressor to influence another (i.e. cause an interaction). Theoretically, this may elucidate contrasting interactions in this study, whereby oxygen transport limitations in both heat and hypoxia interact during combined exposure (hypoxic-heat), while cold

induced changes in biomechanical efficiency combined with hypoxic limitations in oxygen transport do not interact during combined (hypoxic-cold) exposure. However, the present findings should also be considered in light of two recent studies exploring combined hypoxia and thermal (warm/ heat) stress during whole-body exercise (19, 68). In these studies an additive effect on performance was reported by Van Cutsem et al. (68) during a self-paced cycling time trial; while an antagonistic interaction was observed by Girard and Racinais (19) during fixed, moderate intensity cycling to exhaustion. Because the nature of the stressors used in these studies were similar (warm-hypoxic and heat-hypoxic), additional reasons may need to be considered to explain the different interaction types observed during combined stressor exposures.

Another possible modulator of type of interaction may be the impact magnitude of the individual stressors' effects on performance. In this regard, individual stressors with a large influence on exercise capacity could antagonize when combined (19) (e.g. the present combination of moderate hypoxia and severe heat), while combined stressors that evoke milder performance reductions produce more additive effects (1, 31, 68) (e.g. the present combination of moderate hypoxia and mild cold). This ultimately infers a maximum threshold for performance deterioration, whereby performance is only reduced by a specific magnitude before effects of a given stressor are fully antagonized i.e. the effect of one stressor is overruled or entirely cancelled out by the effect of the other. Based on present and past experimental data (1, 19, 31, 68), the magnitude of the stressors impact on performance likely provides a more suitable explanation for interaction type, compared to the pathway of influence (nature) of the two stressors being combined. Importantly, antagonism with increasing stressor impact indicates humans may respond to simultaneous and severe physiological strains based on a 'worst strain takes precedence' principle.

As well as characterizing multi-stressor environments, this novel paradigm may also reveal how multiple limiting factors can be imposed on exercise capacity, as well as clarifying the often contrasting 'cardinal' limitations on exercise performance between studies. For example, in the heat, a reduction in moderate-intensity exercise capacity is frequently associated with the concurrent increases in core (spinal, visceral and cerebral) temperature (19, 42, 68). Yet in the present study, increases in skin and muscle (13, 32) temperature alone imposed severe limitations on high-intensity exercise

performance (Table 2, Fig. 2). Based on the proposed paradigm of stressor antagonism (see above), when simultaneously present, skin, muscle, spinal, visceral and cerebral temperature could each impose their own task specific limitations on exercise capacity, however it is the factor with the greatest impact magnitude that - for a given task - will progressively take precedence over all other factors.

Voluntary activation during brief exertions

The minimal changes in VA in the present study (Fig. 4) could be due to the small variation in T_{core} (Table 2), as well as the moderate level of systemic hypoxemia (>80% SpO_2) (22, 37, 39, 65), both of which should be acknowledged as limitations of the present study. The present findings may also be attributable to the contractions used to measure VA being brief (32, 34, 41). Brief contractions, together with short (~3-s) pauses in central motor drive prior to the MVC (49), reduce the impact of afferent feedback from active, respiratory, cardiac and/or synergistic muscles to conscious moderations in central motor drive (6), therefore facilitating VA to levels beyond what is possible at exhaustion. As such, caution should be taken not to fully discount contributions from central factors to the present reductions in TTE, which occurred during a sustained mental effort and with intact muscle sensory feedback, prior to the brief MVC during which VA was measured.

It is important to note that an additional and unexpected finding in the present study was that MVC force was slightly, though significantly, lower upon immediate exposure to the heated and cold experimental conditions, compared with neutral T_{env}. However, the small changes in MVC force did correlate with changes in VA, and there were no corresponding changes in $Q_{tw,pot}$ amplitude. This indicates the pre-rest measures of MVC force may have been a psychophysiological response to acute exposure to heat and cold (18), not an influence on the participants true ability to produce force or a change in the force transducer sensitivity due to changes in T_{env}.

Perceptual responses to fatigue under environmental stress

In the present study the rates of change in sensed mental effort, fatigue and pain were altered proportionally to the environmental stressors influences on peripheral fatigue development (Fig, 3, Panels F, G, and H). As such, the results appear to indicate that the rise in mental effort was in response to the rise in actual and /or sensed muscle fatigue (10, 33), presumably via a progressive deactivation

of muscle mechanoreceptive feedback for a given central command (52, 70) as well as the progressive activation of metaboreceptive feedback (5, 6, 38, 51). An important exception to this was in the rate of decline in leg muscle pain, which during cold was unchanged from neutral (Table 3) despite changes in TTE. This could be attributed to the attenuated excitability of sensory afferent nerves at lower T_m (32, 34, 56, 58).

Conclusions, perspectives, context and significance of the research

Exposure to real world extreme environments often consists of numerous environmental stressors and thereby multiple physiological strains. While recent studies conducted on small muscle groups (31) and whole-body exercise (1, 19, 68) have begun to address how combined environmental stressors might influence exercise capacity, at present the basis for varied interaction types is unclear. By utilizing a mechanistic fatigue protocol across a variety of single and multi-stressor conditions, the roles of both stressor 'nature' and stressor 'impact magnitude' on the type of multi-stressor interaction expressed were examined.

