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

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1 **Title:**

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

4

5 **Authors:**

6 Alex Lloyd

7 Margherita Raccuglia

8 Simon Hodder

9 George Havenith

10

11 **Affiliation:**

12 Environmental Ergonomics Research Centre, Loughborough University, Loughborough, UK

13

14 **Running Head:**

15 Fatigue in combined hypoxic and thermal stress

16

17 **Corresponding Author:**

18 George Havenith

19 Environmental Ergonomics Research Centre,

20 James France Bldg.,

21 Loughborough,

22 Leicestershire

23 UK

24 LE11 3TU

25 Email: G.Havenith@lboro.ac.uk

26

27

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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_IO₂; 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.

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

60 ABBREVIATIONS

61 ANOVA: Analysis of variance

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

63 CV: Coefficient of variation

64 $F_{I}O_2$: Fraction of inspired oxygen

65 FNS: Femoral nerve stimulation (twitch interpolation)

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

67 HR: Heart rate

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

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

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

71 MVC: Maximal voluntary contraction

72 RFD: Resting twitch rate of force development

73 RFR: Resting twitch rate of relaxation

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

75 O_2 : Oxygen

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

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

78 $Q_{tw,pot}$: Resting potentiated twitch force (peripheral fatigue)

79 $Q_{tw,sup}$: Superimposed twitch force

80 SpO_2 : Oxygen saturation of peripheral blood

81 T_{core} : Core temperature

82 T_{env} : Environmental temperature

83 T_m : Muscle temperature

84 T_{sk} : Skin temperature

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

86 TTE: Time to exhaustion

87 VA: Voluntary activation

88 $\%VO_{2max}$: Relative percentage of maximal aerobic capacity

89

90

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 $ml\ O_2 \cdot min^{-1} \cdot W^{-1}$ or $\%VO_{2max} \cdot W^{-1}$ 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:

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

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

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

147

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

158

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

170

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

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

179

180 MATERIALS AND METHODS

181 **Ethical approval**

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

187

188 **Subjects**

189 Nine healthy, moderately trained male volunteers participated in the study (mean $\pm$ SD, height: $181 \pm$
190 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,
191 resting heart rate during 5-mins supine rest: 64 ± 4 b.min⁻¹). All were right leg dominant with no
192 previous history of cardiovascular, neurological and muscle debility. Participants were requested to
193 preserve their normal exercise routines, but abstain from exercise, caffeine and alcohol 24 hrs. prior to
194 each experimental session. The experiments were conducted in autumn (UK), presumably indicating
195 little or no heat acclimation. Participants were not acclimated to hypoxia prior to participation in the
196 experiment.

197

198 **General Study Overview**

199 Following initial familiarization, participants conducted six experimental sessions at three levels of
200 environmental temperature (T_{env}) and two levels of fraction of inspired oxygen (F_{IO_2}) in an
201 environmental chamber (T.I.S.S. Peak Performance, Series 2009 Climate Chambers). Specifically, the
202 conditions included:

203

204	HYP- COLD	5°C T _{env} & 50% rh in 0.125 F _I O ₂
205	COLD	5°C T _{env} & 50% rh in 0.209 F _I O ₂
206	HYP- NEU	23°C T _{env} & 50% rh in 0.125 F _I O ₂
207	NEU	23°C T _{env} & 50% rh in 0.209 F _I O ₂
208	HYP- HEAT	42°C T _{env} & 70% rh in 0.125 F _I O ₂
209	HEAT	42°C T _{env} & 70% rh in 0.209 F _I O ₂

210

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

221 **[Insert Figure 1 here]**

222

223

224 **Familiarization sessions**

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

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

242

243 **Main experimental sessions**

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

293 The CV in mean force transducer output during dynamic exercise across conditions was also small
294 (3.86%), indicating equal and proportional workloads were successfully applied to the knee extensors.

295

296 **Dynamometer set up**

297 For all experiments, subjects were seated with a hip and knee joint angle of 90 and 100° respectively.

298 The dynamometer was adjusted for each individual's femoral and tibial lengths, as well as their

299 popliteal to patella width. The right leg was secured to a force transducer (Tede- Huntleigh, Model

300 615, Vishay Precision Group, California, USA) using an adjustable, non-compliant harness around

301 ankle malleolus. A layer of padding was applied to the ankle to protect against harness bruising/

302 rubbing during the dynamic component of the exercise protocol. Participants were secured using a

303 waist belt system. Force data was visually displayed on a PC (DataLog software, Biometrics Ltd, UK)

304 via a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK).

305 Baseline noise was less than 0.5N once ambient and force transducer temperature had stabilized. No

306 discernable (over and above baseline noise) differences were observed in force transducer sensitivity at

307 different T_{env} .

308

309 **Knee extension power output calculations**

310 All power outputs were calculated from the kinetic energy measured on the flywheel, plus the power

311 required to move the lower leg, foot, force transducer and crank arm through -20° to +40°

312 (perpendicular to the floor) at an angular velocity of 2.0944 rad.s⁻¹ (i.e. movement through a range of

313 80- 140° knee extension every 0.5 secs). Combined lower leg and foot weight were calculated as total

314 body weight *0.0592 (12).

