- 1 Title: Perception of breakfast ingestion enhances high intensity cycling performance
- 2 Submission Type: Original research

Authors: Stephen A. Mears, Kathryn Dickinson, Kurt Bergin-Taylor, Reagan Dee, Jack
Kay, Lewis J. James

- 5 School of Sport, Exercise and Health Sciences, Loughborough University,6 Loughborough, LE11 3TU.
- 7 Corresponding Author: Stephen A. Mears, School of Sport, Exercise and Health

8 Sciences, Loughborough University, Loughborough, LE11 3TU. Tel: 01509 226391.

- 9 Email: s.a.mears@lboro.ac.uk
- 10 Preferred running head: Breakfast and high intensity cycling
- 11 Abstract word count: 240
- 12 Text-only word count: 3602
- 13 Number of figures and tables: 4 figures

14

15 Abstract

Purpose: To examine the effect on short duration, high intensity cycling time trial
performance when a semi-solid breakfast containing carbohydrate or a taste and texture
matched placebo is ingested 90 minutes pre-exercise compared to a water control.

19 Methods: Thirteen well trained cyclists (25 ± 8 years, 71.1 ± 5.9 kg, 1.76 ± 0.04 m, 383 \pm 46 W_{max}, VO_{2peak} 4.42 \pm 0.53 L·min⁻¹) performed three experimental trials examining 20 21 breakfast ingestion 90 minutes before a 10 minute steady state cycle (60% W_{max}) and a 22 ~20 minute time trial (to complete a workload target of 376 ± 36 kJ). Subjects 23 consumed either water (WAT), a semi-solid carbohydrate breakfast (2 g 24 carbohydrate kg^{-1} body mass; CHO) or a taste and texture matched placebo (PLA). Blood lactate and glucose concentrations were measured periodically throughout the 25 26 rest and exercise periods.

27 Results: The time trial was completed quicker in CHO (1120 \pm 69 s; *P*=0.006) and PLA

28 (1112 \pm 50 s; P=0.030) compared to WAT (1146 \pm 74 s). Ingestion of carbohydrate

29 caused an increase in blood glucose concentration throughout the rest period in CHO

30 (peak at 30 minutes rest: $7.37 \pm 1.10 \text{ mmol} \cdot 1^{-1}$; P < 0.0001) before dropping below

31 baseline levels after the steady state cycling.

32 Conclusion: A short duration cycling time trial was completed quicker when subjects

33 perceived that they consumed breakfast (PLA or CHO) 90 minutes prior to the start of

34 exercise. The improvement in performance is likely attributable to a psychological

35 rather than physiological effect.

36 Key words: carbohydrate, exercise, time trial, fasted, placebo

37 Introduction

38 The benefits of carbohydrate feeding prior to prolonged bouts of endurance exercise are well established $^{1-6}$. When exercise duration is longer than 60 minutes it is generally 39 40 advised that athletes consume carbohydrate in the 1-4 hours before exercise⁷. For 41 exercise lasting less than 45 minutes there appears to be little evidence, if any, to 42 suggest pre-exercise carbohydrate ingestion will enhance performance. It is generally 43 perceived that muscle glycogen depletion is not the limiting factor for short duration 44 exercise and therefore prior ingestion of carbohydrate will serve little benefit^{7,8}. 45 However, for many athletes common practice often dictates consumption of 46 carbohydrate prior to training sessions and competition regardless of the duration and 47 particularly if the training is at a high intensity.

48 Endurance athletes will regularly train in the morning, but for many, the logistics of 49 consuming carbohydrate 1 to 4 hours prior to exercise may be difficult and therefore 50 result in some sessions completed in a fasted state. Training in a fasted state and thereby reducing carbohydrate availability has been shown to potentiate cellular and molecular adaptations to endurance training⁹. This may be of advantage to endurance athletes if 51 52 correctly integrated into a periodised training programme¹⁰, however, other methods of 53 54 reducing carbohydrate availability (i.e. 'sleep low' and 'train low' paradigms) have 55 resulted in reduced self-selected intensity, which might attenuate the training stimulus^{11,12}. From a physiological standpoint, despite small decreases in liver glycogen 56 stores overnight¹³, fasted exercise should not impair short duration performance, and 57 58 therefore any influences on performance or self-selected intensity may be as a result of a 59 placebo effect.

