

Perception of carbohydrate availability augments highintensity intermittent exercise capacity under sleep-low train low conditions

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44 Abstract

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We tested the hypothesis that perception of carbohydrate (CHO) availability augments 46 exercise capacity in conditions of reduced CHO availability. Nine males completed a sleep-47 48 low train-model comprising evening glycogen depleting cycling followed by an exhaustive 49 cycling protocol the next morning in the fasted state (30 minutes steady-state, SS, at 95% 50 lactate threshold followed by 1-min intervals at 80% peak power output until exhaustion). 51 After the evening depletion protocol and prior to sleeping, subjects consumed 1) a known 52 CHO intake of 6 g.kg⁻¹ body mass (TRAIN HIGH), 2) a perceived comparable CHO intake but 0 g.kg⁻¹ body mass (PERCEPTION) or a known train-low condition of 0 g.kg⁻¹ body 53 54 mass (TRAIN LOW). The TRAIN HIGH and PERCEPTION trials were conducted double 55 blind. During SS, average blood glucose and CHO oxidation were significantly higher in 56 TRAIN HIGH $(4.01 \pm 0.56 \text{ mmol}.\text{L}^{-1}; 2.17 \pm 0.70 \text{ g.min}^{-1})$ versus both PERCEPTION (3.30) + 0.57 mmol.L⁻¹; 1.69 + 0.64 g.min⁻¹, P < 0.05) and TRAIN LOW (3.41 + 0.74 mmol.L⁻¹; 1.61 57 58 + 0.59 g.min⁻¹, P < 0.05). Exercise capacity was significantly different between all pairwise 59 comparisons (P<0.05) where TRAIN LOW (8 + 8 min) < PERCEPTION (12 + 6 min) < 12 + 6 minTRAIN HIGH (22 + 9 min). Data demonstrate that perception of CHO availability augments 60 61 high-intensity intermittent exercise capacity under sleep-low, train-low conditions though perception does not restore exercise capacity to that of CHO consumption. Such data have 62 63 methodological implications for future research designs and may also have practical 64 applications for athletes who deliberately practice elements of training in CHO restricted 65 states.

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Keywords: placebo, carbohydrate, train-low, capacity

68 Introduction

69 In addition to its well-documented role as an energy source, it is now recognised that the 70 glycogen granule exerts regulatory roles in modulating skeletal muscle cell signalling and 71 transcriptional responses to acute exercise sessions (Bartlett et al., 2015; Hearris et al., 2018). 72 Accordingly, deliberately commencing and/or recovering from training sessions with reduced 73 CHO availability (the so-called train-low paradigm) increases markers of mitochondrial 74 biogenesis (Hansen et al., 2005; Yeo et al., 2008; Morton et al., 2009) and both whole body 75 and intramuscular lipid oxidation (Yeo et al., 2008; Hulston et al., 2010). In some instances, 76 both exercise capacity (Hansen et al., 2005) and exercise performance (Cochran et al., 2015; 77 Marquet et al., 2016a,b) have also been augmented with short-term (i.e. 3-10 weeks) train-78 low approaches though it is acknowledged that this is not a consistent finding amongst 79 chronic training studies. On this basis, it has therefore been suggested that CHO should be 80 adjusted day-by-day and meal-by-meal in accordance with the goals of both maximising 81 training quality (i.e. ability to sustain the desired workload) and skeletal muscle adaptations 82 (Impey et al., 2018).

Whilst there are multiple research designs used to practically achieve train-low 83 84 conditions (i.e. twice per day training protocols, fasted training and or withholding CHO in the recovery period from acute exercise), the 'sleep-low, train-low' model has emerged as a 85 86 particularly potent strategy for which to prolong the period of CHO restriction (Bartlett et al., 87 2013; Lane et al., 2015). In this approach, participants perform an evening training session, 88 restrict CHO during overnight recovery, and then complete a fasted training session on the 89 following morning. The accumulative time with reduced muscle glycogen could therefore 90 extend to 12–14 h depending on the timing and duration of the training sessions and sleep 91 period. When performed chronically, Marquet et al. (2016a,b) observed that 1-3 weeks of 92 sleep-low training in elite triathletes and cyclists improves cycling efficiency (3.1%), 20 km

93 cycling time-trial performance (3.2%) and 10 km running performance (2.9%) compared with
94 traditional train-high approaches.

