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Carbohydrate mouth rinse and caffeine improves high-intensity interval running capacity when carbohydrate restricted

Andreas M. Kasper[a], Scott Cocking[a], Molly Cockayne[a], Marcus Barnard[a], Jake Tench[a], Liam Parker[a], John McAndrew[a], Carl Langan-Evans[a], Graeme L. Close[a] & James P. Morton[a]
[a] Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK
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ORIGINAL ARTICLE

Carbohydrate mouth rinse and caffeine improves high-intensity interval running capacity when carbohydrate restricted

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Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

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Abstract
We tested the hypothesis that carbohydrate mouth rinsing, alone or in combination with caffeine, augments high-intensity interval (HIT) running capacity undertaken in a carbohydrate-restricted state. Carbohydrate restriction was achieved by performing high-intensity running to volitional exhaustion in the evening prior to the main experimental trials and further refraining from carbohydrate intake in the post-exercise and overnight period. On the subsequent morning, eight males performed 45-min steady-state (SS) exercise (65% $\dot{V}O_{2\,max}$) followed by HIT running to exhaustion (1-min at 80% $\dot{V}O_{2\,max}$ interspersed with 1-min walking at 6 km/h). Subjects completed 3 trials consisting of placebo capsules (administered immediately prior to SS and immediately before HIT) and placebo mouth rinse at 4-min intervals during HIT (PLACEBO), placebo capsules but 10% carbohydrate mouth rinse (CMR) at corresponding time-points or finally, caffeine capsules (200 mg per dose) plus 10% carbohydrate mouth rinse (CAFF + CMR) at corresponding time-points. Heart rate, capillary glucose, lactate, glycerol and NEFA were not different at exhaustion during HIT ($P > 0.05$). However, HIT capacity was different ($P < 0.05$) between all pair-wise comparisons such that CAFF + CMR (65 ± 26 min) was superior to CMR (52 ± 23 min) and PLACEBO (36 ± 22 min). We conclude that carbohydrate mouth rinsing and caffeine ingestion improves exercise capacity undertaken in carbohydrate-restricted states. Such nutritional strategies may be advantageous for those athletes who deliberately incorporate elements of training in carbohydrate-restricted states (i.e. the train-low paradigm) into their overall training programme in an attempt to strategically enhance mitochondrial adaptations of skeletal muscle.

Keywords: *Train-low, HIT, fatigue, mouth rinse*

Introduction

Traditional nutritional approaches for endurance training have typically promoted high carbohydrate availability before, during and after training sessions so as to fuel the energy requirements of high daily training intensities and volumes (Burke, Hawley, Wong, & Jeukendrup, 2011). However, during the past decade, data from our laboratory and others have demonstrated that deliberately training in conditions of *reduced* carbohydrate availability can promote training-induced adaptations of human skeletal muscle (Bartlett, Hawley, & Morton, 2015), as demonstrated by increased maximal mitochondrial enzyme activities and mitochondrial content (Morton et al., 2009; Yeo et al., 2008), increased rates of lipid oxidation (Hulston et al., 2010) and in some instances, improved exercise capacity (Hansen et al., 2005). Such data have led to the concept of 'training-low, but competing-high' whereby selected training sessions are completed in conditions of reduced carbohydrate availability (so as to promote training adaptation) but competition is supported with high carbohydrate availability (Bartlett et al., 2015).

The augmented adaptive responses of skeletal muscle observed with training-low strategies are likely regulated by enhanced activation of key cell signalling kinases (e.g. AMPK, p38MAPK), transcription factors (e.g. p53, PPARδ) and transcriptional co-activators (e.g. PGC-1α) such that a co-ordinated up-regulation of both the nuclear

Correspondence: J. Morton, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Tom Reilly Building, Byrom St. Campus, Liverpool, L3 3AF, UK. E-mail: J.P.Morton@ljmu.ac.uk

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and mitochondrial genomes occur (Bartlett et al., 2013; Cochran, Little, Tarnopolsky, & Gibala, 2010; Psilander, Frank, Flockhart, & Sahlin, 2013; Yeo et al., 2010). It is noteworthy, however, that an up-regulation of the above cell signalling pathways cannot be considered as proxy markers of improved exercise performance given that actual changes in performance (despite increases in mitochondrial enzymes and alterations to substrate metabolism during exercise) have not always been observed. Nonetheless, the capacity of altered carbohydrate availability to enhance activation of the aforementioned signalling pathways forms the theoretical basis for incorporating periods of train-low into an athlete's overall training programme (Hawley & Morton, 2014). Despite the premise promoting train-low, its practical application is limited by potential perturbations to immune function (Gleeson, Nieman, & Pedersen, 2007), increased muscle protein breakdown (Howarth, Moreau, Phillips, & Gibala, 2009) and of course, an inability to maintain the desired training intensity and/or duration (Hulston et al., 2010; Yeo et al., 2008). For this reason, it is therefore essential that sessions of training-low are carefully integrated into a periodised training programme whereby sessions of deliberately training-high provide the platform for those training sessions where training intensity is priority.