Based on the conditions tested in the present study, combined exposure to moderate hypoxia and mild cold stress resulted in **additive** relative (percentage) reductions in times to exhaustion. In contrast, combined moderate hypoxia and severe heat stress resulted in a significant **antagonistic** interaction on time to exhaustion, where the effect of each stressor was attenuated in the presence of the other stressor. The decreases in time to exhaustion during both combined hypoxic-heat and combined hypoxic-cold were consistent with the increased rates of peripheral fatigue development, as well as a faster progression in perceived mental effort and muscle fatigue. Based on the present findings and previous research (19, 31, 68), a novel principle of multifactorial integration is proposed; that the type of interaction between stressors is influenced by the impact magnitude of the individual stressors effect on exercise capacity, in which the greater the stressors impact, the greater the trend for one stressor to cancel out (nullify) the other. This is indicative of an 'antagonistic' or 'worst strain take precedence' model of multifactorial integration.

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TABLE LEGENDS

Table 1: Neuromuscular function and perceptual exercise ratings before (PRE-REST) and after the rest period (POST-REST) and at exhaustion (EXH). From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). MVC, maximal voluntary contraction; VA, voluntary muscle activation; $Q_{tw,sup}$, 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-REST and POST-REST values were averaged for the three maximal voluntary contractions (MVC). Each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled 1-ms prior to delivery of each $Q_{tw,sup}$. All data are presented as mean ± SD. 'T_{env}' indicates a main effect of environmental temperature. 'O_2' indicates a main effect of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the significant pairwise comparisons are displayed in the subsequent brackets (e.g. HEAT, COLD).

Table 2: Temperature recordings and pulse oximetry before (PRE-REST) and after seated rest in the environmental conditions (POST-REST) and at exhaustion (EXH). From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). T_{core}, core

temperature; T_m, vastus lateralis muscle temperature; T_{sk}, skin temperature; HR, heart rate; SpO_2, oxygen saturation of peripheral blood. T_m is displayed as a three depth mean (1, 2 and 3-cm). Mean T_{sk} was calculated using equal weighting from each of the seven measurement sites. All data are presented as mean ± SD. 'T_{env}' indicates a main effect of environmental temperature. 'O_2' indicates a main effect of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the significant pairwise comparisons are displayed in the subsequent brackets (e.g. HEAT, COLD). Where a significant interaction (T_{env} x F_IO_2) has been indicated, the specific ANOVA interactions for hypoxic-cold and hypoxic-heat are also displayed in the subsequent brackets (e.g. HEAT, COLD).

Table 3: Rate of change (%.min^{-1}) in neuromuscular and perceptual variables between the start (POST-REST) and end (exhaustion; EXH) of the exercise protocol. From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). MVC, maximal voluntary contraction; VA, voluntary muscle activation; $Q_{tw,sup}$, superimposed twitch force; mean RFD, resting twitch mean rate of force development; mean RFR, resting twitch mean rate of relaxation. Each set of $Q_{tw,sup}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled 1-ms prior to delivery of each $Q_{tw,sup}$. Pre and post rest neuromuscular assessments are not included in this table. All data represent a reduction over time (except where indicated by a +), and are presented as mean ± SD. 'T_{env}' indicates a main effect of environmental temperature. 'O_2' indicates a main effect of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the pairwise comparisons are displayed in subsequent brackets (e.g. HEAT, COLD).

FIGURE LEGENDS

Figure 1: Overview of the experimental protocol. White boxes indicate the schematic overview of the experimental protocol. Grey boxes indicate the outcome measures. T_{core}, rectal temperature; T_m, muscle temperature; T_{sk}, skin temperature; MVC, maximal isometric voluntary contraction force of knee extensors; HR, heart rate; SpO_2, oxygen saturation of peripheral blood; PRE-REST, pre seated rest in the environmental conditions; REST, rest period; POST-REST, post seated rest in the environmental

