INTERNATIONAL JOURNAL OF
SPORT NUTRITION and
EXERCISE METABOLISM

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

Journal:	*International Journal of Sport Nutrition & Exercise Metabolism*
Manuscript ID	IJSNEM.2019-0275.R1
Manuscript Type:	Original Research
Keywords:	placebo, CHO, train low

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

Sally P. Waterworth[1], Connor C. Spencer[2], Aaron L. Porter[2] and James P. Morton[2]

[1]School of Sport, Rehabilitation and Exercise Sciences
University of Essex
Wivenhoe Park
Colchester
CO4 3SQ
UK

[2]Research Institute for Sport and Exercise Sciences
Liverpool John Moores University
Tom Reilly Building
Byrom St. Campus
Liverpool
L3 3AF
UK

Running title: Perception of CHO and train-low

Address for correspondence:
Professor James Morton
Research Institute for Sport and Exercise Sciences
Liverpool John Moores University
Tom Reilly Building
Byrom St. Campus
Liverpool
L3 3AF
UK
Email: J.P.Morton@ljmu.ac.uk

Abstract

We tested the hypothesis that perception of carbohydrate (CHO) availability augments exercise capacity in conditions of reduced CHO availability. Nine males completed a sleep-low train-model comprising evening glycogen depleting cycling followed by an exhaustive cycling protocol the next morning in the fasted state (30 minutes steady-state, SS, at 95% lactate threshold followed by 1-min intervals at 80% peak power output until exhaustion). After the evening depletion protocol and prior to sleeping, subjects consumed 1) a known 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 mass (TRAIN LOW). The TRAIN HIGH and PERCEPTION trials were conducted double blind. During SS, average blood glucose and CHO oxidation were significantly higher in TRAIN HIGH (4.01 $\pm$ 0.56 $mmol.L^{-1}$; 2.17 $\pm$ 0.70 $g.min^{-1}$) versus both PERCEPTION (3.30 $\pm$ 0.57 $mmol.L^{-1}$; 1.69 $\pm$ 0.64 $g.min^{-1}$, $P<0.05$) and TRAIN LOW (3.41 $\pm$ 0.74 $mmol.L^{-1}$; 1.61 $\pm$ 0.59 $g.min^{-1}$, $P<0.05$). Exercise capacity was significantly different between all pairwise comparisons ($P<0.05$) where TRAIN LOW (8 $\pm$ 8 min) < PERCEPTION (12 $\pm$ 6 min) < TRAIN HIGH (22 $\pm$ 9 min). Data demonstrate that perception of CHO availability augments 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 methodological implications for future research designs and may also have practical applications for athletes who deliberately practice elements of training in CHO restricted states.

Keywords: placebo, carbohydrate, train-low, capacity

Introduction

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

Whilst there are multiple research designs used to practically achieve train-low 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 particularly potent strategy for which to prolong the period of CHO restriction (Bartlett et al., 2013; Lane et al., 2015). In this approach, participants perform an evening training session, restrict CHO during overnight recovery, and then complete a fasted training session on the following morning. The accumulative time with reduced muscle glycogen could therefore extend to 12–14 h depending on the timing and duration of the training sessions and sleep period. When performed chronically, Marquet et al. (2016a,b) observed that 1–3 weeks of sleep-low training in elite triathletes and cyclists improves cycling efficiency (3.1%), 20 km

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

Despite the aforementioned findings, an obvious limitation of the sleep-low, train-low model is that exercise capacity is likely to be significantly impaired during the morning 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) impaired morning exercise capacity at 80% peak power output (PPO) by ~20 to 50% (Hearris et al., 2019). Nonetheless, we acknowledged that lack of blinding between conditions (subjects were aware of CHO availability given that whole foods were consumed) may have influenced subjects' perception of their ability of complete high-intensity workloads. Indeed, placebo effects of CHO availability have been reported in conditions of CHO feeding before (Mears et al., 2018) and during exercise (Clark et al., 2000). To the authors' knowledge, however, the potential placebo effect of CHO availability has not yet been examined under 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 perception of CHO availability augments exercise capacity. To this end, we adopted a sleep-low, train-low model of CHO restriction where recreationally active males commenced an 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 (PERCEPTION) or a known train-low condition during which no CHO was consumed prior to sleeping (TRAIN-LOW). We specifically hypothesised that perception of CHO availability would improve morning exercise capacity compared to known train-low conditions but that perception would not restore exercise capacity to that of true train-high conditions.