In those instances when training-low is the goal, however, it is prudent to implement strategies that attempt to maintain or indeed rescue the capacity to perform the desired training workloads. One strategy may be to target nutritional interventions to the central nervous system so as to potentially restore training capacity. In this regard, Lane, Bird, Burke, and Hawley (2013b) demonstrated that carbohydrate mouth-rinsing (a strategy known to activate reward regions in the brain) improved cycling exercise performance to a greater extent when fasted as opposed to the fed state. Furthermore, the same researchers also observed that caffeine ingestion can partially restore power outputs during cycling based training sessions undertaken in a low glycogen state to that observed when in glycogen loaded conditions (Lane et al., 2013a). Taken together, these data suggest that the combination of both pre-exercise caffeine ingestion and carbohydrate mouth-rinsing during exercise may therefore have additive effects in augmenting measures of training performance during those sessions that are deliberately commenced in conditions of reduced carbohydrate availability. Such a strategy may also be particularly pertinent for running exercise given that running is more dependent on carbohydrate utilisation than when cycling at the same relative exercise intensities (Arkinstall et al., 2004).

Accordingly, the aim of the present study was to therefore test the hypothesis that carbohydrate mouth rinsing, alone or in combination with caffeine intake, augments high-intensity interval (HIT) running capacity undertaken in carbohydrate restricted states. To achieve our model of carbohydrate restriction, we utilised a 'sleep-low, train-low' dietary and exercise protocol recently studied in our laboratory, whereby subjects performed a morning training session after an overnight fast and having also completed a prolonged and exhaustive exercise protocol in the evening prior (Bartlett et al., 2013).

Methods

Subjects

Eight recreationally active males who regularly engaged in running exercise (3–5 times per week) volunteered to participate in the study (mean ± SD: age, 22 ± 2 years; body mass, 70.8 ± 8.1 kg; height, 1.78 ± 0.09 m, $\dot{V}O_{2\,max}$, 57 ± 5 $ml.kg^{-1}.min^{-1}$). All subjects gave written and informed consent after details of the study procedures were fully explained. No subject had a history of smoking or cardiovascular and metabolically related disease and none were under any pharmacological treatment during the course of the study. All subjects refrained from exercise and consuming alcohol and common caffeine containing substances for 24–48 h before each trial. The study was approved by the Ethics Committee of Liverpool John Moores University.

Experimental design

In a randomised, repeated measures and double blind design, subjects performed an exhaustive running exercise protocol in the evening prior to arriving in the laboratory on the subsequent morning in a fasted state where they then performed a steady-state (45 min at 65% $\dot{V}O_{2\,max}$) running exercise protocol followed by a HIT running protocol to exhaustion (1-min bouts at 80% $\dot{V}O_{2\,max}$ interspersed with 1-min bouts walking at 6 km/h). Subjects ingested a standardised caffeine dose (200 mg) or placebo (in capsule format) immediately prior to commencing the steady-state exercise protocol and a further standardised caffeine dose (200 mg) or placebo immediately prior to commencing the HIT protocol. During HIT, subjects also rinsed a 10% carbohydrate mouth rinse or taste matched placebo (for 10-second periods) at 4-min intervals during exercise. In this way, each subject therefore completed three experimental trials consisting of both placebo capsules and placebo mouth rinse (PLACEBO), placebo capsules but carbohydrate mouth rinse (CMR) or caffeine capsules plus carbohydrate mouth rinse (CAFF +