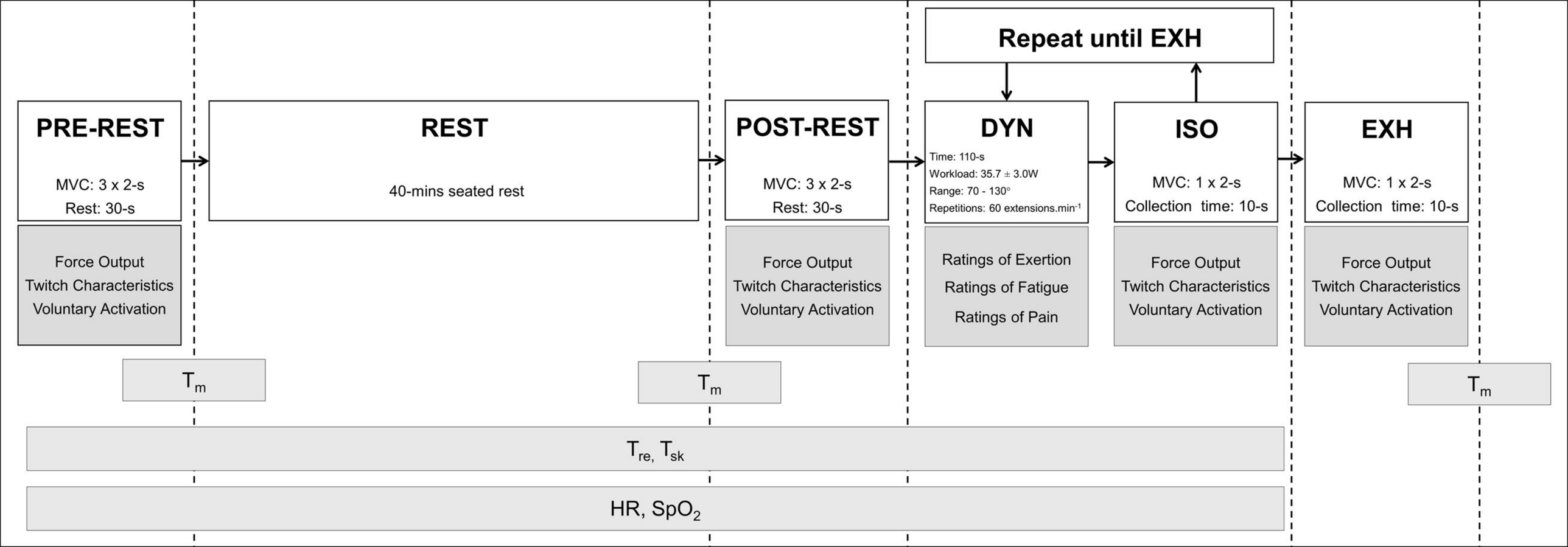
conditions; DYN, fixed intensity dynamic knee extension exercise; ISO, isometric neuromuscular test with femoral nerve stimulation; EXH, exhaustion through task failure or exercise intolerance.

Figure 2: The effect of environmental temperature and hypoxia on time to exhaustion (TTE) and the rate of increase in peripheral fatigue (resting potentiated twitch force, $Q_{tw,pot}$). Panel A shows TTE in seconds across conditions. Panel B shows the rate of peripheral fatigue development in $\%.min^{-1}$ across conditions. Each set of $Q_{tw,pot}$ were averaged for each MVC. Pre and post rest neuromuscular assessments are not included in this figure. From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). *main effect of cold or heated environmental temperature. $^{\#}$main effect of hypoxic oxygen concentration. †interaction between hypoxia and heated environmental temperature. The table insert shows the relative reductions in TTE caused by each individual stressor, with and without the presence of another stressor. Pre and post rest neuromuscular assessments are not included in this figure.

Figure 3: The contribution of resting twitch force ($Q_{tw,pot}$; peripheral fatigue) to time to exhaustion (TTE) as well as maximal voluntary contraction (MVC) force (integrated fatigue) and perceptual exercise ratings (i.e. mental effort, fatigue and pain). Panels A, B, C and D illustrate the increase in peripheral fatigue over time and across conditions. Based on post-hoc observations $Q_{tw,pot}$ was fitted with a 2 order polynomial function. Panels E, F, G and H show the relationship between peripheral fatigue and MVC force, sense of mental effort, sense of leg fatigue and sense of leg pain using linear correlation (least squares method) with the reduction in $Q_{tw,pot}$. Pre and post rest neuromuscular assessments are not included in this figure.

Figure 4: The contribution of voluntary activation (VA; central fatigue) to time to exhaustion (TTE) as well as maximal voluntary contraction (MVC) force (integrated fatigue), sense of mental effort, sense of leg fatigue and sense of leg pain. Panels A, B, C and D illustrate the change in VA over time and across conditions. Based on post-hoc observations VA was fitted with a linear function. Panels E, F, G and H show the relationship between central fatigue and MVC force, sense of mental effort, sense of