Methods

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

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

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

Assessment of lactate threshold, lactate turn point, VO_{2peak} and peak power output. At least 5-7 days prior to the familiarization (FAM) trial, subjects performed a submaximal incremental cycling protocol to determine lactate threshold (LT), lactate turn point (LTP), peak oxygen uptake (VO_{2peak}) and peak power output (PPO) on an electronically braked cycling ergometer (Excalibur Sport; Lode, Groningen, The Netherlands). Following a 5 min warm up at 75 watts (W) at a self-selected cadence, the submaximal test commenced at 125 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 4 min stage. LT (defined as 1 $mmol.L^{-1}$ above resting levels) and LTP (defined as the second inflection point on the lactate curve) were plotted live during the test using Biosen C-Line lactate analyzer (EKF Diagnostics, Barleben, Germany). Heart Rate (HR) (Polar, F10, 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 minutes of each stage using an online gas analysis system (CPX Ultima, Medgraphics,

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

Day 1: Glycogen depletion protocol. On the afternoon of Day 1, subjects arrived at the laboratory (~1500 h) to perform an intermittent bout of cycling to volitional fatigue. Subjects were asked to record and replicate their energy intake in the 24 h period prior to commencing 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 subjects could no longer maintain > 60 rpm, the interval was decreased to 90 seconds, then to 1 min at 90% PPO. Subjects repeated this work to rest ratio at 80% PPO, 70% PPO, and 60% PPO and the exercise protocol was terminated once subjects could no longer maintain > 60 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 al. (1987) that induces glycogen depletion in both type I and type II fibers. Immediately following the cessation of glycogen depleting exercise (~1700 h), subjects consumed 30 g of whey protein isolate (Advanced Whey Isolate, Science in Sport, Nelson, UK) mixed with 250 ml water (in accordance with practical recommendations to promote recovery from 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 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
208 completed 30 min SS cycling at 95% of LT. Breath by breath gas analysis (CPX Ultima,
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).
211 Blood glucose and blood lactates samples were obtained at 15 min and 30 min.
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
217 termination of the HIT protocol.

Familiarization. Eight subjects completed the full experimental protocol described above while adhering to a water only (i.e. no flavoring) familiarization (FAM) condition at least 7 days prior to their first experimental trial (one of the nine subjects withdrew from familiarization after several minutes of the SS exercise protocol having reported feelings of muscle soreness). Upon completion of all three experimental trials, we compared each 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 ± 5 min, PLA = 7 ± 6 min, 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 linear model (GLM) where the within factors were time and condition (TRAIN LOW, PERCEPTION and TRAIN HIGH). Where significant main effects were found, paired samples t-tests with Bonferroni adjustment for multiple comparisons were performed to 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 magnitude based-inferences about the true (large sample) values of outcomes by qualifying 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 $P<0.05$

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***

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

249

250 ***Physiological and perceptual responses during SS exercise***

251 There was no difference (*P*=0.258) in subjects' motivation to exercise prior to commencing
252 the SS protocol (TRAIN HIGH 6.7 ± 2.7 cm; PERCEPTION 6.4 ± 1.7 cm; TRAIN LOW
253 5.1 ± 2.1 cm). Subjects' HR (*P*=0.006) and RPE (*P*<0.001) increased during SS though no
254 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
260 lower (*P*=0.016 and 0.023 respectively). Blood glucose was higher throughout SS and HIT
261 in TRAIN HIGH than in PERCEPTION (*P*=0.002) and TRAIN LOW (P=0.021) and also
262 decreased during exercise (*P*<0.001). Blood lactate rose throughout SS and was significantly
263 increased in TRAIN HIGH compared with both PERCEPTION (*P*=0.016) and TRAIN LOW
264 (*P*= 0.023) after HIT (see Figure 2).

265

Exercise capacity during the HIT test

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

Discussion

Confirming our hypotheses, we provide novel data by demonstrating that perception of CHO availability augments high-intensity intermittent exercise capacity under sleep-low, train-low conditions though perception does not near restore exercise capacity to that of CHO 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-controlled trials. Furthermore, when considering that perception of CHO availability can improve exercise capacity, our data may also have practical applications for those athletes who deliberately practice CHO periodization strategies in an attempt to strategically enhance 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.

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

316 work compared with the known TRAIN LOW trial, despite reporting no significant
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)
319 where CHO has been fed before (Mears et al., 2018) and during (Clark et al., 2000) cycling
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"
333 condition (Hansen et al., 2005; Yeo et al., 2008, 2010; Hulston et al., 2010; Hearris et al.,
334 2019), it is possible that such impairments in performance may also be due, in part, to
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).

In summary, we provide novel data by demonstrating that perception of CHO availability augments 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 implications for future sleep-low train-low research designs by clearly highlighting the requirement for placebo-controlled trials. In addition, our data may also have practical applications for those athletes who deliberately incorporate periods of CHO restriction into their training programmes in an attempt to strategically enhance mitochondrial related adaptations of skeletal muscle.

