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- 20 **Running Head:** Cold Strain and Endurance Capacity
- 21 **Abstract**
- 22

23 **Introduction**: We tested the effects of cold air (0°C) exposure on endurance capacity to 24 different levels of cold strain ranging from skin cooling to core cooling of ∆-1.0°C. **Methods:** 25 Ten males completed a randomized, crossover, control study consisting of a cycling time-to-26 exhaustion (TTE) at 70% of their peak power output following: i) 30-min of exposure to 22°C 27 thermoneutral air (TN), ii) 30-min exposure to 0° C air leading to a cold shell (CS), iii) 0° C air 28 exposure causing mild hypothermia of -0.5°C from baseline rectal temperature (HYPO-0.5°C), 29 and iv) 0° C air exposure causing mild hypothermia of -1.0 $^{\circ}$ C from baseline rectal temperature 30 (HYPO-1.0°C). The latter three conditions tested TTE in 0°C air. **Results:** Core temperature and 31 seven-site mean skin temperature at the start of the TTE were: TN $(37.0 \pm 0.2^{\circ} \text{C}, 31.2 \pm 0.8^{\circ} \text{C})$, 32 CS (37.1 \pm 0.3°C, 25.5 \pm 1.4°C), HYPO-0.5°C (36.6 \pm 0.4°C, 22.3 \pm 2.2°C), HYPO-1.0°C (36.4 $33 \pm 0.5^{\circ}$ C, 21.4 \pm 2.7°C). There was a significant condition effect ($p \le 0.001$) for TTE, which from 34 TN (23.75 ± 13.75 min) to CS (16.22 ± 10.30 min, ∆-30.9 ± 21.5%, *p*=0.055), HYPO-0.5°C 35 (8.50 ± 5.23 min, ∆-61.4 ± 19.7%, *p≤*0.001), and HYPO-1.0°C (6.50 ± 5.60 min, ∆-71.6 ± 36 16.4%, *p≤*0.001). Furthermore, participants had a greater endurance capacity in CS compared to 37 HYPO-0.5°C (*p*=0.046), and HYPO-1.0°C (*p*=0.007), with no differences between HYPO-0.5°C 38 and HYPO-1.0°C (*p*=1.00). **Conclusion:** Endurance capacity impairment at 70% peak power 39 output occurs early in cold exposure with skin cooling, with significantly larger impairments 40 with mild hypothermia up to ∆-1.0°C. 41 **NEW & NOTEWORTHY:** We developed a novel protocol that cooled skin temperature, or

42 skin plus core temperature (∆-0.5°C or ∆-1.0°C), to determine a dose-response of cold exposure

43 on endurance capacity at 70% peak power output. Skin cooling significantly impaired exercise

44 tolerance time by \sim 31%, whereas core cooling led to a further reduction of 30-40% with no

- 45 difference between ∆-0.5°C or ∆-1.0°C. Overall, simply cooling the skin impaired endurance
- 46 capacity, but this impairment is further magnified by core cooling.
- 47 **Keywords:** Core Cooling; Mild Hypothermia; Endurance Capacity; Cold Strain; Heat Debt

48 **Introduction**

49 Athletes, military personal, and occupational workers can train, compete, and work in 50 cold environments where it is important to understand how physical capacity is altered in the 51 cold in order to maintain performance, prevent accidents and injuries, and prevent thermal strain. 52 Exercise in cold air combined with inadequate clothing is physiologically more demanding 53 compared to thermoneutral environments due to changes in cardiorespiratory function (e.g., 54 vasoconstriction, shifts in oxygen dissociation, reduced peak oxygen consumption) (1, 2), 55 increased metabolic demands (if shivering is present) (3, 4), and reduced neuromuscular 56 function, coordination, and contractility (5–7). Despite these physiological changes with cold 57 exposure, data concerning performance changes are equivocal, with time-to-exhaustion (TTE) at 58 \sim 70% of maximal aerobic capacity either being similar between 4 °C and 21 °C air (8) or 59 improved by ~40% in $3^{\circ}C$ (9) compared to 20 $^{\circ}C$. One potential cause of these disparate findings 60 may be a lack of significant skin or core temperature cooling prior to exercise, as these were 61 acute exposure protocols where exercise commenced almost immediately upon entry to a cold 62 environment, likely resulting in little to no change in core or muscle temperature. Recently, 63 studies inducing actual mild hypothermia pre-exercise demonstrate a performance decrement, 64 with ~∆-1.5°C in core temperature via cold-water immersion (10°C) reducing the work 65 completed by \sim 11% during a 20-min self-paced cycling time trial in a thermoneutral 66 environment (23°C) (10). Similarly, an ~∆-0.5°C in core temperature via cold-air exposure 67 impaired 15-km time trial performance in trained cyclists in cold air $(0^{\circ}C)$ (1). Given that ratings 68 of perceived exertion remained similar in the latter study, the $\sim 6\%$ lower average power output 69 suggests a voluntary down-regulation of workload in the face of elevated thermal discomfort,

70 indicating that individuals may not be able to sustain the same absolute workload as 71 thermoneutral environments.

72 In acute cold exposures combined with inadequate clothing, individuals first experience 73 cooling of the overall skin/ outer shell temperature, followed cooling of core temperature if there 74 is insufficient heat production to offset heat loss. It is currently unknown if there is a cold 75 exposure dose response exists between peripheral versus deep core cooling, or the magnitude of 76 core cooling on endurance capacity in the cold. Endurance performance is physiologically 77 determined by mechanical efficiency, anaerobic capacity, and the oxygen transport cascade (11), 78 with the latter known to be regulated in the cold via lower perfusion (capillary vasoconstriction), 79 peripheral resistance, and diffusion (lower intracellular reaction rates and greater kinematic 80 viscosity) capacity (12, 13). Cooling skin or outer shell temperature alone increases peripheral 81 vasoconstriction, and decreases muscle blood flow and oxygenation (14, 15), and increases 82 lactate accumulation while decreasing lactate threshold during exercise (16). Exercise 83 impairment may be caused directly from a cold shell, as using a heated jacket to maintain whole-84 body skin temperature has been shown to improve 2-km rowing time-trial performance following 85 25-min of passive cold air exposure (8°C) (17). Greater cold strain from further core cooling to 86 mild hypothermia elicits shivering and further increase heart rate, thermal discomfort, and 87 vasoconstriction and decreased oxygen availability (1–3, 18), potentially leading to greater 88 impairments in endurance capacity. Peak aerobic capacity during combined arm/leg ergometry is 89 demonstrated to decline \sim 5-6% per °C decrease in core temperature (19). However, the separate 90 and combined effects of cooling skin/shell and core temperature on endurance capacity in cold 91 air is unknown.