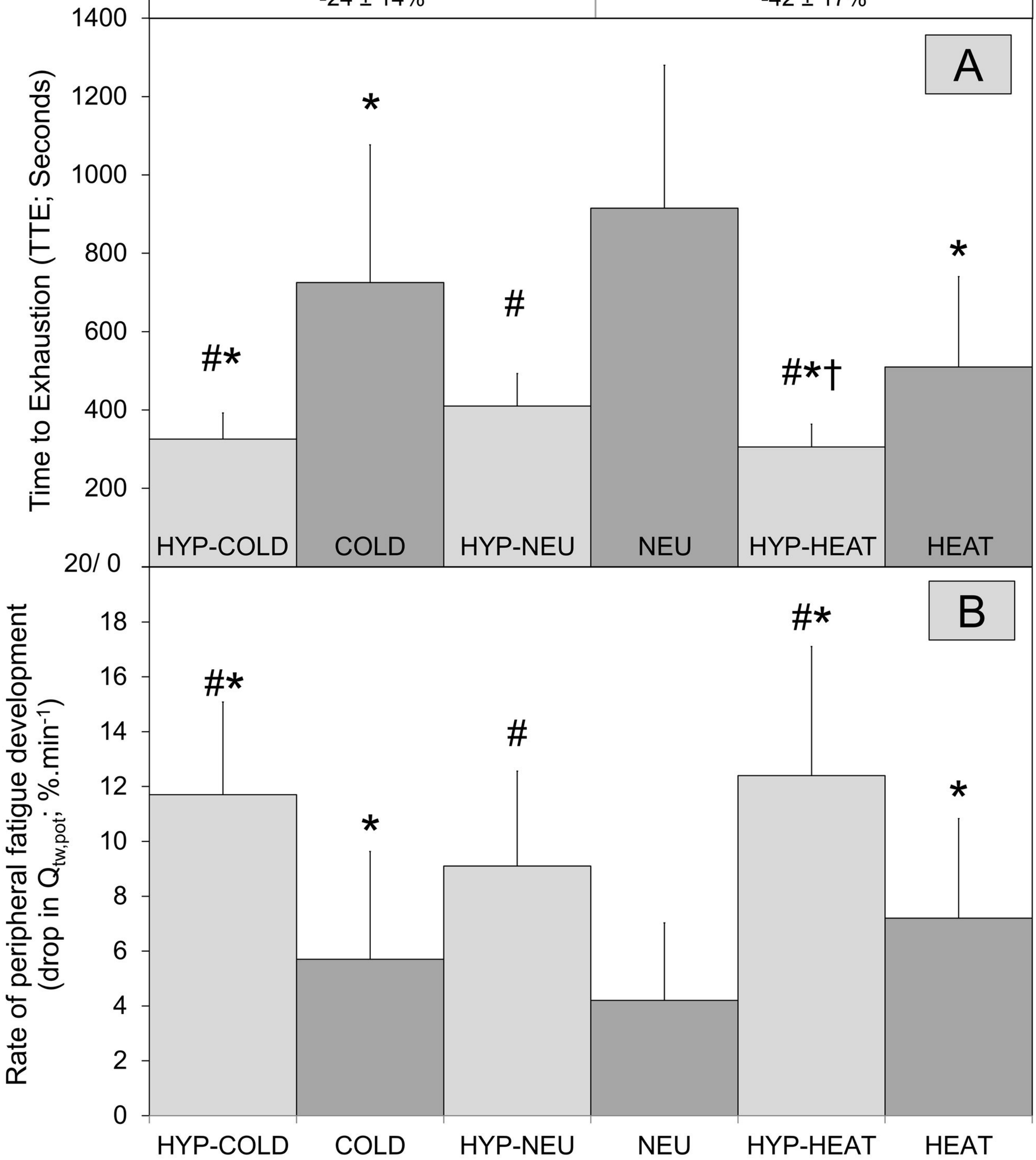
leg fatigue and sense of leg pain using linear correlation (least squares method) with the reduction in VA. Pre and post rest neuromuscular assessments are not included in this figure.