95 Despite the aforementioned findings, an obvious limitation of the sleep-low, train-low 96 model is that exercise capacity is likely to be significantly impaired during the morning 97 training session. Indeed, we recently observed that stepwise reductions in pre-exercise muscle glycogen concentration ~100 mmol.kg⁻¹ dry wt (as achieved by the sleep low model) 98 impaired morning exercise capacity at 80% peak power output (PPO) by ~20 to 50% (Hearris 99 100 et al., 2019). Nonetheless, we acknowledged that lack of blinding between conditions 101 (subjects were aware of CHO availability given that whole foods were consumed) may have 102 influenced subjects' perception of their ability of complete high-intensity workloads. Indeed, 103 placebo effects of CHO availability have been reported in conditions of CHO feeding before 104 (Mears et al., 2018) and during exercise (Clark et al., 2000). To the authors' knowledge, 105 however, the potential placebo effect of CHO availability has not yet been examined under 106 conditions where exercise is commenced with sub-optimal muscle glycogen concentration.

With this in mind, the aim of the present study was to test the hypothesis that 107 108 perception of CHO availability augments exercise capacity. To this end, we adopted a sleep-109 low, train-low model of CHO restriction where recreationally active males commenced an 110 exhaustive morning training session under conditions corresponding to a known prior CHO intake of 6 g.kg⁻¹ body mass (TRAIN-HIGH), a perceived comparable CHO intake 111 112 (PERCEPTION) or a known train-low condition during which no CHO was consumed prior 113 to sleeping (TRAIN-LOW). We specifically hypothesised that perception of CHO availability 114 would improve morning exercise capacity compared to known train-low conditions but that 115 perception would not restore exercise capacity to that of true train-high conditions.

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119 Methods

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121 Subjects. Nine recreationally active males who regularly engaged in exercise training 122 (running, cycling, and intermittent sport) between 3-6 times per week volunteered to 123 participate in the study (mean \pm SD: age, 25 ± 8 years; body mass, 71.6 ± 8.5 kg; height, 1.78 \pm 0.06 m; VO_{2peak}, 55.3 \pm 8.3 ml.kg⁻¹.min⁻¹; peak power output (PPO) 331 \pm 41 watts). All 124 subjects gave written and informed consent after details of the study procedures were 125 126 explained. No subject had a history of smoking, cardiovascular, or metabolically related 127 disease and none were under pharmacological treatment during the study. All subjects 128 refrained from strenuous exercise and alcohol for at least 24 h before each trial. The study 129 was approved by the Ethics Committee of Liverpool John Moores University.

130 Experimental Design. In a randomized, repeated measures design (and after appropriate baseline testing and familiarization), subjects performed three experimental trials consisting 131 132 of a glycogen depleting protocol in the afternoon prior to the main experimental trial the 133 subsequent morning. At the cessation of the glycogen depleting protocol, subjects consumed 1) a known CHO intake of 6 g.kg⁻¹ body mass (TRAIN-HIGH), 2) a perceived comparable 134 CHO intake but 0 g.kg⁻¹ body mass (PERCEPTION) or a known train-low condition of 0 135 136 g.kg⁻¹ body mass (TRAIN-LOW). The TRAIN HIGH and PERCEPTION trials were double 137 blind where blinding of these two solutions were performed by the corresponding author who 138 was not present for any of the exhaustive exercise sessions on Day 2 (with the exception of 139 the familiarisation trials). The following morning subjects arrived at the laboratory in a 140 fasted state where they then performed a steady-state (SS) (30 min at 95% of lactate 141 threshold) cycling exercise protocol followed by a high-intensity intermittent (HIT) cycling 142 protocol to exhaustion (1-min bouts at 80% PPO interspersed with 1-min bouts at 40% PPO). 143 The primary outcome was exercise capacity during the HIT protocol. Respiratory gas

144 exchange, heart rate (HR), rate of perceived exertion (RPE), and fingertip capillary blood 145 samples were also obtained at regular intervals during the SS exercise protocol and 146 immediately following HIT protocol to assess for physiological, metabolic, and perceptual 147 responses to exercise. An overview of the experimental design is shown in Figure 1. The 148 participants were informed that the aim of the study was to compare the effects of two CHO 149 drinks (that differed in composition but not quantity of CHO) on overnight recovery and 150 subsequent morning exercise capacity versus a known non-caloric sugar free drink. Upon 151 completion of the study, all subjects performed an exit interview where they were informed 152 they had been deceived in the PERCEPTION trial. Whilst no formal questionnaires were 153 administered, no subject reported that the drinks tasted differently though 3 subjects did 154 report they felt hungrier in the both the TRAIN LOW and PERCEPTION trials.