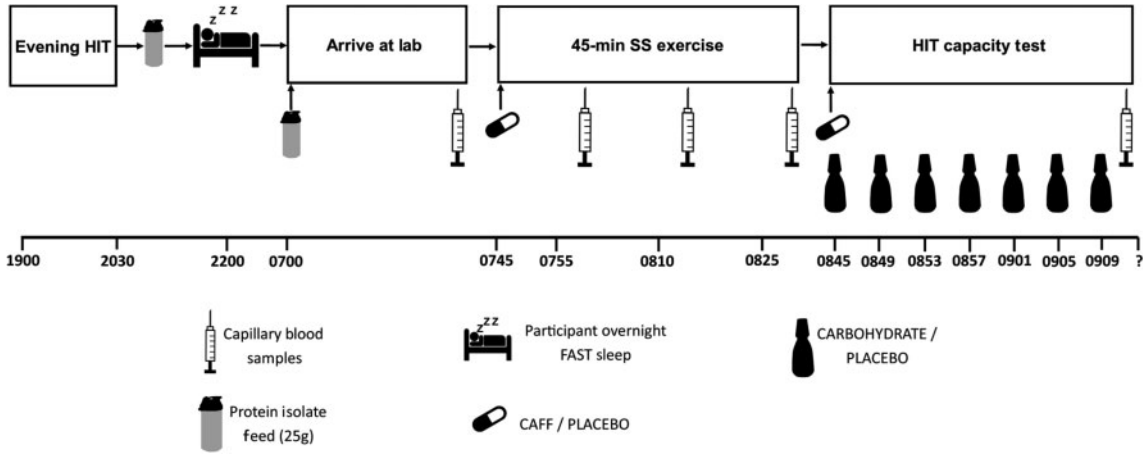

Figure 1. Overview of the experimental design

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CMR). Our primary outcome variable was exercise capacity during the HIT protocol. Heart rate, ratings of perceived exertion and fingertip capillary blood samples were also obtained at regular intervals during the steady-state exercise protocol so as to assess for physiological, metabolic and perceptual responses to exercise. An overview of the experimental design is shown in Figure 1.

Assessment of maximal oxygen uptake

At least 7–10 days prior to the first familiarisation trial, subjects performed a continuous incremental treadmill protocol run to volitional exhaustion on a motorised treadmill (h/p/cosmos – Pulsar, Nussdorf-Traunstein, Germany) for the determination of maximal oxygen uptake ($\dot{V}O_{2\,max}$). The test protocol commenced with a 2 min stage at a treadmill speed of 10 $km{\cdot}h^{-1}$ followed by 2 min stages at 12, 14, 16 and 18 $km{\cdot}h^{-1}$. After completion of the 18 $km{\cdot}h^{-1}$ stage, the treadmill inclined by 2% every 2 min thereafter until volitional exhaustion. Breath-by-breath measurements were obtained throughout exercise using a CPX Ultima series online gas analysis system (Medgraphics, Minnesota, USA) and $\dot{V}O_{2\,max}$ was stated as being achieved by the following end point criteria (1) heart rate within 10 $b{\cdot}min^{-1}$ of age predicted maximum, (2) RER > 1.1 and (3) plateau of oxygen consumption despite increasing workload. The $\dot{V}O_{2\,max}$ relationship with running speed was used to determine the appropriate running speeds for the experimental trials described below.

Carbohydrate restriction protocol

Subjects reported to the laboratory at approximately 1900 h to perform 90-min of HIT running (or to exhaustion if this occurred first) where the aim was to reduce muscle glycogen stores. Following a 5 min self-selected warm-up, participants commenced running for 2 min bouts at a velocity corresponding to 100% $\dot{V}O_{2\,max}$ interspersed with 2 min recovery periods at 60% $\dot{V}O_{2\,max}$. When subjects were unable to complete 2 min work bouts at 100% $\dot{V}O_{2\,max}$, the work-rest ratio was reduced to 1.5 min–2 min and finally, 1 min–2 min. When participants were unable to complete 1-min work bouts at 100% $\dot{V}O_{2\,max}$, the running velocity was reduced to 90% $\dot{V}O_{2\,max}$ and the same pattern of work-rest ratio was completed as described above. The glycogen depletion protocol was performed for 90 min or until the point of exhaustion, the latter defined as inability maintain 1-min work bouts at 70% $\dot{V}O_{2\,max}$. The pattern of exercise completed in subject's initial trial was replicated for their remaining two experimental trials. Subjects were also permitted to consume water *ad libitum* during exercise with the pattern of ingestion replicated for all trials. At 15-min after completion of each trial, subjects ingested 25 g of whey protein isolate (Myprotein®Inc, Northwich, UK) mixed with 500 ml of water.