92 One of the inherent methodological challenges in cold physiology research is normalizing 93 the cold strain between individuals. A set duration protocol (e.g. 120-mins) can lead to wide 94 individual variability in actual core cooling, due to such factors as anthropometrics (body mass, 95 surface-area to mass ratio, fat insulation), age, and sex, which influences cooling rates (For 96 review see (20)). An alternative approach is to cool individuals to a set decrease in baseline core 97 temperature (e.g. Δ -0.5°C) (1, 2, 18) to normalize cold strain. However, this approach can lead to 98 interindividual variability in cooling times, as recently we demonstrated that cooling core 99 temperature to ∆-0.8°C from baseline in cold air (0°C) ranged from 89-173 minutes across 100 participants (Wallace et al., Unpublished Data). The differences in cold exposure/cooling times 101 prior to exercise may introduce additional confounding variables related to cooling that may 102 influence performance. For example, cooling leads to an increase in shivering, which increases 103 metabolic heat production to offset heat loss, thus leading to more energy expended prior to and 104 during exercise. Critically, changes in core temperature are determined by the cumulative 105 imbalance between metabolic heat production and net heat loss (i.e., body heat storage), body 106 mass (i.e., internal heat sink) and body composition (i.e., specific heat capacity of body tissues) (21) . In cold environments, partitional calorimetry is used to calculate the rate of heat storage $(S, 107)$ 108 where positive values indicates heat gain, negative values indicate heat loss), and can be used to 109 estimate heat debt (HD), which represents the cumulative change in whole- body heat content 110 and provides an indication of cold strain (22–24). The use of HD as a physiological measure has 111 primarily been used to measure the thermoregulatory response to cold air following repeated 112 cold water immersion (24, 25) or high intensity interval training (26). This tool can be used to 113 provide an index of cold strain between participants and between different levels of core cooling 114 and can encapsulate confounds of different cooling times based on body surface area, heat

130 **Methods**

131 **Participants -** The experimental protocol was cleared by the Research Ethics Board at 132 Brock University (REB# 19-026) and conformed to the latest revision of the Declaration of 133 Helsinki. Ten healthy male volunteers (See Table 1 for characteristics), who were free from 134 cardiovascular, respiratory, neurological, and cold disorders were recruited from the university 135 and community population. All participants were informed of the experimental protocol and 136 associated risks before participating in this experiment and provided both verbal and written

137 informed consent. Participants were allowed to withdraw their participation at any point and their 138 data up until data collection was completed as data was de-identified.

139 **Experimental Design –** The experiment was a randomized, crossover, control trial consisting of 140 two familiarization sessions and 4 experimental sessions. The first familiarization session 141 involved collecting anthropometric measures, determining peak oxygen consumption, peak 142 power output, and practicing the TTE. The second familiarization provided two further practices 143 of the TTE. The 4 experimental conditions were separated by 3-7 days to minimize the potential 144 of cold acclimation and performed at the same time of day to control for circadian fluctuations in 145 core temperature. Participants were instructed to avoid vigorous exercise and alcohol

146 consumption 24 hours and caffeine 6 hours prior to each session.

147 **[Insert Table 1 About Here]**

148 **Familiarization Trials** – Upon arrival for the 1st familiarization trial, anthropometric 149 measurements of height (cm), mass (kg), body surface area (m^2) , and % body fat from 7-site 150 skinfold were obtained. An incremental test to exhaustion was performed on a cycle ergometer 151 (Velotron, RacerMate Inc, USA) to determine peak oxygen consumption and peak power output 152 (PPO). The test began with a standardized 5-min warm-up at 100 W, followed by workload 153 increase of 25 W each minute until exhaustion. Peak oxygen consumption $(\dot{V}O_{2\text{ peak}})$ was 154 defined as the highest continuous 30-s value measured breath-by-breath from expired gases 155 collected through a soft silicone facemask connected to an inline gas collection system (see 156 details below). The final stage completed was considered PPO (W). Following warm down and 157 ~30-min passive recovery, participants then performed a TTE consisting of a standardized 5-min 158 warm up at 100 W followed by the TTE at 70% of PPO (see details below). Upon arrival for the

 $2nd$ familiarization trial, participants practiced the TTE a total of two times, separated by 25-30 160 minutes.

181 until the participants' rectal temperature (Tre) dropped by ∆-0.3°C from baseline. This design 182 was implemented in order to target a T_{re} decrease of Δ-0.5°C at the start of the TTE with the 183 additional time for transfer to the ergometer along with postural shifts.

184 **HYPO-1.0°C** – Participants remained seated in the environmental chamber as ambient

185 temperature was incrementally decreased to 0°C and wind speed was increased to 0.8-1.2 m/s

186 until the participants Tre dropped by ∆-0.8°C from baseline before transferring to the ergometer

187 and performing the TTE. This design was implemented in order to target a T_{re} decrease of Δ -188 1.0°C for the TTE.

189 For all cold trials, there was an institutional ethical cutoff of core temperature ≤ 35.0 °C and an 190 exposure limit of 150-min following chamber air temperature reaching 0° C in cold trials. Three 191 participants (30%) did not reach the desired ∆-0.8°C Tre within the 150 minutes cutoff limit. 192 Each of these participants started the transition following the cutoff time with a Δ -0.7°C T_{re}. The 193 three cold trials performed the TTE in 0° C air and ~ 0.5 m/s wind speed. Due to the overall 194 challenge of core cooling in cold air, we were unable to time-match environmental exposure for 195 all 4 conditions.