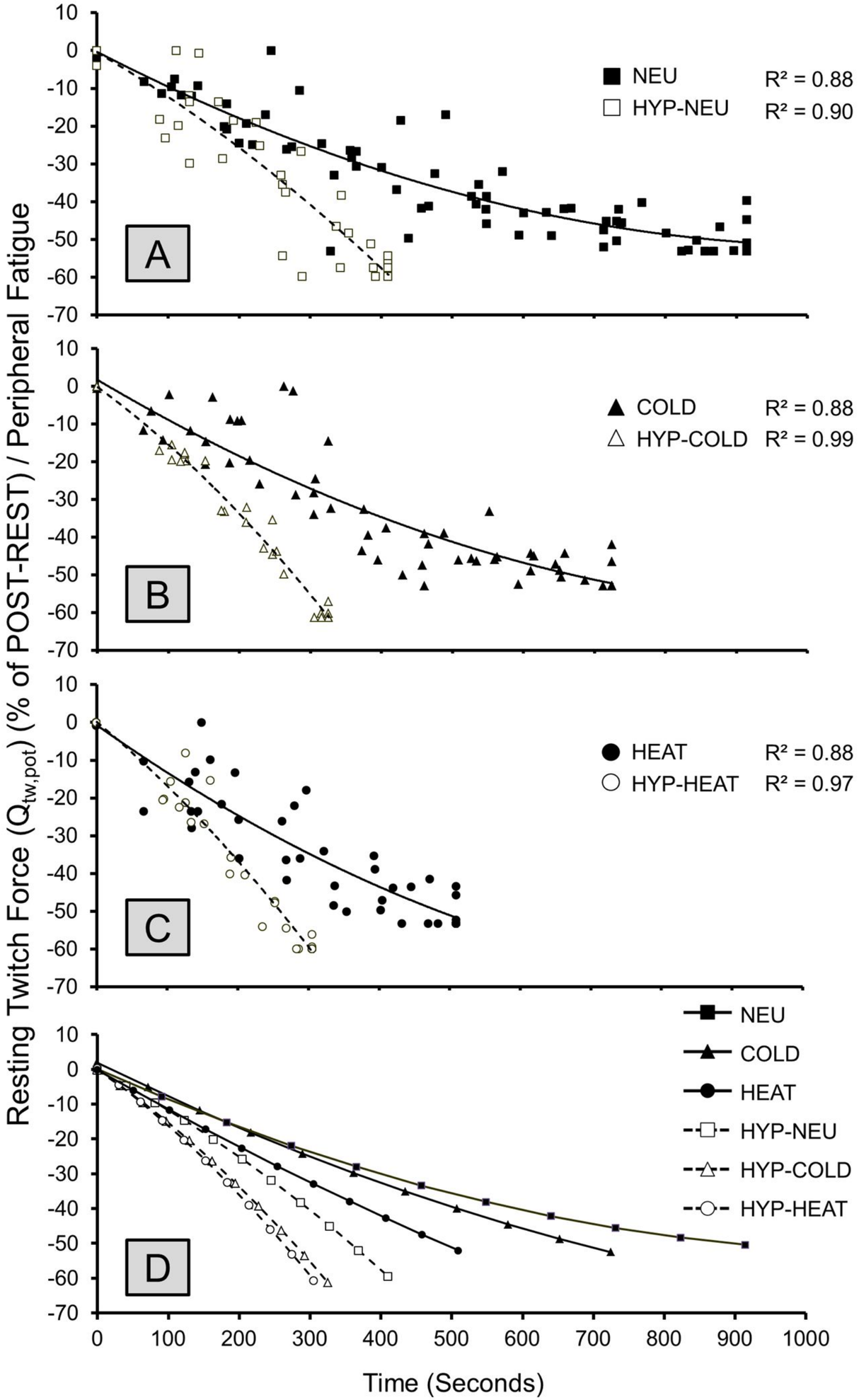

Repeat until EXH
PRE-REST
MVC: 3 x 2-s
Rest: 30-s
REST
40-mins seated rest
POST-REST
MVC: 3 x 2-s
Rest: 30-s
DYN
Time: 110-s
Workload: 35.7 ± 3.0W
Range: 70 - 130°
Repetitions: 60 extensions.min-1
ISO
MVC: 1 x 2-s
Collection time: 10-s
EXH
MVC: 1 x 2-s
Collection time: 10-s
Force Output
Twitch Characteristics
Voluntary Activation
Force Output
Twitch Characteristics
Voluntary Activation
Ratings of Exertion
Ratings of Fatigue
Ratings of Pain
Force Output
Twitch Characteristics
Voluntary Activation
Force Output
Twitch Characteristics
Voluntary Activation
Tm
Tm
Tm
Tre, Tsk
HR, SpO2

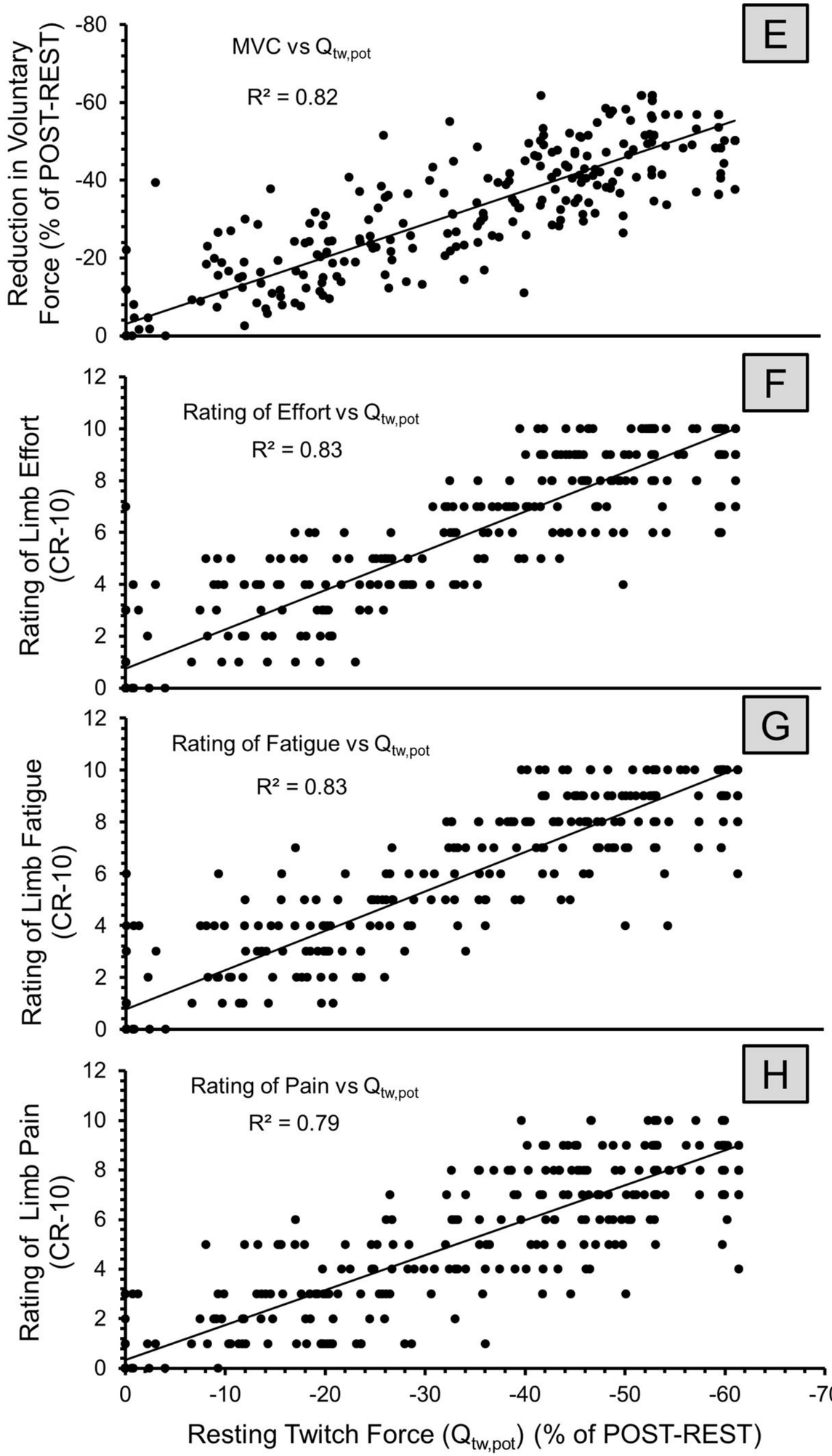
Effect of HYPOXIA on TTE (%)		
In COLD	**In NEUTRAL**	**In HEAT**
-48 ± 19%	-51 ± 16%	-35 ± 16%