155 Assessment of lactate threshold, lactate turn point, VO_{2peak} and peak power output. At least 156 5-7 days prior to the familiarization (FAM) trial, subjects performed a submaximal 157 incremental cycling protocol to determine lactate threshold (LT), lactate turn point (LTP), peak oxygen uptake (VO_{2veak}) and peak power output (PPO) on an electronically braked 158 159 cycling ergometer (Excalibur Sport; Lode, Groningen, The Netherlands). Following a 5 min 160 warm up at 75 watts (W) at a self-selected cadence, the submaximal test commenced at 125 161 W with 25 W increase every 4 min. Twenty µl of fingertip capillary blood samples were collected in a Biosen capillary tube (EKF Diagnostics, Barleben, Germany) at the end of each 162 163 4 min stage. LT (defined as 1 mmol.L⁻¹ above resting levels) and LTP (defined as the second 164 inflection point on the lactate curve) were plotted live during the test using Biosen C-Line 165 lactate analyzer (EKF Diagnostics, Barleben, Germany). Heart Rate (HR) (Polar, F10, 166 Finland) was monitored continuously and recorded during the final 10 seconds of each stage, along with RPE (Borg, 1973). Respiratory gas exchange was recorded during the final two 167 168 minutes of each stage using an online gas analysis system (CPX Ultima, Medgraphics,

169 Minnesota, USA). The submaximal test ended once LTP had been confirmed. Following a 5 170 min recovery period, VO_{2peak} and PPO were assessed. The test to assess VO_{2peak} and PPO 171 commenced at 25 W below each subject's individual LT and consisted of 1-min stages with 172 25 W increments until volitional exhaustion. HR was monitored throughout the test. VO_{2neak} 173 referred to the peak value attained in any 10-second period during the last 60 seconds of data 174 collection and was supported by verification by two or all the following end point criteria (1) 175 heart rate with 10 b.min⁻¹ of age predicted maximum, (2) RER > 1.1 and (3) plateau of 176 oxygen consumption despite increasing workload.

177 Day 1: Glycogen depletion protocol. On the afternoon of Day 1, subjects arrived at the 178 laboratory (~1500 h) to perform an intermittent bout of cycling to volitional fatigue. Subjects 179 were asked to record and replicate their energy intake in the 24 h period prior to commencing 180 the glycogen depletion protocol. Following a 5 min warm up at self-selected intensity, subjects cycled for 2 min at 90% PPO, immediately followed by 2 min at 50% PPO. Once 181 182 subjects could no longer maintain > 60 rpm, the interval was decreased to 90 seconds, then to 183 1 min at 90% PPO. Subjects repeated this work to rest ratio at 80% PPO, 70% PPO, and 60% 184 PPO and the exercise protocol was terminated once subjects could no longer maintain > 60185 rpm at 60% PPO for 1 min. This protocol has been used previously in our laboratory (Bartlett et al., 2013; Taylor et al., 2013; Impey et al., 2016) and is a modification of that of Kuipers et 186 187 al. (1987) that induces glycogen depletion in both type I and type II fibers. Immediately 188 following the cessation of glycogen depleting exercise (~1700 h), subjects consumed 30 g of 189 whey protein isolate (Advanced Whey Isolate, Science in Sport, Nelson, UK) mixed with 250 190 ml water (in accordance with practical recommendations to promote recovery from 191 endurance exercise) before adhering to one of three dietary protocols. In the TRAIN HIGH trial, subjects consumed 1.2 g.kg⁻¹ maltodextrin (Cargill Dry Maltodextrin, UK) mixed with 192 193 500 ml water sugar free squash (Tesco, Hertfordshire, UK) per hour for 5 hours. In the

194 PERCEPTION trial, subjects adhered to an identical feeding frequency and volume protocol 195 but consumed a tasted match placebo solution where they were told contained an identical 196 amount of CHO as that consumed (or to be consumed) in the TRAIN HIGH trial (sugar free 197 squash, Tesco, Hertfordshire, UK). In the TRAIN LOW trial, subjects consumed the same 198 placebo solution as the PERCEPTION trial but were told the solution contained no CHO. All 199 drinks were administered in visually opaque bottles and 2.75 L of fluid was consumed over 200 the 5-hour recovery period in each trial. Subjects remained in the laboratory to complete the 201 first 3 h of the recovery protocol before returning to their homes to complete the last 2 h of 202 recovery (subjects were provided with the additional 2 x 500 ml solutions to take home). 203 Subjects also slept at their own home.