315

316 Since gravity alters the torque requirement at each circular section, a torque decay curve was calculated

317 for each 10° segment moved. In this case, when the crank arm was perpendicular to the floor the

318 additional torque requirement equals zero; whereas this is equal to 100% when parallel to the floor. A

319 correction was then applied to the torque required at each 10° segment to move the lower leg, foot,

320 force transducer and crank arm through the range used in this study, before calculating the total power

321 in watts (W) from flywheel power, angular velocity (rad.s⁻¹) and total corrected torque (N.m⁻¹).

322

323 **Isometric neuromuscular assessments**

324 Peripheral and central fatigue were calculated using the twitch interpolation technique during an MVC
325 (36). To this end, two superimposed twitches ($Q_{tw,sup}$) were evoked over the force plateau of each
326 MVC, each followed by two resting potentiated twitches ($Q_{tw,pot}$) 1-second after muscle relaxation. VA
327 (i.e. central fatigue) was calculated using the following equation (32, 41): $VA = MVC / (MVC + Q_{tw,sup})$.
328 $Q_{tw,pot}$ was used as an index of the mechanical (contractile) properties of the muscle (i.e. peripheral
329 fatigue) (4, 32, 53). The mean rate of force development (RFD) and mean rate of force relaxation
330 (RFR) were calculated for all $Q_{tw,pot}$ (4).

331

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

335

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

348

349 **Temperature, heart rate and pulse oximetry**

350 Rectal temperature (T_{core}), skin temperature (T_{sk}), heart rate (HR) and oxygen saturation of peripheral
351 blood (SpO_2) were logged every 1 min from pre-exposure until exhaustion. Rectal temperature was
352 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 (3×2 ; $T_{env} \times F_iO_2$) 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 ($\% \cdot \text{min}^{-1}$) 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 ($T_{env} \times F_{I}O_2$) was observed in conjunction with significant main effects of heat, cold as well as $F_{I}O_2$, an additional two-way (2×2 ; HEAT or COLD vs NEU $\times F_{I}O_2$) repeated measures ANOVA was conducted to examine the interaction type expressed during hypoxic-heat and hypoxic-cold (31).

391

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 $\pm$ SD.

399

400 RESULTS

401 **Pre-rest measures**

Prior to the seated rest, there was no main effect of T_{env} or $F_{I}O_2$ 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).

410 **[Insert Table 1 here]**

411

412 **Temperature, heart rate and pulse oximetry**

Table 2 shows the temperature recordings and pulse oximetry before and after the rest period, as well as at exhaustion. T_{core} was unaffected by condition ($p > 0.2$) except at the post-exercise time point where T_{core} was 0.25°C higher ($p = 0.017$) in the heated conditions compared to neutral. Conversely, after the rest period, right leg quadriceps T_m and mean T_{sk} decreased ($p < 0.002$) by $3.8 \pm 1.8^{\circ}\text{C}$ and $5.4 \pm 0.6^{\circ}\text{C}$ in cold conditions, increased ($p < 0.001$) by $2.2 \pm 1.4^{\circ}\text{C}$ and $5.1 \pm 1.1^{\circ}\text{C}$ in heated conditions, and decreased by $0.8 \pm 1.2^{\circ}\text{C}$ and $0.3 \pm 0.5^{\circ}\text{C}$ in neutral conditions. Rest in hypoxia did not affect exercising quadriceps T_m ($p = 0.234$); however mean T_{sk} was marginally increased ($p < 0.001$) by $0.4 \pm 0.5^{\circ}\text{C}$ in hypoxic conditions. The dynamic exercise protocol increased exercising T_m in all conditions; however exercising T_m remained significantly different ($p < 0.001$) in the same order across environmental temperatures at exhaustion.

Across the whole exposure, mean SpO_2 was significantly ($p < 0.001$) reduced to $85 \pm 4\%$ in hypoxia compared to $99 \pm 1\%$ in normoxia. SpO_2 remained significantly ($p < 0.001$) lower in hypoxia at exhaustion. HR was significantly increased after the rest period in heated ($p = 0.002$) and hypoxic ($p = 0.013$) conditions.

There was no significant interaction ($p > 0.1$) between $F_{\text{I}\text{O}_2}$ and T_{env} on any dependent variable immediately after the rest period. At exhaustion however, there was a trend ($p = 0.084$) for synergistic increases in T_{sk} , and a trend ($p = 0.062$) for antagonistic decreases in SpO_2 , when hypoxia and cold were combined. In addition, hypoxia significantly ($p = 0.039$) antagonized non-exercising T_m in heated conditions compared to neutral conditions; however this is most likely explained by the higher non-exercising T_m at the start of the exercise in the hypoxic-thermoneutral condition (Table 2).