60 The placebo effect has been commonly observed in exercise performance settings, arising from the belief that one is receiving a treatment or product that will result in a 61 62 favourable outcome¹⁴. In exercise lasting approximately 1 hour, Clark and collegues¹⁴ observed a 4% improvement in cycling performance when a placebo drink thought to be 63 64 containing carbohydrate was consumed during exercise, yet for longer periods of exercise (\sim 3h), no placebo effect has been reported¹⁵. Although these results are based 65 66 on feeding during exercise, it appears that there is more likely to be a placebo effect 67 when the exercise bout is short in duration and muscle glycogen use is not the limiting 68 factor. For a cycling time trial lasting ~20 minutes (i.e. comparable to a 10-mile time 69 trial), a placebo effect may therefore be of substantial significance, with those 70 consuming carbohydrate or breakfast potentially perceiving this to be advantageous and 71 increasing self-selected intensity.

72 Therefore the aim of this study was to examine the effect of pre-exercise carbohydrate 73 intake (i.e. breakfast) on cycling time trial performance compared to a taste and flavour-74 matched placebo and a water control. A semi-solid breakfast was used to enhance the 75 perception of energy/ nutrient intake and facilitate blinding. It was hypothesised that a 76 carbohydrate breakfast would have no effect on performance compared to the placebo, 77 but both would be advantageous compared to water.

78

79 Methods

Thirteen well-trained male cyclists (age 25 ± 8 years, body mass (BM) 71.1 ± 5.9 kg, 80 height 1.76 ± 0.04 m, maximum power output 383 ± 46 W, $VO_{2peak} 4.42 \pm 0.53$ L·min⁻¹) 81 82 were recruited to take part in three trials, undertaken in a randomised order. The study 83 protocol was explained to all subjects both verbally and in writing before they provided 84 written informed consent. The study was approved by the Loughborough University 85 Ethics Approvals (Human Participants) Sub-Committee and conformed to the 86 Declaration of Helsinki. It was estimated that 12 subjects were required to detect a 2.5% 87 (30 sec) difference between trials based on an a of 0.05 and a statistical power of 0.8. 88 Thirteen were recruited to provide adequate power and account for dropouts.

- Subjects visited the laboratory on 5 occasions: a VO_{2peak} test, a time trial familiarisation
 and three experimental trials (water (CON), placebo (PLA) and carbohydrate (CHO);
 figure 1). Subjects were recruited on the premise that the investigation was examining
 two breakfast drinks. Trials were performed in a randomised cross-over design. The
 PLA and CHO trials were administered in a double-blind manner, although it was
 impossible to blind the WAT trial from either experimenters or subjects.
- 95 During the first visit, subjects performed a VO_{2peak} test using a continuous incremental 96 protocol on an electronically braked cycle ergometer (Lode Excalibur; Lode BV, 97 Groningen, Netherlands). Commencing at 95 W, subjects completed three minutes 98 stages increasing by 35 W until volitional exhaustion. Maximal power output (W_{max}) 99 was calculated as the power of the last completed stage plus the fraction of time spent in 100 the next stage multiplied by the intensity increment. W_{max} values determined 60% W_{max} 101 used for the experimental trials. During the final minute of each stage and the final 102 minute of the test, expired gases were collected into a Douglas bag and analysed for 103 oxygen and carbon dioxide concentration (Servomex 1400 Oxygen and Carbon Dioxide 104 Gas Analyser; Servomex, Crowborough, UK). Using a Harvard dry gas meter (Harvard 105 Apparatus Ltd., Edenbridge, UK) and thermometer (Edale Digital Thermometer D515: 106 Edale Instruments Ltd., Cambridge, UK), gas volumes and temperature were measured, 107 respectively and corrected to STPD (standard temperature and pressure, dry). Following 108 the VO_{2peak} test, subjects performed at least 50% of the time trial protocol used in the 109 experimental trials to initially familiarise with the method. During the second visit, 110 subjects performed a familiarisation of the exercise portion of the experimental trial. This involved a 10 minute bout at 60% $W_{\text{max}},\,5$ minute rest, and a cadence dependent 111 112 linear-factor time trial (similar to that used by Hulston and Jeukendrup¹⁵), where 113 subjects were asked to reach a target workload, based on cycling at 80% W_{max} for 20 114 minutes, as quickly as possible. The following formula was used to calculate work 115 required:

$$Target \, kJ = \frac{(Wmax \times 0.8 \times 1200s)}{1000}$$

Subjects were able to see work completed and received verbal notification upon completion of 25, 50 and 75% of the time trial. The time trial was completed in silence, in an enclosed area of the laboratory with no additional feedback provided. Time to complete each 25% segment and heart rate at every 25% were recorded. No food or fluid was ingested during either the steady-state exercise or TT.