204 Day 2: Steady state (SS) and HIT exercise capacity test. Subjects arrived at the laboratory 205 between 0800 and 0830h the following morning after an overnight fast. Body mass (Seca, 206 Hamburg, Germany), motivation to train (using a visual analogue scale, VAS, McCormack et 207 al., 1988), resting blood lactate and blood glucose were initially measured. Subjects then then completed 30 min SS cycling at 95% of LT. Breath by breath gas analysis (CPX Ultima, 208 209 Medgraphics, Minnesota, USA) was measured for 2 min during 8-10 min, 18-20 min, and 28-210 30 min and substrate utilization was assessed according to Jeukendrup and Wallis (2005). Blood glucose and blood lactates samples were obtained at 15 min and 30 min. 211 212 Measurements of HR (Polar, F10, Finland) and RPE (Borg, 1973) were recorded at 10 min 213 intervals during the SS exercise. Following completion of SS exercise, subjects were 214 provided with 3 min active recovery at 50 W and subsequently commenced the HIT exercise 215 capacity test consisting of 1 min bouts at 80% PPO interspersed with 1 min bouts at 40% 216 PPO until volitional exhaustion. A final capillary blood sample was collected at the termination of the HIT protocol. 217

218 Familiarization. Eight subjects completed the full experimental protocol described above 219 while adhering to a water only (i.e. no flavoring) familiarization (FAM) condition at least 7 220 days prior to their first experimental trial (one of the nine subjects withdrew from 221 familiarization after several minutes of the SS exercise protocol having reported feelings of 222 muscle soreness). Upon completion of all three experimental trials, we compared each 223 subject's exercise capacity during the FAM trial and the TRAIN LOW trial and observed no significant difference, as evidenced by a *t*-test for paired samples (FAM = $5 \pm 5 \text{ min}$, PLA = 224 225 $7 \pm 6 \min_{0.25}$ P=0.25).

Blood analyses. Blood samples were obtained via finger prick capillary sampling using a 1.8
mm sterile safety-lancet (Sarstedt AG & Co, Nümbrecht, Germany) after sterilization using a
pre-injection medical swab (Medlock Medical Ltd., Oldham). A 20µl blood sample was
collected in a Biosen capillary tube (EKF Diagnostics, Barleben, Germany) and analyzed
using Biosen C-Line for blood glucose and lactate concentrations (EKF Diagnostics,
Barleben, Germany).

Statistical Analysis. Data were analysed using one or two-way repeated measures general 232 233 linear model (GLM) where the within factors were time and condition (TRAIN LOW, PERCEPTION and TRAIN HIGH). Where significant main effects were found, paired 234 235 samples t-tests with Bonferroni adjustment for multiple comparisons were performed to 236 identify differences. In relation to our primary outcome variable of exercise capacity, we also report uncertainty of outcomes as 95% confidence intervals (95% CI) and make probabilistic 237 238 magnitude based-inferences about the true (large sample) values of outcomes by qualifying 239 the likelihood that the true effect represents a substantial change, according to (Batterham & Hopkins, 2006). All data in text, tables and figures are expressed as means + SD with P < 0.05240

- 241 indicating statistical significance. Statistical analyses were performed using Statistics
- 242 Package for the Social Sciences (SPSS) for Windows (version 24, SPSS Inc, Chicago, IL).

243

- 244 **Results**
- 245 *Glycogen depletion protocol*

There was no difference (P=0.71) in time to exhaustion during the glycogen depletion protocol between the TRAIN HIGH (79 \pm 20 min), PERCEPTION (75 \pm 16 min) or TRAIN LOW (79 \pm 22 min) trials.

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250 *Physiological and perceptual responses during SS exercise*

There was no difference (P=0.258) in subjects' motivation to exercise prior to commencing the SS protocol (TRAIN HIGH 6.7 ± 2.7 cm; PERCEPTION 6.4 ± 1.7 cm; TRAIN LOW 5.1 ± 2.1 cm). Subjects' HR (P=0.006) and RPE (P<0.001) increased during SS though no difference was apparent between conditions (P=0.299 and 0.273 respectively, see Table 1).

255

256 Metabolic responses during SS exercise and HIT capacity test

257 During SS, RER (P < 0.001) and CHO oxidation rate (P < 0.001) decreased while fat oxidation 258 increased (P<0.001). Average CHO oxidation was higher throughout SS in TRAIN HIGH 259 than both PERCEPTION (P=0.019) and TRAIN LOW (P=0.012) while fat oxidation was lower (P=0.016 and 0.023 respectively). Blood glucose was higher throughout SS and HIT 260 261 in TRAIN HIGH than in PERCEPTION (P=0.002) and TRAIN LOW (P=0.021) and also decreased during exercise (P<0.001). Blood lactate rose throughout SS and was significantly 262 263 increased in TRAIN HIGH compared with both PERCEPTION (P=0.016) and TRAIN LOW 264 (P=0.023) after HIT (see Figure 2).