Steady-state (SS) exercise protocol and HIT exercise capacity test

Subjects arrived in the laboratory at 0700 h on the subsequent morning after an overnight fast of approximately 10 h. Subjects were immediately provided with an additional 25 g of whey protein isolate (Myprotein®Inc, Northwich, UK) mixed with 500 ml of water at 45 min prior to completing the SS exercise protocol, the latter consisting of 45 min at 65% $\dot{V}O_{2\,max}$. Subjects also consumed 200 mg of caffeine (Myprotein®Inc, Northwich, UK) or visually identical placebo capsules (Whey Protein Isolate, Myprotein®Inc, Northwich, UK) immediately prior to and immediately upon completion of the SS exercise protocol. Given that peak plasma caffeine typically occurs 45 min post-ingestion (Graham & Spriet, 1995), we deliberately chose to administer

caffeine immediately prior to SS exercise so that peak plasma caffeine levels would be observed immediately prior to commencing the HIT protocol. Furthermore, this standardised dose of caffeine of 200 mg corresponds to approximately 3 mg/kg for the body mass of our subjects and is therefore commensurate with known doses that are ergogenic (Spriet, 2014). Additionally, given that caffeine consumed during prolonged exercise is also ergogenic (Cox et al., 2002) and on the basis of our exercise capacity observed during familiarisation trials, we also chose to administer a further 200 mg dose immediately prior to the HIT protocol in an attempt to further augment ergogenic effects. Measurements of heart rate (Polar, S610i, Finland) and ratings of perceived exertion (RPE, Borg, 1973) were also obtained at 15-min intervals during SS exercise. After completion of the SS protocol, subjects walked for 2 min at 6 km/h and subsequently commenced a HIT exercise capacity test consisting of 1-min bouts at 80% $\dot{V}O_{2\,max}$ interspersed with 1-min bouts walking at 6 km/h until volitional exhaustion. Subjects rinsed 25 ml of a 10% CHO beverage or a taste matched (orange) and visually identical placebo solution (Robinsons Squash, Britvic Orange Soft Drinks© PLC, Hertfordshire, UK) with/without maltodextrin (Myprotein®Inc, Northwich, UK), respectively, for 10-second periods every 4-min during exercise before expectorating. Capillary blood samples were also collected at 15-min intervals during the SS protocol and at the point of exhaustion during the HIT capacity test. Water ingestion was permitted *ad libitum* during both exercise protocols with the pattern of ingestion replicated across all experimental trials. Immediately after completion of the third main experimental trial, each subject was asked to identify the sequence of treatments they had received. Only one subject from the eight studied was able to correctly identify the sequence of their treatments administered.

Familiarisation

All eight subjects completed the full experimental protocol described above whilst adhering to the PLACEBO conditions at least 5–7 days prior to commencing their first experimental trial, thereby serving as a familiarisation trial (FAM). Upon completion of all three experimental trials, we compared each subject's exercise capacity during the FAM trial and the PLACEBO trial of the main experimental trials and observed no significant difference between trials, as evidenced by students-*t*-test for paired samples (FAM = 33 ± 15, PLACEBO = 36 ± 22, $P = 0.67$).

Blood analyses

Blood glucose and lactate concentrations were obtained via finger prick capillary sampling using a 1.8 mm safety lancet (Sarstedt, Aktiengesellschaft & Co., Sweden) after sterilisation using a pre-injection medical swab (Medlock Medical Ltd., Oldham). Five µl of whole blood was immediately analysed for glucose and lactate concentration using an automated glucose analyser (HemoCue Glucose 201$^+$ Analyser, Ängelholm, Sweden) and lactate analyser (Lactate Pro, Arkray, Shiga, Japan), respectively. Additionally, approximately 200 µl of capillary blood was collected in EDTA tubes, centrifuged at 1500 g for 10 min at 4°C and plasma was stored at −80°C until later analysis. Plasma glycerol and NEFA were determined using commercially available kits (Randox, Daytona, UK).

Statistical analyses

Data were analysed by one-way and/or two-way within subject General Linear Models (GLMs) with repeated measures (version 18 for Windows, SPSS Inc, Chicago, IL, USA). Prior to analysis, all data were analysed for normal distribution using the Shapiro-Wilks test. Differences in exercise related variables (i.e. physiological, perceptual and metabolic variables) were analysed using a two-way repeated measures GLM where the within factors were time (i.e. exercise) and condition (i.e. PLACEBO v CMR v CAFF + CMR). Differences in exercise capacity (i.e. time to exhaustion) between conditions were analysed using a one-way repeated measures GLM. Where there was a significant difference, a paired *t*-test using Bonferonni corrections for multiple comparisons was employed for post hoc analysis. All values are expressed as means and SD, with the statistical level of significance established at $P < 0.05$. 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 and Hopkins (2006).