121 In the 24 h prior to the first experimental trial, subjects recorded all food and fluid 122 intake, and any low-intensity habitual physical activity, and repeated these patterns before the two remaining trials. Subjects arrived overnight fasted between 0700 and0900 h, with the specific time standardised for each individual.

125 On arrival, subjects provided a urine sample, and had nude body mass measured. A 126 heart rate monitor (Polar Vantage; Kempele, Finland) was fitted before the subject sat 127 for 5 minutes and resting heart rate recorded. At the end of the rest period a capillary 128 fingertip blood sample (20 µl) was collected and later analysed for whole blood lactate 129 and glucose concentrations. Subjects were also asked to rate their gastrointestinal (GI) 130 comfort (1 = neutral; 12 = painful). Subjects were then asked to consume one of three breakfasts within 5 minutes: CON (7 ml·kg body mass(BM)⁻¹ water), PLA (6 ml·kg 131 BM⁻¹ water, 1 ml·kg BM⁻¹ orange squash (Robinson's, Britvic, Hemel Hempstead, UK), 132 0.67 g·kg BM⁻¹ xanthan gum (Doves Farm, Hungerford, UK) and 0.067 g·kg BM⁻¹ 133 artificial sweetener (Canderel, Merisant, High Wycombe, UK)) and CHO (6 ml·kg BM⁻ 134 ¹ water, 1 ml·kg BM⁻¹ orange squash, 2 g·kg BM⁻¹ maltodextrin (MyProtein, Northwich, 135 UK), 0.67 g·kg BM⁻¹ xanthan gum and 0.067 g·kg BM⁻¹ artificial sweetener). PLA and 136 137 CHO were matched for taste and texture. Xanthan gum was used to produce a semi-138 solid meal and increase the perception of 'energy intake'. When provided with either 139 the PLA or CHO breakfast, subjects were told, "this is one of the two breakfast drinks". 140 At 15, 30, 60 and 90 minutes post-ingestion, heart rate and GI comfort were measured 141 and blood samples were collected.

142 Subjects then completed 10 minutes at 60% W_{max}. During the final minute of exercise, 143 heart rate and rating of perceived exertion were measured, and a sample of expired gas 144 was collected. On completion, a blood sample was collected and subjects rated GI 145 comfort. Following a 5 minute period of rest, subjects began the time trial. Final 146 measurements (blood and GI comfort) were collected at the end of the time trial. After 147 the final trial, subjects were asked a series of questions: "Was there a difference 148 between the drinks?", "If so, can you identify this difference?", "One of the drinks contained carbohydrate, which one was it?" and "Do you ever complete aspects of your 149 150 training in the morning after an overnight fast?"

151 Sample analysis

Each 20 μl whole blood sample was collected in a capillary tube and placed in an
Eppendorf containing 1 ml of haemolysing solution (EKF Diagnostics, Cardiff, UK).
This was stored on ice until analysis for glucose and lactate concentrations (Biosen CLine, EKF Diagnostics). Urine samples were analysed in duplicate for osmolality by
freezing-point depression (Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec,
Berlin, Germany).

158 Statistical analysis

159 Data were checked for normality of distribution using Shapiro-Wilks tests. All data 160 were normally distributed. A one-way repeated measures ANOVA was used to analyse 161 data containing one factor (performance time, urine osmolality, expired air and substrate 162 use). Data with two factors (pacing, blood lactate/ glucose concentrations, heart rate and 163 scales) were analysed using a two-way repeated measures ANOVA. If a significant 164 ANOVA was observed, paired samples t-test with Holm-Bonferroni correction were 165 used to identify where the difference occurred. Statistical significance was accepted 166 when P < 0.05. Data is expressed as mean \pm standard deviation (SD). Statistical 167 Package for the Social Sciences for Windows, version 22.0 (SPSS Inc., Chicago, IL, 168 USA) was used to conduct the statistical analysis.