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266 *Exercise capacity during the HIT test*

267 High-intensity intermittent exercise capacity was different between conditions (P < 0.001), whereby TRAIN HIGH ($22 \pm 9 \text{ min}$; P=0.005: 95% CI for differences = 3 to 16 min, almost 268 269 *certainly beneficial*) was greater than both PERCEPTION ($12 \pm 6 \text{ min}$) and TRAIN LOW (8) \pm 8 min; P=0.001: 95% CI for differences = 7 to 20 min, almost certainly beneficial). 270 271 Exercise capacity was also greater in PERCEPTION compared with TRAIN LOW (P=0.025: 272 95% CI for differences = 1 to 8 min, very likely beneficial: see Figure 3A). Seven subjects 273 completed more intervals in PERCEPTION than TRAIN LOW, whilst all nine subjects 274 completed more intervals in the TRAIN HIGH compared with both non-CHO trials (see 275 Figure 3B). There was no trial order effect (P=0.849).

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277 Discussion

Confirming our hypotheses, we provide novel data by demonstrating that perception of CHO 278 279 availability augments high-intensity intermittent exercise capacity under sleep-low, train-low 280 conditions though perception does not near restore exercise capacity to that of CHO 281 consumption. We therefore consider our data to have methodological implications for future sleep-low train-low research designs by clearly highlighting the requirement for placebo-282 283 controlled trials. Furthermore, when considering that perception of CHO availability can 284 improve exercise capacity, our data may also have practical applications for those athletes 285 who deliberately practice CHO periodization strategies in an attempt to strategically enhance 286 oxidative adaptations of skeletal muscle.

To achieve our sleep low, train low model of CHO restriction, we employed a similar glycogen depletion and re-synthesis protocol to that recently studied in our laboratory (Hearris et al., 2019). Whilst we acknowledge that we did not directly assess muscle glycogen, evaluations of substrate utilisation during the SS exercise protocol are consistent

with differences in CHO availability between the TRAIN HIGH trial and the non-CHO trials. On the basis of the fitness levels of the present subjects (i.e. VO_{2peak} , 55.3 ± 8.3 ml.kg⁻¹.min⁻¹) and absolute CHO intake (i.e. 6 g.kg⁻¹), we estimate from our previous data (Hearris et al., 2019) and a recent meta-analysis (Areta & Hopkins, 2018) that muscle glycogen concentration in TRAIN HIGH was in the region of 300-350 mmol.kg⁻¹ dw, as opposed to 100-150 mmol.kg⁻¹ dw in the PERCEPTION and TRAIN LOW trials.

297 Consistent with the well-documented effect of muscle glycogen availability on 298 exercise capacity (Bergstrom et al., 1967; Hawley et al., 1997; Impey et al., 2016; Hearris et 299 al., 2019), it is unsurprising that all nine subjects were able to exercise for significantly longer 300 during the TRAIN HIGH trial compared with the non-CHO trials. The magnitude of 301 improvement observed here (i.e. ~15 minutes) agrees favorably with our recent data (Hearris 302 et al., 2019) where we observed that small differences in pre-exercise muscle glycogen 303 concentration (~100 mmol.kg⁻¹ dw) improves high-intensity intermittent exercise capacity at 304 80% PPO between $\sim 20\%$ and 50% (8–18 min). In our previous study, however, we 305 acknowledged that lack of blinding between trials may have influenced subjects' motivation 306 and perceived ability to complete high-intensity workloads (Hearris et al., 2019). To 307 overcome the issue of subjects being visually aware of the quantity of CHO rich foods 308 consumed (Mears et al., 2018), we deliberately chose to blind CHO availability in the present 309 study by using taste matched beverages delivered in opaque bottles.

When comparing subjects' exercise capacity between the TRAIN LOW and water only FAM trial, it is noteworthy that no significant differences in exercise capacity were observed. Such data highlight that when subjects were aware that no prior CHO had been consumed (despite differences in taste between the TRAIN LOW and FAM trials), exercise capacity was not affected. However, when subjects perceived they had consumed CHO before sleeping in the PERCEPTION trial, 7 of the 9 subjects performed significantly more

work compared with the known TRAIN LOW trial, despite reporting no significant 316 317 differences in their motivation to exercise. A placebo effect of CHO availability has been 318 documented previously (in conditions of normal pre-exercise muscle glycogen concentration) where CHO has been fed before (Mears et al., 2018) and during (Clark et al., 2000) cycling 319 320 time trials equating to durations of approximately 20 and 60 minutes, respectively. In 321 contrast, no placebo effect of CHO feeding is evident when exercise duration extends beyond 322 3 hours, likely due to near glycogen depletion and that the metabolic requirement for CHO 323 dominates over central drive (Hulston & Jeukendrup, 2009). Nonetheless, the present data 324 demonstrate that a placebo effect of prior CHO ingestion may also manifest in those 325 conditions where short term-high intensity intermittent exercise is commenced with 326 considerably reduced pre-exercise muscle glycogen concentration.