196 **Time to Exhaustion –** The TTE started with a standardized 'warmup' of 5-min at 100 W 197 followed by the TTE at 70% of PPO. Participants freely choose their cadence, and the test was 198 performed to volitional fatigue or when cadence dropped below 60 rpm for 5 consecutive 199 seconds. No feedback or verbal motivation was provided except for one verbal warning if 200 cadence dropped below 60 rpm. Due to differences in completion times between participants and 201 trials, comparison of physiological responses were averaged over 30-s at isolated percentages 202 (ISO) of 0%, 25%, 50%, 75%, and 100% of each completed TTE. Therefore, the ISO-timepoints 203 compared are different based on the TTE in each condition and are different between trials.

204 **Clothing –** During TN trials, participants wore a cotton t-shirt or cycling jersey, cycling bib 205 shorts, socks, athletic/ cycling shoes, and metabolic mask $($ \sim 0.26 clo ensemble). In all cold 206 trials, participants wore the same ensemble as TN at baseline with the inclusion of track pants 207 (~0.48 clo ensemble). Upon commencement of cooling the chamber, participants were fitted 208 with earmuffs, winter gloves, and a fleece blanket around their shoes (~0.63 clo ensemble). Prior 209 to the TTE, the blanket was removed $(\sim 0.57 \text{ clo ensemble})$. The additional clothing during the 210 cold trials was deemed necessary during pilot testing to offset extreme discomfort of extremities 211 during cooling and minimize the risk of participant dropout.

212 **Perceptual Measurements –** Prior to performing the TTE, motivation was taken using a 0-4 213 scale (27), as well as shivering intensity measured by the experimenter on a 0-4 scale ($0 = no$) 214 shivering, $1 = \text{occasional mild termor of the jaw and neck}$, $2 = \text{intense terms of the chest}$, $3 = \text{interference of the chest}$ 215 intermittent vigorous generalized tremor, continuous violent muscle activity)**.** Subjective 216 assessments of the environmental conditions were assessed using a 1-4 scale to measure thermal 217 comfort and a 1-7 scale for thermal sensation (28), and ratings of perceived exertion (6-20) (29) 218 at ISO0% and ISO100%.

219 **Physiological Measurements –** Prior to baseline, participants self-instrumented with a flexible 220 thermocouple (RET-1, Physitemp Instruments, USA) 15 cm beyond the anal sphincter to 221 measure T_{re} (°C) sampled at 4 Hz. Weighted mean skin temperature ($\overline{T}_{\text{skin}}$, °C) and mean heat 222 flux (HF, $W \cdot m^{-2}$) were measured using heat flux sensors with an integrated thermistor (Concept 223 Engineering, Old Saybrook, USA) sampled on seven sites (30):

 $\overline{T}_{\text{skin}}$ and HF = 0.07_{forehead} + 0.14_{forearm} + 0.05_{hand} + 0.35_{abdomen} + 0.19_{thigh} + 0.13_{shin} $+0.07_{foot}$

224 Water vapor pressure of the skin was measured using a temperature and humidity sensor 225 (HMP60-L, Vaisala, FN) sampled at four sites: upper arm, chest, thigh, and calf. Heart rate was 226 calculated using R-R intervals using a standard three-lead electrocardiogram (MLA2340, AD 227 Instruments; USA). Participants were fitted with a soft silicone facemask (Hans Rudolph, USA) 228 connected to a 4.7 L gas mixing chamber where gas volume was measured using a pneumotach 229 (MTL 1000L, AD Instruments; USA; Pneumotach Amplifier Series 1110, Hans Rudolph Inc., 230 USA) and gas concentrations with a gas analyzer (ML206 Gas Analyzer, AD Instruments, USA). 231 Measures of expired ventilation (\dot{V}_E , L·min⁻¹), oxygen consumption ($\dot{V}O_2$, L·min⁻¹), carbon 232 dioxide expiration ($\dot{V}CO_2$, L·min⁻¹), and respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$) were used 233 to calculate metabolic heat production and heat loss from the respiratory tract. In order to index 234 workload, VO_2 was normalized to body mass (mL·kg·min^{-1}) during the TTE. Calculations were 235 adjusted based on barometric pressure (mmHg) and mixing chamber air temperature $(^{\circ}C,$ 236 sampled at 1 kHz) to account for changes in body temperature influencing gas volumes through 237 changes in expired air temperature. The metabolic cart was calibrated following the 238 manufacturer's instruction using air tanks containing 16% oxygen and 5% carbon dioxide. 239 **Partitional Calorimetry Calculations –** Heat storage using partitional calorimetry was 240 calculated each minute during the thermoneutral and cooling periods prior to the TTE and

241 normalized to body surface area using the following equation (31):

$$
\dot{S} = \dot{M} - \dot{W}_{K} \pm \dot{R} \pm \dot{C}_{skin} \pm \dot{K} - \dot{E}_{skin} - (\dot{E}_{resp} + \dot{C}_{resp}) [W \cdot m^{-2}]
$$

242 Where: \dot{S} = heat storage, \dot{M} = metabolic heat production, \dot{W}_{K} = energy used for work, \dot{R} = 243 Radiation, \dot{C}_{skin} = convection of skin, \dot{K} = conduction, \dot{E}_{skin} = evaporation from skin, \dot{E}_{resp} = 244 evaporation from respiratory tract, and \dot{C}_{resp} = convection from respiratory tract. \dot{W}_{K} is

245 considered 0 in this study as participants were at rest. \dot{K} is assumed to be at 0 in this experiment. 246 Combined $\dot{R} \pm \dot{C}_{skin}$ was determined through weighted HF. One-minute averages of each 247 component were taken from baseline and over the course of the environmental condition prior to 248 performing the TTE.