169 Results

170 Pre-trial urine osmolality was similar between trials (WAT: $670 \pm 195 \text{ mOsmol} \cdot \text{kg}^{-1}$, 171 PLA: $801 \pm 199 \text{ mOsmol} \cdot \text{kg}^{-1}$, CHO: $754 \pm 226 \text{ mOsmol} \cdot \text{kg}^{-1}$; P = 0.158), suggesting 172 subjects arrived in a similar state of hydration.

173 *Performance measures*

174 Time to complete the time trial was quicker in both the CHO (1120 \pm 69 s; *P* = 0.005)

and PLA (1112 \pm 50 s; *P* = 0.030) trials compared to the WAT trial (1146 \pm 74 s; figure 2), however, there was no difference in performance between the CHO and PLA trial (*P*

2), however, there was no difference in performance between the = 0.544). No trial order effect was observed (P = 0.841).

TTT = 0.544). No that older effect was observed (T = 0.641).

178 Analysis of pacing strategy showed a time effect, with the first 25% TT section of all 179 trials completed quicker than the 25-50% and 50-75% sections (P < 0.0001; figure 3) 180 but similar to the final 25% (P = 0.141). The second 25% section was also completed 181 faster than the third section (P = 0.004). There was a significant trial effect (P < 0.0001) 182 but no interaction effect (P = 0.298).

Heart rate was similar between trials at baseline $(61 \pm 11 \text{ beat} \cdot \text{min}^{-1}; \text{ grouped mean and}$ SD of trials; P = 0.780), during the rest period $(58 \pm 10 \text{ bpm}; \text{ grouped mean and SD of}$ trials; P = 0.316) and in the 10 minute steady state period of cycling $(140 \pm 19 \text{ bpm};$ grouped mean and SD of trials; P = 0.312). Mean heart rate was slightly lower during the TT in the WAT trial $(174 \pm 8 \text{ bpm})$ compared to the PLA $(175 \pm 6 \text{ bpm}; P = 0.006)$

- 188 and CHO (177 \pm 9 bpm; *P* = 0.003) trials.
- 189 Blood analysis

190 A significant time, trial and trial x time interaction effect was observed for blood 191 glucose concentrations (P < 0.0001; figure 4a). Following breakfast in the CHO trial, 192 blood glucose concentrations increased above baseline and remained elevated until 90 193 minutes, before dropping below baseline concentrations following the 10 minute steady 194 state cycling. Blood glucose concentrations then increased above baseline following 195 completion of the TT. This increase following the TT also occurred in the WAT and 196 PLA trials. In the CHO trial, blood glucose concentrations were greater at 15, 30, 60 197 and 90 minutes and lower following steady state compared to the corresponding 198 samples in both the WAT and PLA trials (P < 0.05).

199 Blood lactate concentrations were not influenced by trial (P = 0.088), however there 200 was a time effect with concentrations peaking following the completion of the TT (P <201 0.0001; figure 4b).

202 Substrate utilisation

203 Due to problems with expired gas analysis, respiratory exchange ratio (RER) and 204 substrate utilisation during the period of steady state cycling were only available for 10 205 out of 13 subjects. RER was greater in the CHO trial (0.94 ± 0.03) compared to the PLA 206 trial (0.89 \pm 0.04; P <0.0001). RER during the WAT trial was not different to the other trials (0.92 \pm 0.03; P = 0.112 v PLA; P = 0.117 v CHO). Carbohydrate oxidation was 207 208 greater in the CHO trial $(3.10 \pm 0.17 \text{ g} \cdot \text{min}^{-1})$ compared to the PLA trial $(2.41 \pm 0.53 \text{ min}^{-1})$ g·min⁻¹; P < 0.0001), however during the WAT trial (2.82 ± 0.48 g·min⁻¹), carbohydrate 209 210 oxidation was similar to the two other trials (P = 0.088 v PLA; P = 0.148 v CHO). Fat oxidation was greater in the PLA trial $(0.52 \pm 0.23 \text{ g.min}^{-1})$ compared to the CHO trial 211

212 $(0.26 \pm 0.17 \text{ g} \cdot \text{min}^{-1}; P = 0.003)$, but similar in the WAT trial compared to the two other 213 trials $(0.36 \pm 0.16 \text{ g} \cdot \text{min}^{-1}; P = 0.108 \text{ v PLA}; P = 0.121 \text{ v CHO}).$

There was no difference in GI comfort between trials (time x trial interaction, P = 0.446) and no rise from baseline values of 1 ± 1 (WAT), 1 ± 1 (PLA) and 1 ± 1 (CHO, all P > 0.05) throughout the trials.