327 Whilst we acknowledge that the magnitude of effect with perception was less than 328 that of actual CHO consumption (~5 versus 15 minutes at 80% PPO), the present data are of 329 practical relevance for reasons related to both research design and practical application with 330 athletic populations. Indeed, when considering that previous studies reporting decrements in 331 power output or exercise capacity during acute train-low training sessions (using the twice 332 per day or sleep low models) have not blinded subjects to the "low CHO availability" condition (Hansen et al., 2005; Yeo et al., 2008, 2010; Hulston et al., 2010; Hearris et al., 333 2019), it is possible that such impairments in performance may also be due, in part, to 334 335 psychological reasons as opposed to physiological factors per se. Similarly, given that 336 Marquet et al. (2016b) observed that just one week of a sleep-low training intervention 337 (incorporating only 3 train-low sessions) improved 20 km cycling time trial performance by 338 3.2%, it is possible that such improvements were simply due to subjects beliefs that the sleep 339 low protocol would lead to superior improvements in performance, as opposed to 340 physiological or metabolic adaptations. In relation to practical application, the placebo effect of prior CHO intake may also extend the effects of caffeine (Lane et al., 2013) and
CHO mouth rinse (Kasper et al., 2016) as potential tools for which to increase exercise
capacity for those athletes who deliberately practice CHO restriction in an attempt to amplify
training adaptations (Impey et al., 2018).

345 In summary, we provide novel data by demonstrating that perception of CHO 346 availability augments high-intensity intermittent exercise capacity under sleep-low, train-low 347 conditions though perception does not restore exercise capacity to that of CHO consumption. 348 Such data have implications for future sleep-low train-low research designs by clearly 349 highlighting the requirement for placebo-controlled trials. In addition, our data may also have 350 practical applications for those athletes who deliberately incorporate periods of CHO 351 restriction into their training programmes in an attempt to strategically enhance mitochondrial related adaptations of skeletal muscle. 352

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354

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361 References

- Areta, J. L., & Hopkins, W. G. (2018). Skeletal Muscle Glycogen Content at Rest and During
 Endurance Exercise in Humans: A Meta-Analysis. Sports Med, 48(9), 2091-2102.
 doi:10.1007/s40279-018-0941-1
- Bartlett, J. D., Hawley, J. A., & Morton, J. P. (2015). Carbohydrate availability and exercise
 training adaptation: too much of a good thing? *Eur J Sport Sci*, 15(1), 3-12.
 doi:10.1080/17461391.2014.920926
- 368 Bartlett, J. D., Louhelainen, J., Igbal, Z., Cochran, A. J., Gibala, M. J., Gregson, W., . . . 369 Morton, J. P. (2013). Reduced carbohydrate availability enhances exercise-induced 370 p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. 371 Physiol Regul Integr Comp Physiol. 304(6), R450-458. Am Jdoi:10.1152/ajpregu.00498.2012 372
- Batterham, A. M., & Hopkins, W. G. (2006). Making meaningful inferences about
 magnitudes. *Int J Sports Physiol Perform*, 1(1), 50-57. doi: 10.1123/ijspp.1.1.50
- Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and
 physical performance. Acta Physiol Scand, 71(2 3), 140-150. doi:10.1111/j.17481716.1967.tb03720.x
- Borg, G. A. (1973). Perceived exertion: a note on "history" and methods. Med Sci Sports,
 5(2), 90-93
- Clark, V. R., Hopkins, W. G., Hawley, J. A., & Burke, L. M. (2000). Placebo effect of
 carbohydrate feedings during a 40-km cycling time trial. *Med Sci Sports Exerc*, 32(9),
 1642-1647. doi:10.1097/00005768-200009000-00019
- Cochran, A. J., Myslik, F., MacInnis, M. J., Percival, M. E., Bishop, D., Tarnopolsky, M. A.,
 & Gibala, M. J. (2015). Manipulating Carbohydrate Availability Between TwiceDaily Sessions of High-Intensity Interval Training Over 2 Weeks Improves TimeTrial Performance. Int J Sport Nutr Exerc Metab, 25(5), 463-470.
 doi:10.1123/ijsnem.2014-0263
- Hansen, A. K., Fischer, C. P., Plomgaard, P., Andersen, J. L., Saltin, B., & Pedersen, B. K.
 (2005). Skeletal muscle adaptation: training twice every second day vs. training once daily. J Appl Physiol (1985), 98(1), 93-99. doi:10.1152/japplphysiol.00163.2004
- Hawley, J. A., Palmer, G. S., & Noakes, T. D. (1997). Effects of 3 days of carbohydrate
 supplementation on muscle glycogen content and utilisation during a 1-h cycling
 performance. Eur J Appl Physiol Occup Physiol, 75(5), 407-412.
 doi:10.1007/s004210050180
- Hearris, M. A., Hammond, K. M., Fell, J. M. & Morton, J. P. (2018). Regulation of Muscle
 Glycogen Metabolism during Exercise: Implications for Endurance Performance and
 Training Adaptations. *Nutrients*, 10 (3), 298. doi:10.3390/nu10030298
- 398 Hearris, M. A., Hammond, K. M., Seaborne, R. A., Stocks, B., Shepherd, S. O., Philp, A., ... 399 Louis, J. B. (2019). Graded reductions in preexercise muscle glycogen impair exercise 400 capacity but do not augment skeletal muscle cell signaling: implications for CHO 401 periodization. Physiol (1985), 126(6), 1587-1597. JAppl doi:10.1152/japplphysiol.00913.2018 402
- Hulston, C. J., & Jeukendrup, A. E. (2009). No placebo effect from carbohydrate intake
 during prolonged exercise. *Int J Sport Nutr Exerc Metab*, 19(3), 275-284.
 doi:10.1007/s00421-008-0838-z
- Hulston, C. J., Venables, M. C., Mann, C. H., Martin, C., Philp, A., Baar, K., & Jeukendrup,
 A. E. (2010). Training with low muscle glycogen enhances fat metabolism in welltrained cyclists. *Med Sci Sports Exerc, 42*(11), 2046-2055.
 doi:10.1249/MSS.0b013e3181dd5070