Acknowledgement, Authorships, Declarations

The study was designed by JPM, SPW, CS and AP. Data were collected and analysed by SPW, CS, AP. Data interpretation and manuscript preparation were undertaken by JM, SPW, CS and AP. All authors approved the final version of the paper. JPM is a consultant for Science in Sport (SiS). His previous research on glycogen metabolism and exercise has been funded by GlaxoSmithKline (GSK), Lucozade Ribena Suntory (LRS) and SiS.

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

Bartlett, J. D., Louhelainen, J., Iqbal, Z., Cochran, A. J., Gibala, M. J., Gregson, W., . . . Morton, J. P. (2013). Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. *Am J Physiol Regul Integr Comp Physiol, 304*(6), R450-458. doi:10.1152/ajpregu.00498.2012

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.1748-1716.1967.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 Twice-Daily Sessions of High-Intensity Interval Training Over 2 Weeks Improves Time-Trial 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

Hearris, M. A., Hammond, K. M., Seaborne, R. A., Stocks, B., Shepherd, S. O., Philp, A., . . . Louis, J. B. (2019). Graded reductions in preexercise muscle glycogen impair exercise capacity but do not augment skeletal muscle cell signaling: implications for CHO periodization. *J Appl Physiol (1985), 126*(6), 1587-1597. doi:10.1152/japplphysiol.00913.2018

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 well-trained 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, 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*, S28-37. 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.0000000000000823

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

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 shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. *J Appl Physiol (1985), 106*(5), 1513-1521. 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

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

463

464 **Figure 1** – Overview of the experimental design.

465

466 **Figure 2** – (A) RER, (B) CHO oxidation, (C) lipid oxidation, (D) blood glucose and (E)
467 blood lactate concentration during the SS exercise protocol (as completed on the morning of
468 Day 2). *denotes significant difference between TRAIN HIGH and PERCEPTION and
469 TRAIN LOW trials, $P<0.05$. a denotes significant difference from 10, b denotes significant
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 ± SD) and (B) individual subject's exercise capacity
474 during the TRAIN LOW, PERCEPTION and TRAIN HIGH trials. *denotes significant
475 difference from TRAIN LOW, # denotes significant difference from PERCEPTION, both
476 $P<0.05$.

477
478

Table 1 – Heart rate, VO_2 (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.

	Time (min)		
	10	**20**	**30**
HR (b.min^{-1})			
TRAIN LOW	145 ± 16	147 ± 17	150 ± 19 ^{ab}
PERCEPTION	146 ± 11	149 ± 11	151 ± 14 ^{ab}
TRAIN HIGH	142 ± 14	143 ± 14	146 ± 15 ^{ab}
% VO_{2peak}			
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 $P<0.05$.

514

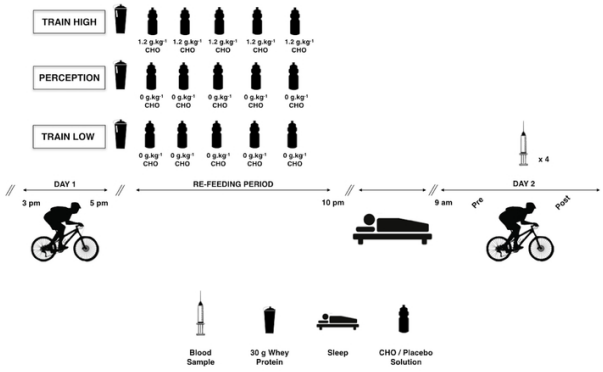

Figure 1 – Overview of the experimental design.

99x56mm (300 x 300 DPI)