217 *Questionnaire data*

218 Out of thirteen subjects, five stated they felt there was a difference in the drinks, with all 219 of these subjects correctly identifying the CHO trial as either containing 'carbohydrate' 220 or 'energy'. Of these five subjects, two subjects performed better on the PLA trial (by 221 38 s and 83 s), one performed better on the CHO trial (by 50 s) and two had very similar performance times (both 5 s faster on the CHO trial). Of the remaining eight subjects, 222 223 four correctly guessed the order of the PLA and CHO trials. Seven subjects completed 224 little to none of their training in a fasted state, with the remaining six subjects 225 performing a fraction (1-2 rides per week) of their training in a fasted state.

226 Discussion

The aim of the study was to examine the effect of a pre-exercise carbohydrate intake in the form of maltodextrin (i.e breakfast) on a short duration high intensity cycling time trial, compared to a placebo and water control. Performance was improved in both the CHO and PLA trials suggesting there was a placebo effect of ingesting breakfast. This would indicate that with the length and intensity of the exercise used in the current study, nutritional intake may be of psychological benefit, rather than physiological.

- 233 The main result of the study was the placebo effect observed on performance. The placebo effect has been observed in 60 minute performance trials¹⁴ when perhaps the 234 235 metabolic and psychological effects of carbohydrate ingestion may cross. However, 236 when exercise is of longer duration and the metabolic benefits of carbohydrate intake are clearer, no placebo effect was observed¹⁵. The interesting aspect of this study was 237 the short duration nature of the time trial in combination with a water control to 238 239 maximise the perception of carbohydrate/ breakfast consumption. Pre-exercise 240 carbohydrate studies tend to compare a carbohydrate drink with a taste-matched placebo^{5,8}, but have not increased the viscosity to create the perception of ingesting a 241 Few include a water control¹⁵, preventing the investigation of knowingly 242 meal. 243 ingesting nothing which may dampen any placebo effect. Palmer and colleagues⁸ 244 provided a 6.8% carbohydrate drink or a coloured and flavoured placebo 10 minutes 245 prior to a cycle test of similar duration to the current study (20 km) but did not have a 246 water control. Whilst no performance difference was observed between the trials, this 247 does not discount the positive effect that both drinks may have had on performance. In 248 the current study, it is possible the perception of nutritional intake resulted in an 249 anticipatory effect encouraging increased self-selected intensity as evidenced by an 250 increased HR in the PLA and CHO trials. Although more commonly practiced during 251 exercise, this effect is not too dissimilar from the suggested mechanistic action of 252 carbohydrate mouth rinsing, where oral sensing of carbohydrate has enhanced endurance performance^{16,17}. It has been proposed that there is an increase in central 253 motor drive rather than any metabolic effects¹⁶. In the present study the increased 254 255 viscosity of the drink may have contributed to the sensing or perception of substrate and 256 an increase in central motor drive.
- The general trend of the time trials were to start quickly, slow in the second and third quarters before a tendency to speed up at the end. The lack of difference between segments in trials, particularly between the PLA and CHO trials suggests that substrate availability, or rather the increased availability from maltodextrin ingestion did not contribute to pacing. In addition, although non-significant, small differences in time to complete the first two WAT segments appeared to contribute to the overall slower time compared to the perception of breakfast.
- 264 Ingestion of maltodextrin increased carbohydrate oxidation during the steady state 265 exercise in CHO compared to PLA and likely during the time trial. Maltodextrin ingestion would have stimulated insulin release and in combination with high blood 266 glucose would decrease fatty acid oxidation¹⁸, as well as increasing glucose uptake into 267 268 the muscle¹⁹. In the current study, this did not appear to influence performance, either 269 because the time trial was too short in duration for substrate utilisation to have a 270 meaningful influence or the placebo effect of breakfast was greater than any metabolic 271 effects and had greater regulation over pacing.

One of the main aims of a carbohydrate meal after an overnight fast is to replenish liver glycogen¹³. In the current study this did not appear to enhance performance as there was no difference between the PLA and CHO performance times. The absence of a difference is likely explained by the short duration of the time trial, where less glycogen availability is required compared to longer performance tests in which differences have been observed⁵.