- Impey, S. G., Hammond, K. M., Shepherd, S. O., Sharples, A. P., Stewart, C., Limb, M., ...
 Morton, J. P. (2016). Fuel for the work required: a practical approach to amalgamating train-low paradigms for endurance athletes. *Physiol Rep, 4*(10). doi:10.14814/phy2.12803
- Impey, S. G., Hearris, M. A., Hammond, K. M., Bartlett, J. D., Louis, J., Close, G. L., &
 Morton, J. P. (2018). Fuel for the work required: a theoretical framework for
 carbohydrate periodization and the glycogen threshold hypothesis. *Sports Medicine*,
 417 48(5), 1031-1048. doi:10.1007/s40279-018-0867-7
- Jeukendrup, A. E., & Wallis, G. A. (2005). Measurement of substrate oxidation during
 exercise by means of gas exchange measurements. *Int J Sports Med*, *26 Suppl 1*, S2837. doi:10.1055/s-2004-830512
- Kasper, A. M., Cocking, S., Cockayne, M., Barnard, M., Tench, J., Parker, L., ... Morton, J.
 P. (2016). Carbohydrate mouth rinse and caffeine improves high-intensity interval running capacity when carbohydrate restricted. *Eur J Sport Sci*, *16*(5), 560-568. doi:10.1080/17461391.2015.1041063
- Lane, S. C., Areta, J. L., Bird, S. R., Coffey, V. G., Burke, L. M., Desbrow, B., . . . Hawley,
 J. A. (2013). Caffeine ingestion and cycling power output in a low or normal muscle
 glycogen state. *Med Sci Sports Exerc*, 45(8), 1577-1584.
 doi:10.1249/MSS.0b013e31828af183
- Lane, S. C., Camera, D. M., Lassiter, D. G., Areta, J. L., Bird, S. R., Yeo, W. K., ... Hawley,
 J. A. (2015). Effects of sleeping with reduced carbohydrate availability on acute
 training responses. J Appl Physiol (1985), 119(6), 643-655.
 doi:10.1152/japplphysiol.00857.2014
- Marquet, L. A., Brisswalter, J., Louis, J., Tiollier, E., Burke, L. M., Hawley, J. A., &
 Hausswirth, C. (2016a). Enhanced Endurance Performance by Periodization of
 Carbohydrate Intake: "Sleep Low" Strategy. *Med Sci Sports Exerc, 48*(4), 663-672.
 doi:10.1249/mss.0000000000823
- Marquet, L. A., Hausswirth, C., Molle, O., Hawley, J. A., Burke, L. M., Tiollier, E., &
 Brisswalter, J. (2016b). Periodization of Carbohydrate Intake: Short-Term Effect on
 Performance. *Nutrients*, 8(12). doi:10.3390/nu8120755
- McCormack, H. M., Horne, D. J., & Sheather, S. (1988). Clinical applications of visual
 analogue scales: a critical review. Psychol Med, 18(4), 1007-1019.
 doi:10.1017/s0033291700009934
- Mears, S. A., Dickinson, K., Bergin-Taylor, K., Dee, R., Kay, J., & James, L. J. (2018).
 Perception of Breakfast Ingestion Enhances High-Intensity Cycling Performance. *Int J Sports Physiol Perform, 13*(4), 504-509. doi:10.1123/ijspp.2017-0318
- 446 Morton, J. P., Croft, L., Bartlett, J. D., Maclaren, D. P., Reilly, T., Evans, L., . . . Drust, B. (2009). Reduced carbohydrate availability does not modulate training-induced heat 447 448 shock protein adaptations but does upregulate oxidative enzyme activity in human 449 skeletal muscle. JAppl Physiol (1985),106(5), 1513-1521. 450 doi:10.1152/japplphysiol.00003.2009
- Taylor, C., Bartlett, J. D., van de Graaf, C. S., Louhelainen, J., Coyne, V., Iqbal, Z., . . .
 Morton, J. P. (2013). Protein ingestion does not impair exercise-induced AMPK signalling when in a glycogen-depleted state: implications for train-low compete-high. *Eur J Appl Physiol*, *113*(6), 1457-1468. doi:10.1007/s00421-012-2574-7
- Yeo, W. K., Paton, C. D., Garnham, A. P., Burke, L. M., Carey, A. L., & Hawley, J. A.
 (2008). Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *J Appl Physiol (1985), 105*(5), 1462-1470. doi:10.1152/japplphysiol.90882.2008