Effect of COLD on TTE (%)	
In HYPOXIA	**In NORMOXIA**
-21 ± 22%	-20 ± 9%

Effect of HEAT on TTE (%)	
In HYPOXIA	**In NORMOXIA**
-24 ± 14%	-42 ± 17%




A
NEU
HYP-NEU
R² = 0.88
R² = 0.90
B
COLD
HYP-COLD
R² = 0.88
R² = 0.99
C
HEAT
HYP-HEAT
R² = 0.88
R² = 0.97
D
NEU
COLD
HEAT
HYP-NEU
HYP-COLD
HYP-HEAT
Resting Twitch Force ($Q_{tw,pot}$) (% of POST-REST) / Peripheral Fatigue
Time (Seconds)


E
MVC vs $Q_{tw,pot}$
R² = 0.82
Reduction in Voluntary Force (% of POST-REST)
F
Rating of Effort vs $Q_{tw,pot}$
R² = 0.83
Rating of Limb Effort (CR-10)
G
Rating of Fatigue vs $Q_{tw,pot}$
R² = 0.83
Rating of Limb Fatigue (CR-10)
H
Rating of Pain vs $Q_{tw,pot}$
R² = 0.79
Rating of Limb Pain (CR-10)
Resting Twitch Force ($Q_{tw,pot}$) (% of POST-REST)

Voluntary Activation (%) / Central Fatigue

A
NEU R² = 0.02
HYP-NEU R² = 0.06

B
COLD R² = 0.01
HYP-COLD R² = 0.38

C
HEAT R² = 0.03
HYP-HEAT R² = 0.01

D
NEU
COLD
HEAT
HYP-NEU
HYP-COLD
HYP-HEAT

Time (Seconds)

Reduction in Voluntary Force (% of POST-REST)

E
MVC vs VA
R² = 0.02

Rating of Limb Effort (CR-10)

F
Rating of Effort vs VA
R² = 0.00

Rating of Limb Fatigue (CR-10)

G
Rating of Fatigue vs VA
R² = 0.00

Rating of Limb Pain (CR-10)

H
Rating of Pain vs VA
R² = 0.00

Reduction in Voluntary Activation (VA) (%)