278

279 The pre-exercise feeding recommendation for exercise greater than 60 minutes is to ingest carbohydrate in the 1-4 hours before exercise⁷. The 90 minutes pre-exercise 280 281 breakfast ingestion in the current study found similar results to Galloway and colleagues²⁰ when ingestion occurred 2 hours before a shorter exercise capacity test 282 (~7.5-9.0 minutes). The amount of carbohydrate provided by Galloway et al.²⁰ used was 283 284 32 g (the present study used approximately 142 g) and there was no water control to 285 determine if there was a placebo effect. In an interesting caveat, in the same study, a 286 performance difference was observed when the 32 g of carbohydrate was ingested 30 287 minutes before exercise compared to a placebo. This was attributed to an increase in 288 glucose uptake and oxidation in the early stages of exercise as well as possible non-289 metabolic effects such as positive alterations in mood and arousal. When carbohydrate 290 is ingested close to exercise there is the possibility of rebound hypoglycaemia during 291 the initial stages of exercise. Although the general results are mixed, the effect on performance has been largely refuted (reviewed by ²¹). Whilst hypoglycaemia did not 292 293 occur in the present study there was a small decrease in blood glucose concentrations 294 following the 10 minute steady state. It therefore seems that for short duration exercise 295 carbohydrate ingestion close to exercise may improve performance through a partial 296 metabolic effect, however, when ingested around 90 minutes before exercise the 297 improvement in performance is likely psychological hence the similar performance observed between the PLA and CHO trials. 298

The present research was conducted in well-trained cyclists, however experience of fasted training was limited to only 6 subjects and this largely comprised of low intensity or short duration rides. As the results appeared to be driven by psychological determinants it would be of interest to study a chronic effect of fasted high intensity training to determine if cyclists could become accustomed to the effort and alter their self-selected intensity without the perception of ingesting a CHO or PLA drink.

305 Practical Applications

306 Many athletes will complete some training sessions in a fasted state, however these are 307 often limited to recovery and low intensity sessions. Typically, athletes will ingest a 308 pre-exercise meal or source of carbohydrate prior to engaging in high quality and intense bouts of exercise, even if guidelines do not necessarily suggest consumption 309 when exercise duration is less than 60 minutes⁷. The results of this study suggest that 310 311 from a physiological perspective this is not necessary; however the act of ingesting a 312 perceived breakfast improved performance regardless of energy content. Studies 313 examining alternative methods of low carbohydrate availability (i.e. training after an 314 overnight fast or in a depleted state – in both situations the athlete is not blinded to the condition) have repeatedly demonstrated a reduction in self-selected intensity^{11,12}, yet 315 316 also beneficial cell signalling responses and the increasing of mitochondrial biogenesis^{9,12,22}. This study poses the question of the possibility that the benefits of both 317

318 high (maintained self-selected intensity) and low (increased cellular adaptations) 319 carbohydrate availability can be achieved through a placebo breakfast. The placebo 320 effect is unlikely to last chronically so a carefully planned approach by the coaching 321 team is required to maximise adaptations by selecting key sessions for acute 322 implementation.

323 Conclusion

324 In conclusion, subjects in this study were able to complete a short duration 325 (approximately 20 minutes) cycling time trial quicker when they consumed a PLA or 326 CHO breakfast 90 minutes prior to the start of exercise compared to a water control. 327 The improvement in performance was due to a psychological rather than physiological 328 cause, with the subjects perceiving the ingestion of breakfast and nutrients as beneficial, 329 resulting in an increased self-selected intensity.

330 Acknowledgements

Thank you to Mr Nessan Costello, Mr Luke Hillier and Miss Ciara Noble (all School of
Sport, Exercise and Health Sciences, Loughborough University, Loughborough) for
their assistance with some of the preliminary data collection.

334 **References**

335 1. Gleeson M, Maughan RJ, Greenhaff PL. Comparison of the effects of pre-exercise
336 feedings of glucose, glycerol and placebo on endurance and fuel homeostasis in man.
337 *Eur J Appl Physiol.* 1986;55:646-653.

328 2. Neuffer PD, Costill DL, Flynn MG, Kirwan JP, Mitchell JB, Houmard J.
339 Improvements in exercise performance: effects of carbohydrate feedings and diet. J
340 Appl Physiol. 1987;62:983-988.