249 **Metabolic Heat Production –** Heat production was calculated using indirect calorimetry of 250 expired gases using the following equation if RER was < 1.00 (31)**:**

$$
\dot{M} = \left(\dot{V} O_2 \cdot \frac{\left[\left(\left(\frac{\text{RER} - 0.7}{0.3} \right) \cdot 21.13 \right) + \left(\left(\frac{1.0 - \text{RER}}{0.3} \right) \cdot 19.62 \right) \right]}{60} \times 1000 \right) / A_D \left[W \cdot m^{-2} \right]
$$

251 Where, $\dot{V}O_2$ is in L·min⁻¹, RER is the respiratory exchange ratio, and is normalized to A_D is body 252 surface area calculated using the following equation:

$$
A_D = 0.202 \times (Height)^{0.425} \times (mass)^{0.725} [m^2]
$$

253 Where, height is in m and mass is in kg.

254 Indirect calorimetry assumes that metabolic heat production is due to oxidative, rather than non-255 oxidative (anerobic) energy sources (31), however during passive cold exposure, RER has the 256 potential to ≥ 1 due to increased reliance on glycogen and carbohydrates to fuel shivering 257 thermogenesis (32) and/or through increased lactate production and hyperventilation leading to 258 increase carbon dioxide expired (33). If RER \geq 1, the following equation was used to account for 259 the energy equivalent for carbohydrates only (31)**:**

$$
\dot{M} \text{ (RER } \geq 1.0) = \left(\dot{V}O_2 \cdot \frac{21.13}{60} \times 1000 \right) / A_D \text{ [W} \cdot \text{m}^{-2}]
$$

260 Energy expenditure was calculated as Kcals expended from the start of baseline until the

261 commencement of the TTE by taking the integral of \dot{M} in W divided by 70 to convert to Kcals 262 (22).

263 **Evaporative heat loss from the skin surface –** The following equation was used to determine

 264 \dot{E}_{skin} from the relative humidity sensors and environmental factors (31, 34):

$$
\dot{E}_{skin} = h_e \cdot \omega \cdot (P_{skin} - P_a) [W \cdot m^{-2} \cdot {}^{\circ}C]
$$

265 Where, h_e = heat transfer coefficient for evaporative heat loss, ω = skin wittedness of participant, 266 assumed to be minimal at 0.06 due to no regulatory sweating, P_{skin} = saturated vapor pressure of 267 the skin, P_a = partial vapor pressure of the air.

268 The heat transfer coefficient for evaporative heat loss (h_e) is calculated by re-arranging the Lewis 269 relation equation:

$$
Lewis Relation = \frac{h_c}{h_e}
$$

270 Where, the Lewis relation is assumed to be $16.5 \text{ °C} \cdot \text{kpa}^{-1}$, h_c = convective heat transfer

271 coefficient, and h_e = heat transfer coefficient for evaporative heat loss. The convective heat

272 transfer coefficient was calculated with the following equation (31):

$$
h_c = 8.3 v^{0.6} [W \cdot m^2 \cdot K^{-1}]
$$

273 Where v is air velocity in m⋅s⁻¹. This equation is used for air velocities between 0.2-4.0 m⋅s⁻¹. 274 Wind speed was recorded using a handheld anemometer (Kestrel 1000, ITM Instruments, CAN) 275 for convective heat loss at the level of xyphoid process of the participants at baseline and every 276 15-min.

277 Saturated vapor pressure of the skin was calculated using Antoine's equation by using mean skin 278 temperature:

$$
P_{skin} = \frac{\exp\left(18.956 - \frac{4030.18}{\overline{T}_{skin} + 235}\right)}{10} [\text{kpa}]
$$

279 Where, $\overline{T}_{\text{skin}}$ = mean skin temperature (°C), division by 10 is to convert P_{skin} from mb to kPa. 280 The partial vapor pressure in the air (P_a) and saturated vapor pressure of water (P_{sa}) were 281 derived based on their relationship with relative humidity (∅, fractional %) using temperature 282 and humidity measurements from sensors with the following equations:

$$
P_a = \emptyset P_{sa} [kPa]
$$

283 Saturated vapor pressure at the skin was calculated for each site, then weighted using the

284 following equation which was originally derived for mean skin temperature (35):

Weighted Relative Humidity or $T_{amb \, skin} = 0.3_{arm} + 0.3_{chest} + 0.2_{thigh} + 0.2_{calf}$

285 **Respiratory Heat Loss –** Combined convective and evaporative heat loss from the respiratory 286 tract was the summation of the following equations (31)**:**

$$
\dot{C}_{resp} = \frac{\left(0.001516 \cdot \dot{M} \left(28.56 + \left(0.641 \cdot P_{a\,\text{air}}\right) - \left(0.885 \cdot T_{amb}\right)\right)\right)}{A_D} \left[W \cdot m^2\right]
$$
\n
$$
\dot{E}_{resp} = \frac{\left(0.00127 \cdot \dot{M} \left(59.34 + \left(0.53 \cdot P_{a\,\text{air}}\right) - \left(11.63 \cdot T_{amb}\right)\right)\right)}{A_D} \left[W \cdot m^2\right]
$$

287 Where M is in W, $P_{a \text{ air}}$ is the vapor pressure of inspired air in kPa, and T_{amb} is ambient 288 temperature of inspired air in $\rm{^{\circ}C}$. Ambient temperature (T_{amb,} $\rm{^{\circ}C}$) and relative humidity (%) were 289 measured using a hand-held hygrometer and thermometer (Pocket DewPoint, VWR, USA) for

290 respiratory heat loss at the level of xyphoid process of the participants at baseline and every 15- 291 min.

292 **Heat Debt -** The change in body heat content over time or HD was obtained by taking the 293 integral of heat storage and converting to kJ with the following equation (23, 31)**:**

$$
\Delta HD = \int_{t=0}^{t} \dot{S} * A_{D} * dt / 1000 \text{ [k]}
$$

294 Where, the rate of heat storage is converted to W by multiplying by A_D , then multiplied by 295 exposure time (dt) in seconds (s) and divided by 1000 to convert W to kJ. HD was calculated 296 every minute from when cooling the chamber started until prior to commencing the TTE. 297 **Statistical Analysis** – All physiological data are presented as mean ± SD with statistical 298 significance set a $p \le 0.05$. All data analyses of physiological variables were conducted in R 299 (version 4.2.2) using the RStudio environment (Version 2023.03.1.446) (36). Data were analyzed 300 using a linear mixed model (lmer) with a fixed effect for condition and timepoint (if necessary) 301 and random effect for participant using the R package l*me4* (37). Data were normally distributed 302 determined through visual inspection of Q-Q plots and using the Shapiro-Wilks test (in *car* 303 package) (38). Homoscedasticity was confirmed through visual inspection of the residuals 304 plotted over the fitted linear mixed model and using a Levine's test for homogeneity of variance 305 (in *car* package) (38). Three types of linear mixed models were performed (depending on 306 variable) including; a 1 x 4 condition (TN vs CS vs HYPO-0.5 °C HYPO-1.0 °C), or a 4 307 (condition) x 6 (timepoint; Baseline vs ISO0% vs ISO25% vs ISO50% vs ISO75% vs 308 ISO100%), or a 4 (condition) x 5 (timepoint; ISO0% vs ISO25% vs ISO50% vs ISO75% vs 309 ISO100%). A repeated measures ANOVA was performed on each linear mixed model, when