Yeo, W. K., McGee, S. L., Carey, A. L., Paton, C. D., Garnham, A. P., Hargreaves, M., &
Hawley, J. A. (2010). Acute signalling responses to intense endurance training
commenced with low or normal muscle glycogen. *Exp Physiol*, 95(2), 351-358.
doi:10.1113/expphysiol.2009.049353

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464 Figure 1 – Overview of the experimental design.

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- 466 Figure 2 – (A) RER, (B) CHO oxidation, (C) lipid oxidation, (D) blood glucose and (E) blood lactate concentration during the SS exercise protocol (as completed on the morning of 467 468 Day 2). *denotes significant difference between TRAIN HIGH and PERCEPTION and TRAIN LOW trials, P<0.05. a denotes significant difference from 10, b denotes significant 469 470 difference from 20, c denotes significant difference from 0, d denotes significant difference 471 from 15 and 30, all *P*<0.05. Exh, exhaustion. 472
- 473 Figure 3 - (A) Exercise capacity (means \pm SD) and (B) individual subject's exercise capacity 474 during the TRAIN LOW, PERCEPTION and TRAIN HIGH trials. *denotes significant difference from TRAIN LOW, # denotes significant difference from PERCEPTION, both 475 Review 476 *P*<0.05.

477 478 Table 1 – Heart rate, VO₂ (as % of VO_{2peak}) and RPE during the SS exercise protocol (as completed on the morning of Day 2) in the TRAIN LOW, PERCEPTION and TRAIN HIGH trials.

	<u>Time (min)</u>		
	10	20	30
HR (b.min ⁻¹)			
TRAIN LOW	145 <u>+</u> 16	147 ± 17	$150 \pm 19^{\text{ ab}}$
PERCEPTION	146 <u>+</u> 11	149 <u>+</u> 11	151 <u>+</u> 14 ^{ab}
TRAIN HIGH	142 ± 14	143 ± 14	146 ± 15^{ab}
% VO _{2neak}			
TRAIN LOW	61 ± 9	63 ± 7	61 ± 6
PERCEPTION	63 + 8	64 + 7	62 ± 6
TRAIN HIGH	64 ± 9	61 ± 9	63 ± 6
RPE (AU)			
TRAIN LOW	12 ± 2	14 ± 2	16 ± 3^{ab}
PERCEPTION	12 ± 2	13 ± 3	15 ± 3 ab
TRAIN HIGH	12 ± 2	14 ± 2	15 ± 3 ab

a denotes significant difference from 10, b denotes significant difference from 20, both *P*<0.05. ie perez

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Figure 1 – Overview of the experimental design.

99x56mm (300 x 300 DPI)



-O- TRAIN LOW -- PERCEPTION -- TRAIN HIGH

Figure 2 – (A) RER, (B) CHO oxidation, (C) lipid oxidation, (D) blood glucose and (E) blood lactate concentration during the SS exercise protocol (as completed on the morning of Day 2). *denotes significant difference between TRAIN HIGH and PERCEPTION and TRAIN LOW trials, P<0.05. a denotes significant difference from 10, b denotes significant difference from 20, c denotes significant difference from 0, d denotes significant difference from 15 and 30, all P<0.05. Exh, exhaustion.

167x215mm (300 x 300 DPI)



Figure 3 – (A) Exercise capacity (means ± SD) and (B) individual subject's exercise capacity during the TRAIN LOW, PERCEPTION and TRAIN HIGH trials. *denotes significant difference from TRAIN LOW, # denotes significant difference from PERCEPTION, both P<0.05.

80x194mm (300 x 300 DPI)