- 341 3. Schabort EJ, Bosch AN, Weltan SM, Noakes TD. The effect of a pre-exercise meal
 342 on time to fatigue during prolonged cycling exercise. *Med Sci Sports Exerc.*343 1999;31:464-471.
- 344 4. Sherman WM, Brodowicz G, Wright DA, Allen WK, Simonsen JC, Dernbach A.
 345 Effects of 4 h preexercise carbohydrate feedings on cycling performance. *Med Sci*346 *Sports Exerc.* 1989;21:598-604.
- 5. Sherman WM, Peden MC, Wrigh DA. Carbohydrate feedings 1 h before exercise
 improves cycling performance. *Am J Clin Nutr.* 1991;54:866-870.
- 6. Wright DA, Sherman WM, Dernbach AR. Carbohydrate feedings before, during, andin combination improves cycling performance. *J Appl Physiol*. 1991;71:1082-1088.
- 351 7. Burke LM, Hawley JA, Wong SHS, Jeukendrup AE. Carbohydrates for training and
 352 competition. *J Sports Sci.* 2011;29(S1):S17-S27.
- 8. Palmer GS, Clancy MC, Hawley JA, Rodger IM, Burke LM, Noakes TD.
 Carbohydrate ingestion immediately before exercise does not improve 20km time trial
 performance in well trained cyclists. *Int J Sports Med.* 1998;19:415-418.
- 356 9. Van Proeyen K, Szlufcik K, Nielens H, Ramaekers M, Hespel P. Beneficial
 357 metabolic adaptations due to endurance exercise training in the fasted state. *J App*358 *Physiol.* 2011;110:236-245.
- 359 10. Burke LM. Fueling strategies to optimize performance: training high or training low?
 360 *Scand J Med Sci Sports.* 2010;20:48-58.
- 11. Hulston CJ, Venables MC, Mann CH, Martin C, Philp A, Baar K, Jeukendrup AE.
 Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med Sci Sports Exerc.* 2010;42:2046-2055.
- 364 12. Yeo WK, Paton CD, Garnham AP, Burke LM, Carey AL, Hawley JA. Skeletal
 365 muscle adaptation and performance responses to once a day versus twice every second
 366 day endurance training regimens. *J Appl Physiol.* 2008;105:1462-1470.
- 367 13. Taylor R, Magnusson I, Rothman DL, Cline GW, Caumo A, Cobelli C, Shulman GI.
 368 Direct assessment of liver glycogen storage by 13C nuclear magnetic resonance
 369 spectroscopy and regulation of glucose homeostasis after a mixed meal in normal
 370 subjects. *J Clin Invest.* 1996;97:126-132.
- 14. Clark VR, Hopkins WG, Hawley JA, Burke LM. Placebo effect of carbohydrate
 feedings during 40-km cycling time trial. *Med Sci Sports Exerc*. 2000;32:1642-1647.

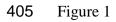
- 373 15. Hulston CJ, Jeukendrup AE. No placebo effect from carbohydrate intake during
 374 prolonged exercise. *Int J Sport Nutr Exerc Metab.* 2009;19:275-284.
- 16. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1h cycle time trial performance. *Med Sci Sports Exerc*. 2004;36:2107-2111.
- 377 17. Rollo I. Cole M, Miller R, Williams C. Influence of mouth rinsing a carbohydrate
 378 solution on 1-h running performance. *Med Sci Sports Exerc.* 2010;42:798-804.
- 379 18. Bonen A, Malcolm SA, Kilgour RD, MacIntyre KP, Belcastro AN. Glucose
 380 ingestion before and during intense exercise. J Appl Physiol Respir Environ Exerc
 381 Physiol. 1981;50:766-771.
- 382 19. Douen A, Ramlal T, Rastogi S, Bilan P, Cartee G, Vranic M, Holloszy J, Klip A.
 383 Exercise induces recruitment of the 'insulin-responsive glucose transported'. Evidence
 384 for distinct intracellular insulin- and exercise-recruitable transported pool in skeletal
 385 muscle. *J Biol Chem.* 1990;265:13427-13430.
- 386 20. Galloway SDR, Lott MJE, Toulouse LC. Preexercise carbohydrate feeding and
 387 high-intensity exercise capacity: effects of timing of intake and carbohydrate
 388 concentration. *Int J Spor Nutr Exerc Metab.* 2014;258-266.21. Jeukendrup AE, Killer
 389 SC. The myths surrounding pre-exercise carbohydrate feeding. *Nutr Metab.*390 2010;57:18-25.
- 391 22. Morton JP, Croft L, Bartlett JD, MacLaren DPM, Reilly T, Evans L, McArdle A,
 392 Drust B. Reduced carbohydrate availability does not modulate training-induced heat
 393 shock protein adaptations but does upregulate oxidative enzyme activity in human
 394 skeletal muscle. *J App Physiol.* 2009;106:1513-1521.