310 significant (*p* ≤ 0.05), a Bonferroni *post hoc* analysis corrected for multiple comparisons was 311 used to test for specific main effects between conditions and timepoints using the *emmeans* 312 package (39). If there was a significant interaction (*p* ≤ 0.05), a 1 x 4 condition Bonferroni *post* 313 *hoc* analysis performed at each specific timepoint to compare differences between conditions. 314 Cohen's *d* (40) was used to calculate effect sizes for TTE data between conditions where 315 descriptors of magnitude (41) are very small 0.01, small 0.2, medium 0.5, large 0.8, and very 316 large 1.2.

317 Perceptual data (RPE, TC, TS) were analyzed using 4 (condition) x 2 ISO-timepoint 318 (ISO0%, ISO100%) repeated measures ANOVAs. As data was not normally distributed and 319 ordinal data, post hoc comparisons between conditions were also performed using a Wilcoxon-320 Signed Rank test at ISO0% and ISO100%. Motivation and shivering intensity were assessed 321 using a 1 x 4 (condition) Friedman's ANOVA with a Wilcoxon-Signed Rank test for post-hoc 322 analysis to compare between conditions. To reduce the likelihood of Type 1 error due to multiple 323 comparisons, α value was revised based on number of comparisons (total 6), therefore $p \le 0.008$ 324 was set for significance. All perceptual analyses are expressed as median (quartile 1 – quartile 3) 325 and were performed using SPSS statistics for Windows.

326 **Results**

327 **Thermal Manipulations –** Cooling times prior to performing the TTE were as follows: CS (30.0 328 ± 1.1 min), HYPO-0.5°C (116.0 \pm 39.2 min) and HYPO-1.0°C (160.3 \pm 32.3 min). We were

- 329 successful at creating a CS group (neutral core, cooled skin/shell) and two mild hypothermia
- 330 groups (reduced T_{re} and cold skin) compared to TN. There was a significant condition,
- 331 timepoint, and interaction effect (all $p < 0.001$) for T_{re} (Figure 1A), relative ΔT_{re} (Figure, 1B) and
- 332 \bar{T}_{skin} (Figure 1C) where pairwise comparisons demonstrated no difference at Baseline for each

333 variable (all $p = 1.00$). For absolute T_{re} , at ISO0%, both TN and CS were significantly different

- 353 ($p = 0.055$). Heat storage was significantly (all $p \le 0.018$) reduced compared to TN (-23.4 \pm 12.9
- 354 W⋅m²) in all cooling conditions. Heat storage was significantly (both $p \le 0.001$) lower in CS (-
- 355 87.0 ± 13.6 W⋅m²) compared to HYPO-0.5°C (-54.0 ± 17.9 W⋅m²) and HYPO-1.0°C (-41.0 ±

372 **[Insert Figure 2 About Here]**

373 **Cardiorespiratory and Cadence Responses** – Data for heart rate is reduced to $n = 9$ due to 374 poor signal quality. There was a significant condition, timepoint, and interaction (all $p < 0.001$) 375 for heart rate (Figure 3A) and $\dot{V}O_2$ (Figure 3B). Pairwise comparisons demonstrated a non-376 uniform difference of responses between conditions, where significant differences (*p* < 0.05) are 377 displayed in Figure 3A and 3B. There was a significant timepoint effect (*p* < 0.001), but no

378 condition (*p* = 0.074) or interaction (*p* = 0.970) for cadence, where cadence declined over the 379 course of the TTE and was lower in ISO100% (all *p* < 0.05) compared to all other ISO 380 timepoints (Figure 3C).

381 **[Insert Figure 3 About Here]**

382 Perceptual Variables – There was a significant condition, and interaction (all $p < 0.05$) for RPE, 383 TS, and TC (Table 2). There was a significant iso-timepoint effect (both *p* < 0.05), where RPE 384 and TS increased over the course of the TTE. However, there was no condition effect for TC (*p* = 385 0.399). Post-hoc comparisons are displayed in Table 2. RPE was significantly higher at ISO0% 386 in HYPO-1.0°C compared to TN, with no differences at ISO100% between conditions. Thermal 387 sensation was lower in all cold conditions compared to TN at ISO0% (all *p* < 0.007), while TS 388 remained lower at ISO100% in both core cooling conditions compared to TN and CS (all $p <$ 389 0.007). Thermal comfort was higher (i.e., more uncomfortable) in both core cooling conditions 390 compared to TN (both $p = 0.004$) at ISO0%. Thermal comfort approached significance between 391 TN and CS $(p = 0.013)$ and CS and HYPO-0.05°C $(p = 0.020)$ at ISO0%, with no differences 392 between (all *p* > 0.007) at ISO100%. There was a significant condition effect (*p* < 0.001) for 393 shivering intensity where shivering was higher in the two core cooling conditions, with no 394 differences between TN and CS ($p = 0.062$, Table 2). There was a significant condition effect (p $395 \leq 0.001$) for motivation to perform TTE, however post-hoc comparisons determined there were 396 no difference between conditions (all $p \ge 0.011$) (Table 2).