[Insert Table 2 here]

Post-rest neuromuscular measures

After the rest period, there was no main effect of $F_{\text{I}\text{O}_2}$ on MVC force, VA, $Q_{\text{tw},\text{sup}}$, $Q_{\text{tw},\text{pot}}$, mean RFD, mean RFR, perceived mental effort, perceived leg fatigue or perceived leg pain. Additionally there was no main effect of T_{env} on mean RFD, mean RFR, perceived mental effort, perceived leg fatigue or perceived leg pain. However MVC force and VA were significantly reduced during heated exposures

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

446

447 **Time to exhaustion**

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

454 **[Insert Figure 2 here]**

455

456 During combined hypoxic-cold, exercise time was reduced further (-589-s) compared to thermoneutral
457 normoxia ($-60 \pm 14\%$); however there was no significant interaction between stressors ($p = 0.198$; Fig.
458 2, Panel A). While the reduction in TTE in absolute terms did not visually appear additive of the two
459 stressors (suggesting antagonism) (Fig. 2, Panel A), the relative percentage reductions in TTE caused
460 by cold and hypoxia were similar irrespective whether this was during combined or individual stressor
461 exposure (Fig. 2, Table Insert). This suggests an additive relative effect (percentage reduction) when
462 cold and hypoxia are combined. Conversely, combined hypoxic-heat reduced exercise time by 609-s
463 compared to thermoneutral normoxia ($-63 \pm 13\%$), with a significant antagonistic interaction between
464 stressors ($p = 0.003$). The relative influences of hypoxia and heat each were different in the presence of
465 the other stressor (Fig. 2, Table Insert) supporting a significant antagonism between heat and hypoxia
466 on TTE when combined. The interaction types expressed at group level varied slightly between
467 participants during both hypoxic-cold (6 additive, 2 antagonistic, 1 synergistic) and hypoxic-heat (2
468 additive, 7 antagonistic).

469

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 $\% \cdot \text{min}^{-1}$ 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 $\% \cdot \text{min}^{-1}$) 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 $0.4\% \cdot \text{min}^{-1}$ (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. $\%VO_{2max} \cdot W^{-1}$), 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 $ml\ O_2 \cdot min^{-1} \cdot W^{-1}$) (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

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

629

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

643

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

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

654

655 **Voluntary activation during brief exertions**

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

666

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

674

675 **Perceptual responses to fatigue under environmental stress**

676 In the present study the rates of change in sensed mental effort, fatigue and pain were altered
677 proportionally to the environmental stressors influences on peripheral fatigue development (Fig. 3,
678 Panels F, G, and H). As such, the results appear to indicate that the rise in mental effort was in response
679 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 $\pm$ 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

898 temperature; T_m , vastus lateralis muscle temperature; T_{sk} , skin temperature; HR, heart rate; SpO_2 ,
 899 oxygen saturation of peripheral blood. T_m is displayed as a three depth mean (1, 2 and 3-cm). Mean T_{sk}
 900 was calculated using equal weighting from each of the seven measurement sites. All data are presented
 901 as mean $\pm$ SD. ' T_{env} ' indicates a main effect of environmental temperature. ' O_2 ' indicates a main effect
 902 of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the significant
 903 pairwise comparisons are displayed in the subsequent brackets (e.g. HEAT, COLD). Where a
 904 significant interaction ($T_{env} \times F_{IO_2}$) has been indicated, the specific ANOVA interactions for hypoxic-
 905 cold and hypoxic-heat are also displayed in the subsequent brackets (e.g. HEAT, COLD).

906

907 Table 3: Rate of change ($\% \cdot \text{min}^{-1}$) in neuromuscular and perceptual variables between the start (POST-
 908 REST) and end (exhaustion; EXH) of the exercise protocol. From left to right the conditions are:
 909 hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic-thermoneutral (HYP-NEU), normoxic-
 910 thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). MVC, maximal
 911 voluntary contraction; VA, voluntary muscle activation; $Q_{tw, sup}$, superimposed twitch force; mean RFD,
 912 resting twitch mean rate of force development; mean RFR, resting twitch mean rate of relaxation. Each
 913 set of $Q_{tw, sup}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled
 914 1-ms prior to delivery of each $Q_{tw, sup}$. Pre and post rest neuromuscular assessments are not included in
 915 this table. All data represent a reduction over time (except where indicated by a +), and are presented as
 916 mean $\pm$ SD. ' T_{env} ' indicates a main effect of environmental temperature. ' O_2 ' indicates a main effect of
 917 environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the pairwise
 918 comparisons are displayed in subsequent brackets (e.g. HEAT, COLD).

919

920 FIGURE LEGENDS

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

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

928

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

940

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

949

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

955 leg fatigue and sense of leg pain using linear correlation (least squares method) with the reduction in
956 VA. Pre and post rest neuromuscular assessments are not included in this figure.
957

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%

Resting Twitch Force ($Q_{tw,pot}$) (% of POST-REST) / Peripheral Fatigue

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