Variable	Time Point	HYP-COLD	COLD	HYP-NEU	NEU	HYP-HEAT	HEAT	Main Effects
MVC Force (N)	PRE-REST	855 ± 140	835 ± 172	878 ± 144	915 ± 199	803 ± 161	861 ± 194	T_{env} (HEAT, COLD)
	POST-REST	869 ± 139	844 ± 120	903 ± 131	907 ± 191	770 ± 142	845 ± 159	T_{env} (HEAT)
	EXH	461 ± 133	436 ± 89	409 ± 122	395 ± 100	433 ± 139	436 ± 113	T_{env} (COLD)
VA (%)	PRE-REST	93.3 ± 3.0	90.2 ± 6.7	91.3 ± 4.9	92.1 ± 6.0	89.3 ± 6.6	90.9 ± 6.1	-
	POST-REST	93.2 ± 3.1	92.8 ± 3.6	93.7 ± 3.2	94.1 ± 3.7	88.8 ± 7.6	91.1 ± 4.5	T_{env} (HEAT)
	EXH	95.4 ± 5.9	91.7 ± 11.5	92.0 ± 9.6	91.0 ± 9.5	90.7 ± 14.1	91.9 ± 5.5	T_{env}
$Q_{tw,sup}$ (N)	PRE-REST	79 ± 34	84 ± 50	81 ± 43	72 ± 47	90 ± 46	81 ± 48	-
	POST-REST	60 ± 25	63 ± 25	59 ± 30	54 ± 30	93 ± 59	80 ± 36	T_{env} (HEAT)
	EXH	18 ± 20	39 ± 52	36 ± 48	38 ± 44	40 ± 59	42 ± 36	O_2
$Q_{tw,pot}$ (N)	PRE-REST	339 ± 45	349 ± 80	368 ± 90	345 ± 60	350 ± 68	370 ± 76	-
	POST-REST	322 ± 49	329 ± 63	337 ± 63	332 ± 49	344 ± 61	354 ± 48	T_{env}
	EXH	129 ± 44	167 ± 80	143 ± 67	166 ± 68	144 ± 66	177 ± 80	O_2
Mean RFD ($N.ms^{-1}$)	PRE-REST	5.05 ± 0.98	5.08 ± 0.83	5.34 ± 1.36	4.92 ± 0.60	5.06 ± 0.91	5.33 ± 0.86	-
	POST-REST	4.19 ± 1.18	4.28 ± 1.20	4.87 ± 1.12	3.91 ± 1.18	4.96 ± 1.18	4.52 ± 1.03	-
	EXH	1.63 ± 0.76	2.38 ± 1.26	1.78 ± 1.09	2.48 ± 1.28	1.90 ± 1.22	2.45 ± 1.39	O_2
Mean RFR ($N.ms^{-1}$)	PRE-REST	1.35 ± 0.54	1.43 ± 0.50	1.61 ± 0.78	1.46 ± 0.41	1.31 ± 0.48	1.58 ± 0.58	-
	POST-REST	1.28 ± 0.53	1.32 ± 0.27	1.50 ± 0.79	1.56 ± 0.82	1.39 ± 0.47	1.47 ± 0.57	-
	EXH	0.33 ± 0.19	0.65 ± 0.42	0.52 ± 0.46	0.69 ± 0.50	0.44 ± 0.33	0.65 ± 0.50	O_2
Sense of Effort (CR-10)	PRE-REST	-	-	-	-	-	-	-
	POST-REST	0.11 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.44	0.00 ± 0.00	-
	EXH	8.89 ± 1.27	9.44 ± 0.73	9.78 ± 0.44	9.78 ± 0.44	9.22 ± 1.30	9.78 ± 0.44	T_{env}
Sense of Leg Fatigue (CR-10)	PRE-REST	-	-	-	-	-	-	-
	POST-REST	0.11 ± 0.33	0.11 ± 0.33	0.11 ± 0.33	0.00 ± 0.00	0.22 ± 0.44	0.00 ± 0.00	-
	EXH	9.11 ± 1.26	9.56 ± 0.73	9.56 ± 0.73	9.56 ± 0.73	9.44 ± 0.73	9.56 ± 0.88	-
Sense of Leg Pain (CR-10)	PRE-REST	-	-	-	-	-	-	-
	POST-REST	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-
	EXH	8.00 ± 0.73	8.56 ± 0.88	9.00 ± 0.87	9.00 ± 1.12	9.11 ± 0.60	9.00 ± 0.87	T_{env}