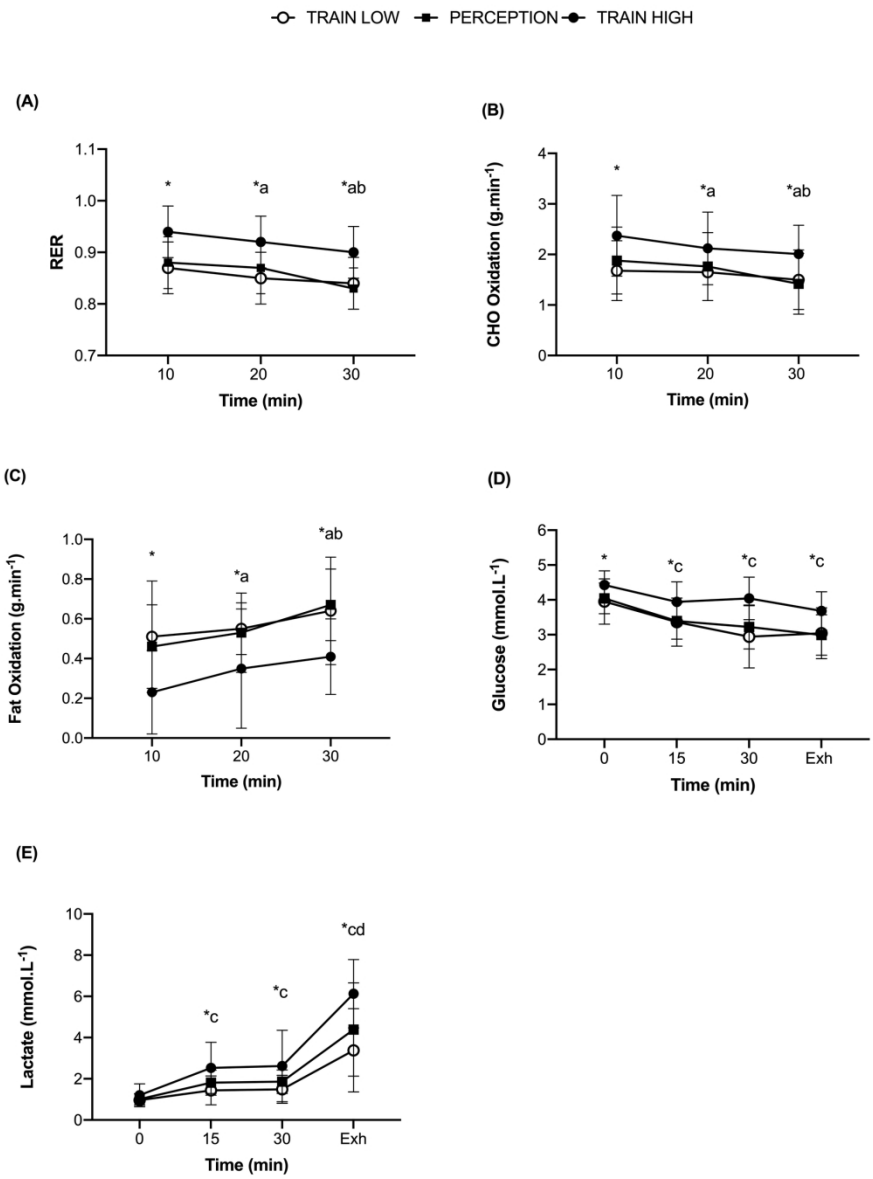

Figure 2 – (A) RER, (B) CHO oxidation, (C) lipid oxidation, (D) blood glucose and (E) blood lactate concentration during the SS exercise protocol (as completed on the morning of Day 2). *denotes significant difference between TRAIN HIGH and PERCEPTION and TRAIN LOW trials, P<0.05. a denotes significant 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)

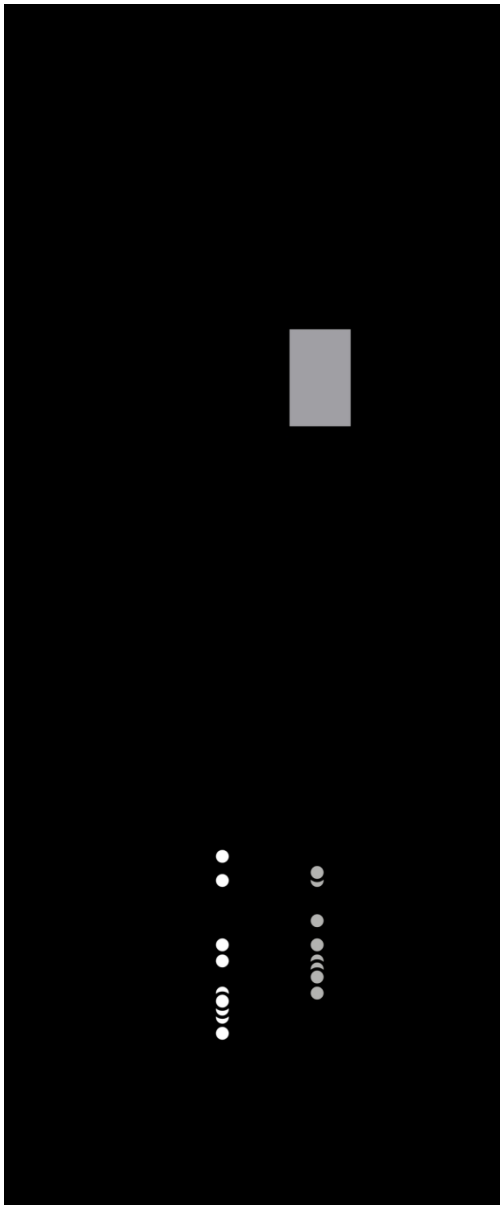

Figure 3 – (A) Exercise capacity (means ± SD) and (B) individual subject's exercise capacity during the TRAIN LOW, PERCEPTION and TRAIN HIGH trials. *denotes significant difference from TRAIN LOW, # denotes significant difference from PERCEPTION, both P<0.05.

80x194mm (300 x 300 DPI)