397 **[Insert Table 2 About Here]**

Endurance Capacity - There was a significant condition effect ($p \le 0.001$, partial eta² = 0.66) 399 for TTE (Figure 4A) where endurance capacity decreased from TN (23.75 \pm 13.75 min) in

411 **[Insert Figure 4 About Here]**

412 **Discussion**

413 In real-life scenarios such as acute exposure or survival situations in the cold, the first 414 experience faced by an individual is a reduction in skin temperature, occurring well before 415 significant changes to core temperature. If cold exposure continues, eventually core temperature 416 drops along with further skin cooling. Therefore, we aimed to determine if there was a dose-417 response of cold exposure on endurance capacity in cold $(0^{\circ}C)$ air; this was done by separating 418 and isolating the effects of a cold outer shell - without changes in core temperature - compared to 419 two levels of core cooling. Our first hypothesis was accepted as cooling just the shell by itself 420 without any core cooling was sufficient to increase physiological strain and caused a medium to 421 large reduction in endurance capacity by \sim 30% compared to thermoneutral. Our second 422 hypothesis was accepted as mild cooling of the core led to a very large impairment in capacity

423 with a further \sim 30-40% reduction compared to skin cooling alone. Our third hypothesis was 424 rejected as there were no differences between the two core cooling conditions. While we 425 attempted to have two distinct doses of core cooling, the drop in core temperature and actual heat 426 debt incurred were similar, and this may have contributed to the similar endurance capacity. 427 Consensus for whether cold air by itself impairs exercise capacity is equivocal (20), as most 428 studies initiate exercise directly upon cold exposure. Thus, actual skin cooling and heat debt is 429 minimized and offset by the large and immediate endogenous metabolic heat production from 430 exercise. In the CS condition of the current study, participants were exposed to cold air for \sim 30 431 minutes before initiating the TTE, inducing redistribution of peripheral blood to core, as well as 432 significant reductions in $\overline{T}_{\text{skin}}$ (~-7.6°C), and likely superficial muscle temperature (Quad skin 433 temperature of 23.1 \pm 2.1 °C in CS versus 28.9 \pm 1.1 °C in TN at ISO0%). Even though core 434 temperature did not significantly decrease, heat debt decreased \sim 200 kJ more than thermoneutral, 435 suggesting that cooling did occur. Furthermore, the rate of heat storage (S) was the lowest of all 436 three cooling conditions, as there was a large decrease in $\overline{T}_{\text{skin}}$ with initial cold exposure (due to 437 vasoconstriction and $\dot{R} \pm \dot{C}_{\rm skin}$ heat loss) compared to a relatively minor increase in shivering 438 thermogenesis (M) (42). Overall, there was a significant reduction in TTE time by \sim 31% 439 indicating CS alone and exercising in inadequate clothing can limit overall endurance capacity in 440 cold air. Impairment was not uniform, with a wide range of responses from -64% for one 441 participant to another improving performance by $+6\%$, however, these two individuals were $2/3$ 442 lowest TTEs in TN, which may explain their variability in this condition. There was likely strong 443 vasoconstriction with our average $\overline{T}_{\text{skin}}$ of ~25.2°C at ISO0%, as maximal vasoconstriction 444 occurs at $\overline{T}_{\text{skin}}$ of ~29.5-30°C (32). This likely impaired performance through decreased blood 445 flow to working muscles and superficial muscle cooling. For example, it has previously been

446 reported that 15-min of 12°C cold-water leg immersion decreased maximal power (13.7%) and 447 average power (9.5%) during a 30-s cycling sprint in thermoneutral conditions (43). Our data 448 thus highlight the importance of preventing shell cooling, supported by observations that wearing 449 a heated vest for 25-min of rest in cold air $(8^{\circ}C)$ prevented core and skin temperature decreases 450 compared to wearing a tracksuit, eliciting $a \sim 1.1\%$ improvement in subsequent rowing time trial 451 performance (17). Together, these results indicate that skin/shell cooling by itself combined with 452 inadequate clothing can impair endurance capacity in cold air, though the magnitude of this 453 response may vary widely across individuals.

454 With continued cold exposure, core cooling itself can occur and may further negatively 455 impact exercise capacity. In the current study, core cooling led to a large impairment in 456 endurance capacity beyond just cooling the shell alone. The overall absolute magnitude of core 457 temperature cooling was relatively small (ranged 35.6°C to 36.9°C at ISO0% in HYPO-1.0°C) 458 which is above clinical hypothermia (\leq 35°C core temperature) and in some individuals within a 459 normothermic range. However, it is clear that individuals were cold strained as relative to TN 460 and CS, both core cooling conditions induced significant reductions in $\overline{T}_{\text{skin}}$, relative T_{re} , and 461 thermal discomfort, increased shivering, along with prolonged negative heat storage and greater 462 heat debt prior to exercise. Pre-exercise shivering – measured as average M in the partitional 463 calorimetry calculations – was about two-fold greater in both core cooling conditions than in TN 464 or CS. At the end of the cooling periods, the relative intensity of \dot{M} was ~19% and 21% of peak 465 oxygen consumption in HYPO-0.5°C and HYPO-1.0°C, respectively, compared to ~10% in TN. 466 Thus, one potential mechanism for impairment may be reduced motor coordination or altered 467 motor unit recruitment strategies within musculature from the asynchronous shivering 468 contractions. Shivering primarily occurs in trunk and thigh muscles where continuous low

469 intensity shivering $\langle \sim 2-5\%$ maximal voluntary contraction) recruit primarily Type I muscle 470 fibers, while high intensity bursts $\left(\frac{27-15}{6}\right)$ of maximal voluntary contraction) recruit Type II 471 muscle fibers (For review see: (44)) and were very likely similar muscle types required for our 472 submaximal test workload. Further, local cooling of muscles decreased maximal voluntary force 473 while altering motor unit contractile characteristics and recruitment patterns (7). Collectively, the 474 pre-exercise shivering may have impaired endurance capacity through a direct influence on 475 muscle capacity. However, future studies are needed measuring muscle temperature, muscle 476 activity/recruitment (e.g., using electromyography), or biomechanical analyses of the pedal 477 stroke to fully elucidate this mechanism. Beyond colder muscles alone or changes in motor 478 coordination from shivering, another mechanism of impairment may be a competition between 479 metabolic demands of exercise itself versus that from shivering. Comparing pre-cooling to a 480 sustained 40% of peak shivering versus no pre-cooling, Gagnon et al. (3) reported a reduction in 481 treadmill speed in order to maintain a constant metabolic demand of either light or moderate 482 exercise intensities of 50 or 70% peak oxygen uptake, respectively. In both Gagnon et al. (3) and 483 the current study, endogenous heat production from exercise appears insufficient to compensate 484 for the large heat debt, and shivering throughout subsequent exercise likely contributed to further 485 decreases in exercise capacity in both core cooling conditions compared to the cold shell 486 condition. Lastly, another potential mechanism for impaired performance with core cooling is 487 decreased oxygen availability caused by a reduction in muscle blood flow and oxygen diffusion 488 due to a leftward shift of the oxygen disassociation curve $(1, 2, 14)$. We have previously 489 determined that the use of hyperoxia (40% oxygen) can counter declines in 15 km cycling time-490 trial performance in cold air with a 0.5°C reduction in core temperature. However, it is currently