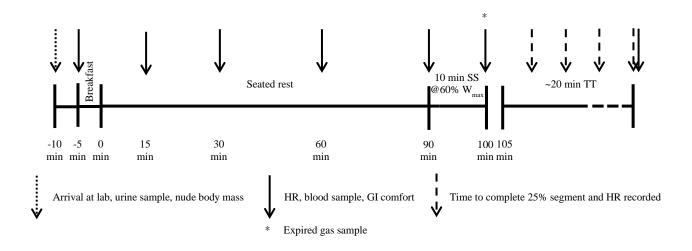
395 List of figures

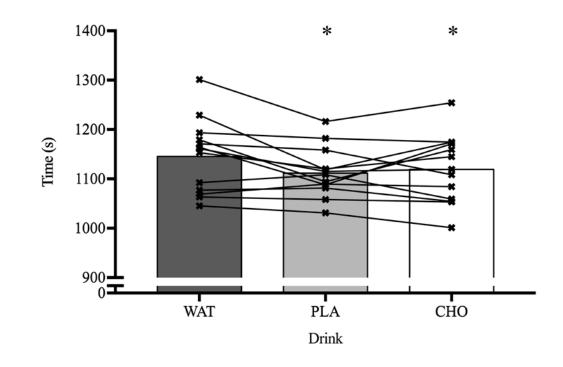
- **396** Figure 1. Schematic overview of the experimental trial
- Figure 2. Time to complete the time trial. Lines denote individual performances. * denotes different to WAT trial (P < 0.05). Mean \pm SD

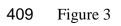
Figure 3. Time splits for each 25% segment. * denotes quicker completion of segment compared to 50-75%. # denotes quicker completion of segment compared to 25-50% (P 401 < 0.05). Mean ± SD

402Figure 4. Blood (a) glucose and (b) lactate concentrations during the recovery and403exercise periods. * denotes different to WAT and PLA trials. # denotes different to404baseline in CHO trial. \$ denotes different to baseline in all trials (P < 0.05). Mean ± SD

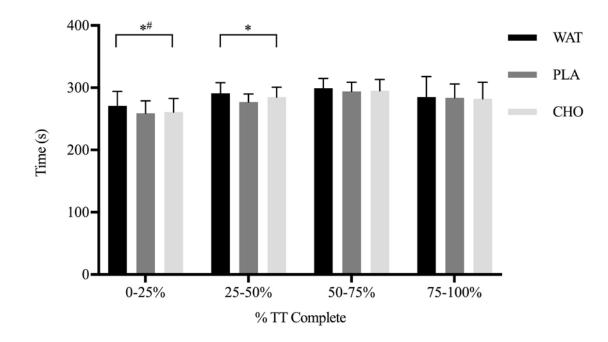






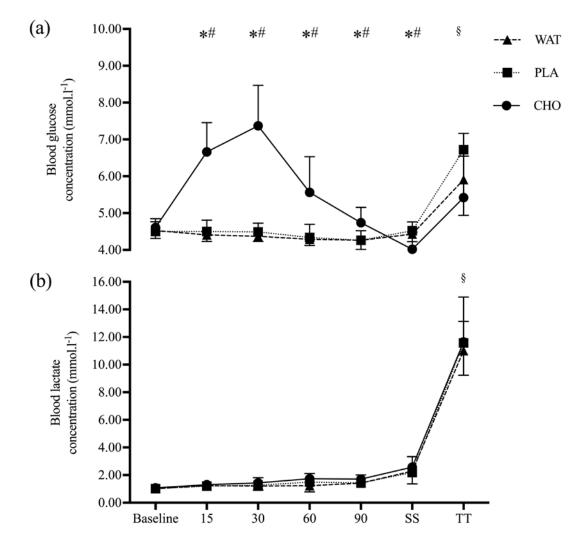








412 Figure 4



Sample