Variable	Time Point	HYP-COLD	COLD	HYP-NEU	NEU	HYP-HEAT	HEAT	Main Effects
Rectal T_{core} (°C)	PRE-REST	37.24 ± 0.26	37.26 ± 0.34	37.31 ± 0.24	37.36 ± 0.16	37.28 ± 0.23	37.28 ± 0.37	-
	POST-REST (Δ)	-0.13 ± 0.20	0.00 ± 0.58	-0.18 ± 0.17	-0.15 ± 0.10	0.02 ± 0.17	0.03 ± 0.18	-
	EXH (Δ)	-0.22 ± 0.23	-0.08 ± 0.64	-0.14 ± 0.16	-0.09 ± 0.11	0.12 ± 0.18	0.14 ± 0.16	T_{env} (HEAT)
Whole Body T_{sk} (°C)	PRE-REST	32.1 ± 0.9	31.9 ± 0.9	32.3 ± 0.7	31.9 ± 0.8	31.8 ± 1.0	31.9 ± 1.3	-
	POST-REST	26.8 ± 0.9	26.3 ± 0.7	32.4 ± 0.7	31.9 ± 0.7	37.0 ± 0.2	36.8 ± 0.3	T_{env} (HEAT, COLD); O_2
	EXH	26.6 ± 1.0	25.9 ± 0.7	32.6 ± 0.6	32.5 ± 0.8	37.1 ± 0.3	37.1 ± 0.4	T_{env} (HEAT, COLD); O_2; T_{env} x O_2
Exercising Vastus Lateralis T_m (°C)	PRE-REST	34.1 ± 0.6	34.1 ± 1.4	33.8 ± 0.9	34.4 ± 1.3	33.7 ± 1.7	34.4 ± 0.8	O_2
	POST-REST	30.5 ± 1.9	30.2 ± 1.5	33.5 ± 0.7	33.1 ± 1.0	36.2 ± 0.5	36.4 ± 0.3	T_{env} (HEAT, COLD)
	EXH	34.4 ± 1.2	34.2 ± 1.8	36.2 ± 0.5	36.3 ± 0.6	37.6 ± 0.5	37.9 ± 0.5	T_{env} (HEAT, COLD)
Non-Exercising Vastus Lateralis T_m (°C)	PRE-REST	34.3 ± 0.6	33.9 ± 1.0	33.7 ± 0.8	34.5 ± 1.0	33.7 ± 1.3	34.1 ± 0.6	-
	POST-REST	30.6 ± 1.3	29.9 ± 1.6	33.3 ± 0.8	32.7 ± 1.3	35.9 ± 0.5	36.0 ± 0.4	T_{env} (HEAT, COLD)
	EXH	29.1 ± 1.6	28.0 ± 1.6	32.9 ± 0.9	32.2 ± 1.3	36.1 ± 0.5	36.3 ± 0.6	T_{env} (HEAT, COLD); O_2; T_{env} x O_2 (HEAT)
SpO_2(%)	PRE-REST	99.7 ± 0.7	99.7 ± 0.5	99.6 ± 0.7	99.4 ± 0.7	99.6 ± 0.7	99.7 ± 0.7	-
	POST-REST	83.1 ± 2.8	99.2 ± 0.7	86.4 ± 4.6	98.8 ± 1.0	87.7 ± 4.8	98.4 ± 0.5	O_2
	EXH	87.3 ± 5.6	96.8 ± 4.6	90.3 ± 4.4	96.6 ± 3.9	92.4 ± 3.7	99.2 ± 0.4	T_{env}; O_2; T_{env} x O_2
HR ($b.min^{-1}$)	PRE-REST	70.6 ± 6.1	73.0 ± 7.3	72.6 ± 11.2	72.0 ± 4.9	72.7 ± 7.2	72.7 ± 9.0	-
	POST-REST	84.3 ± 15.2	81.1 ± 11.0	94.3 ± 11.8	78.9 ± 9.9	108.8 ± 12.1	100.6 ± 15.1	T_{env} (HEAT); O_2
	EXH	116.4 ± 14.6	124.2 ± 18.9	141.0 ± 30.6	132.6 ± 28.1	149.3 ± 11.5	157.8 ± 13.7	T_{env}

Variable	Time Point	HYP-COLD	COLD	HYP-NEU	NEU	HYP-HEAT	HEAT	Main Effects
MVC Force (%.min^{-1})	POST-REST to EXH Rate of Change	9.03 ± 2.86	4.71 ± 2.07	8.39 ± 2.63	4.23 ± 2.20	8.86 ± 2.80	6.42 ± 2.38	T_{env}, O_2
VA (%.min^{-1})	POST-REST to EXH Rate of Change	+0.45 ± 0.90	+0.13 ± 0.90	0.16 ±1.13	0.16 ±0.53	+0.51 ± 1.54	+0.31 ± 0.69	T_{env} (HEAT, COLD)
$Q_{tw,sup}$ (%.min^{-1})	POST-REST to EXH Rate of Change	14.07 ± 6.14	6.04 ± 8.06	7.33 ± 7.40	2.68 ± 5.03	13.95 ± 6.80	7.44 ± 7.49	T_{env} (HEAT, COLD), O_2
Mean RFD (%.min^{-1})	POST-REST to EXH Rate of Change	11.99 ± 4.33	5.23 ± 4.43	9.54 ± 5.18	2.78 ± 5.01	12.97 ± 5.70	6.52 ± 4.87	T_{env} (HEAT), O_2
Mean RFR (%.min^{-1})	POST-REST to EXH Rate of Change	14.40 ± 4.36	6.28 ± 5.25	10.38 ± 3.35	4.56 ± 3.29	13.93 ± 5.19	7.69 ± 4.54	T_{env} (HEAT, COLD), O_2
Sense of Effort (%.min^{-1})	POST-REST to EXH Rate of Change	16.76 ± 4.39	9.63 ± 4.76	14.86 ± 3.25	7.40 ± 3.09	17.93 ± 2.94	13.11 ± 4.18	T_{env} (HEAT), O_2
Sense of Leg Fatigue (%.min^{-1})	POST-REST to EXH Rate of Change	17.18 ± 4.38	9.69 ± 4.84	14.33 ± 3.25	7.24 ± 3.14	18.52 ± 3.00	12.76 ± 4.12	T_{env} (HEAT, COLD), O_2
Sense of Leg Pain (%.min^{-1})	POST-REST to EXH Rate of Change	15.48 ± 5.36	8.84 ± 4.53	13.55 ± 3.21	7.01 ± 3.41	18.43 ± 3.50	12.35 ± 4.68	T_{env} (HEAT), O_2