491 unknown if manipulation of oxygen availability can also influence endurance capacity in the 492 cold (1).

493 Our previous study reported an approximate 6% reduction in average wattage in 15 km 494 cycling time trial performance with a 0.5°C decrease in core temperature (1), and we aimed to 495 extend this range with a dose response of core cooling. Across a range of core cooling, Bergh 496 and Ekblom (15) reported a $20\% \,^{\circ}C^{-1}$ linear reduction in maximal work time below a threshold 497 esophageal and muscle temperature of 37.5°C and 38°C, respectively through to absolute core 498 temperature reductions to \sim 35°C. We extend their findings by demonstrating an average 499 reduction of TTE by 72% with a 1°C decrease in core temperature. However, we did not find a 500 similar linear decrease with core temperature. Despite our pre-experimental target of a 0.5° C T_{re} 501 difference between the two core cooling conditions, there was no statistical significance in HD at 502 the end of cooling, nor in core temperature or skin temperature at ISO0%. The lack of HD 503 differences may be due to continued core cooling increasing shivering drive, as M progressively 504 increases and is near maximal at a core temperature of ~35 \degree C (45), while reductions in $\overline{T}_{\text{skin}}$ 505 decreases the thermal gradient between the skin and environment reducing convective heat loss 506 during prolonged cooling (34). Though these individual partitional calorimetry components 507 averaged over the cooling period were non-significant in our calculations, they may still have 508 been sufficient to moderate any heat storage differences and slow down the further accumulation 509 of HD between the two core cooling conditions in the later portion of the cooling period. The use 510 of partitional calorimetry was advantageous in the study beyond thermometry measures to index 511 cold strain, as HD and TTE impairment was similar between the core cooling conditions, 512 indicating that the amount of cold accumulated prior to exercise influenced exercise capacity as 513 opposed to cooling time per say (38% longer in HYPO-1.0°C compared to HYPO-0.5°C). More

514 research is needed on testing partitional calorimetry tools and calculations as a majority of 515 research occurs in hot environments (31) to provide a better understanding of heat balance in the 516 cold.

517 There are several considerations and limitations in the current study limiting the 518 understanding of cold on performance. Peak power output was determined with an incremental 519 step protocol as opposed to an incremental ramp protocol where peak power output may have 520 been underestimated (46). Furthermore, we did not calculate critical power or functional 521 threshold power for our participants. This information would be valuable in determining which 522 intensity threshold individuals were cycling in and could aid in explaining the wide inter-523 individual variability in TTE. Future work is needed to determine how endurance capacity at 524 different exercise intensity is affected by cold stress, where potential lower intensity exercise is 525 less affected and may better increase core temperature following cooling (47). Data collection 526 was performed over the winter and spring months (November to May), where potential cold 527 acclimation may have influenced cooling responses, however, based on participants' activity 528 history is unlikely that they were cold acclimated. Any potential cold acclimation may not 529 significantly impacted exercise performance, as recently Jones et al. (10) found that cold 530 acclimation following 7 days of cold-water immersion (controlled by time and change in core 531 temperature) did not mitigate the decrements in 20-min self-paced time-trial performance in 532 thermoneutral conditions induced by a reduction in core temperature by ~∆-1.5°C. We 533 demonstrated an average T_{re} afterdrop of \triangle -0.2-0.3°C during the TTEs in the core cooling 534 conditions likely caused from the skeletal muscle pump moving cooler blood from the periphery 535 to the core and warmer blood from the core towards the working muscle (20, 34). However, 536 there is potential that the afterdrop was underestimated, where esophogeal temperature would be 537 more accurate representation of organ temperature and can better respond to changes in core 538 temperature compared to rectal temperature (48). The cardiovascular fluid shift is challenging to 539 model (34) and we cannot account for if this shift caused an independent effect on TTE 540 performance (e.g., through systemic vasoconstriction, decreased brain temperature). 541 Furthermore, no blood measures were collected in this study, and it is unknown how plasma 542 glucose and lactate levels changed in response to the cooling protocols that may have influenced 543 TTE performance. Lastly, this study is limited to males as no females were used in the current 544 study to control against fluctuations in resting core temperature during the menstrual cycle. On 545 average, females have a lower body mass, height, body surface area, and greater body fat 546 percentage compared to males and have a higher core temperature during the luteal phase that 547 may influence cutaneous vasoconstriction, shivering and non-shivering thermogenesis (49) 548 leading to potential sex-related differences in cooling times. However, based on the current 549 study, regardless of cooling time or starting core temperature, core cooling impaired endurance 550 capacity, potentially indicating that these sex-related differences may not influence endurance 551 impairment. However, future research is needed to determine sex-related differences and if the 552 menstrual cycle influences whole-body cooling and endurance capacity in the cold.

553 In summary, we determined a dose-response for cooling and endurance capacity where 554 cooling of the shell reduced mean endurance capacity by \sim 30% compared to thermoneutral, and 555 core cooling further reduced capacity by and additional \sim 30-40%. From an applied perspective, 556 these data give insight into the magnitude of impairment from cold that may be useful for 557 modeling work capacity or survival and indicate that individuals should prevent declines in shell 558 and/or core temperature prior to performing sustained work or exercise in the cold. Furthermore, 559 impairments in endurance capacity occur with relatively mild core cooling, well before

560 individuals reach clinical hypothermia (core temperature $\leq 35^{\circ}$ C). Future research is needed to

561 investigate the high inter-individual variability in both cooling response and exercise tolerance.

562 The current results improve our understanding of exercise responses in the cold and may help

563 develop effective countermeasures to improve exercise and capacity in the cold.

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572 **Conflict of Interest**

- 573 The authors declare that the research was conducted in the absence of any commercial or
- 574 financial relationships that could be construed as a potential conflict of interest.

575 **Author Contributions**

- 576 All authors contributed to the conception and design of the research study; PJW, JGN, JL, and
- 577 NS piloted and performed the experiments. PJW and GLH performed the statistical analysis. All
- 578 authors interpreted the results of the study. PJW and SSC drafted the manuscript. All authors
- 579 edited, revised, and approved the final version of the manuscript.

580 **List of Figures**

- 581 **Figure 1** Thermoregulatory responses for absolute rectal temperature (Panel A), delta rectal
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- 584 variable was analyzed using a 6 Time X 4 Condition linear mixed model repeated measures
- 585 ANOVA. All variables demonstrated a significant interaction where pairwise comparisons can
- 586 be interpreted as $a =$ difference between TN and CS, $b =$ difference between TN and HYPO-
- 587 0.5°C, c = difference between TN and HYPO-1.0°C, $d =$ difference between CS and HYPO-
- 588 0.5°C, e = difference between CS and HYPO-1.0°C, $f =$ difference between HYPO-0.5°C and
- 589 HYPO-1.0°C. Legend: $TN =$ thermoneutral, $CS =$ Cold Skin/Shell, HYPO-0.5°C = mild core 590 cooling (hypothermia) of ∆-0.5°C from baseline, HYPO-1.0°C = mild core cooling
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- 591 (hypothermia) of ∆-1.0°C from baseline.
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- 597 mean \pm SD, while individual values are plotted with a unique symbol for each participant
- 598 consistent between figures. Each variable was analyzed using a 1 X 4 Condition linear mixed
- 599 model repeated measures ANOVA. There was a significant condition effect, where pairwise
- 600 comparisons can be interpreted as: $TN =$ different from TN, $CS =$ different from CS, HYPO-601 0.5°C = different from HYPO-0.5°C and HYPO-1.0°C = HYPO-1.0°C. Legend: TN =
- 602 thermoneutral, CS = Cold Skin/Shell, HYPO-0.5°C = mild core cooling (hypothermia) of Δ -
- 603 0.5°C from baseline, HYPO-1.0°C = mild core cooling (hypothermia) of ∆-1.0°C from baseline.
- 604 **Figure 3** Cardiorespiratory responses for heart rate (Panel A, *n* = 9 males), oxygen
- 605 consumption (Panel B, $n = 10$ males) and cadence (Panel C, $n = 10$ males). All data presented as
- 606 mean \pm SD. Each variable was tested with a 5 Time X 4 Condition linear mixed model repeated
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- 609 difference between TN and HYPO-1.0°C, $d =$ difference between CS and HYPO-0.5°C, $e =$
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- 611 1.0 $^{\circ}$ C. If a significant time effect occurred, pairwise comparisons can be interpreted as: 1 =
- 612 different from ISO0%, 2 = different from ISO25%, 3 = different from ISO50%, 4 = different
- 613 from ISO75%, and 5 different from ISO100%. Legend: TN = thermoneutral, CS = Cold
- 614 Skin/Shell, HYPO-0.5°C = mild core cooling (hypothermia) of ∆-0.5°C from baseline, HYPO-
- 615 1.0°C = mild core cooling (hypothermia) of Δ -1.0°C from baseline.
- 616 **Figure 4** Time to exhaustion (Panel A) and % change in TTE (Panel B) over the 4
- 617 experimental conditions (both $n = 10$ males). The bar data are presented as mean \pm SD, while
- 618 individual values are plotted with a unique symbol for each participant consistent between
- 619 figures. Each variable was analyzed using a 1 X 4 Condition linear mixed model repeated
- 620 measures ANOVA. There was a significant condition effect for both variables, where pairwise
- 621 comparisons can be interpreted as: $TN =$ different from TN, $CS =$ different from CS, HYPO-
- 622 0.5°C = different from HYPO-0.5°C and HYPO-1.0°C = HYPO-1.0°C. Legend: TN =
- 623 thermoneutral, CS = Cold Skin/Shell, HYPO-0.5°C = mild core cooling (hypothermia) of Δ -
- 624 0.5°C from baseline, HYPO-1.0°C = mild core cooling (hypothermia) of ∆-1.0°C from baseline.

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Experimental Conditions

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Table 1- Participant characteristics presented as mean ± SD.

Table 2 – Perceptual responses collected during the TTE at ISO0% and ISO100% presented as median (Quartile 1 – Quartile 3) for the four experimental conditions (all $n = 10$ males). TN – Thermoneutral, CS = Cold Skin/Shell, HYPO-0.5°C = mild hypothermia of ∆-0.5°C from Baseline, HYPO-1.0°C = mild hypothermia of Δ -1.0°C from Baseline. * indicates a significant condition effect ($p < 0.05$) using a 2 X 2 repeated measures ANOVA or 1 X 4 repeated measures ANOVA for motivation. Post-hoc comparisons using Wilcoxon signed rank tests are -at can be interpreted as: a significantly different ($p < 0.008$) from TN, b significantly different from CS, c significantly different from HYPO-0.5°C, ^d significantly different from HYPO-1.0°C.

Dose-Dependent Impact of Skin/ Core Cooling on Endurance Capacity $\sqrt{6}$ $25₁$ XXX tio $-25-$ ∇⊡ $-50 \blacktriangle \triangledown$ Tim Δ $-75 \circ \bullet \bullet^{\diamond}$ $\overline{\square}$ щ. $\begin{array}{c}\n\mathbf{g} - 100 \\
\mathbf{g} - 1\n\end{array}$ \mathbf{CS} TN $\overline{\mathbf{C}}$ -125 TPOM-S POINSC

Skin cooling by itself impaired exercise capacity ~30%; core cooling impaired another ~30-40%

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