Results

Physiological and perceptual responses during SS exercise and HIT capacity test

Subjects' heart rate and RPE during the SS and HIT protocols are displayed in Table I. Both heart rate and RPE increased during exercise (both $P < 0.01$) though neither variable was different between trials ($P = 0.42$ and 0.94, respectively). Subjects' running

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Table I. Heart rate and RPE during the SS exercise protocol and at exhaustion following the HIT capacity test in the PLACEBO, CMR and CAFF + CMR experimental trials

	Time (min)			
	15	30	45	Exhaustion
Heart rate (b.min^{-1})				
PLACEBO	141 ± 16	149 ± 20	151 ± 23*	159 ± 11*
CMR	144 ± 16	153 ± 15	153 ± 14*	166 ± 9*
CAFF + CMR	138 ± 21	148 ± 21	155 ± 16*	166 ± 12*
RPE (AU)				
PLACEBO	12 ± 3	14 ± 2	14 ± 3*	19 ± 1*
CMR	12 ± 2	14 ± 2	14 ± 2*	19 ± 1*
CAFF + CMR	12 ± 1	13 ± 1	14 ± 1*	19 ± 1*

*denotes significant difference from 15, $P < 0.05$.

velocity corresponding to 80 and 65% $\dot{V}O_{2\,max}$ was 14.2 ± 1.7 and 11.6 ± 0.3 km.h^{-1}, respectively.

Metabolic responses during SS exercise and HIT capacity test

Blood glucose, blood lactate, plasma glycerol and plasma NEFA are displayed in Table II. Glucose, lactate, glycerol and NEFA all displayed significant changes during exercise (all $P < 0.01$) though there were no significant differences between trials (P = 0.41, 0.24, 0.18 and 0.56, respectively). Specifically, glucose significantly decreased from pre-exercise following completion of the SS protocol ($P = 0.04$) and at the point of exhaustion during the HIT protocol ($P < 0.01$). In contrast, changes in blood lactate were only significant at the point of exhaustion upon completion of the HIT capacity test ($P < 0.01$). Plasma glycerol and NEFA showed progressive increases during exercise such that each time-point was significantly different from the preceding time-point, after the point at which significance from pre-exercise values were first detected (all $P < 0.05$).

Exercise capacity during HIT test

Exercise capacity during the HIT test is displayed in Figure 2a,b where both group means and individual responses are shown, respectively. There was a significant main effect between trials ($P < 0.01$) where both CMR (52 ± 23 min; $P = 0.06$) and CAFF + CMR (65 ± 26 min; $P < 0.01$) were different from PLACEBO (36 ± 22 min). Seven of the eight subjects ran longer in both the CMR (95% CI for differences = −1.6 to +33 min, *possibly beneficial*) and CAFF + CMR conditions (95% CI for differences = +10 to +46 min, *beneficial*) versus the PLACEBO trial. Furthermore, all eight subjects ran longer in the CAFF + CMR trial (95% CI for differences = +8 to +17 min, *beneficial*) compared with the CMR only trial ($P < 0.01$).

Discussion

Confirming our hypothesis, we provide novel data by demonstrating that carbohydrate mouth rinsing improves HIT running capacity undertaken in conditions of carbohydrate restriction when compared with rinsing a placebo solution. We further demonstrate that carbohydrate mouth rinsing with co-ingestion of caffeine before and during exercise augments exercise capacity compared to carbohydrate mouth rinse

Table II. Blood glucose, blood lactate, plasma glycerol and plasma NEFA during the SS exercise protocol and at exhaustion following the HIT capacity test in the PLACEBO, CMR and CAFF + CMR experimental trials

	Time (min)				
	Pre-	15	30	45	Exhaustion
Glucose (mmol.L^{-1})					
PLACEBO	4.5 ± 0.7	3.9 ± 0.7	4.0 ± 0.7	3.7 ± 0.7	3.6 ± 0.6*
CMR	4.4 ± 0.5	4.2 ± 0.5	4.2 ± 0.3	3.9 ± 0.4	3.7 ± 0.6*
CAFF + CMR	4.5 ± 0.4	4.1 ± 0.3	4.3 ± 0.2	4.3 ± 0.3	3.7 ± 0.5*
Lactate (mmol.L^{-1})					
PLACEBO	0.9 ± 0.2	1.0 ± 0.3	1.1 ± 0.3	1.3 ± 0.4	1.8 ± 0.7*
CMR	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	1.7 ± 0.5*
CAFF + CMR	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.4	1.2 ± 0.4	2.4 ± 0.9*
Glycerol (μmol.L^{-1})					
PLACEBO	58 ± 51	173 ± 52*	309 ± 102*	351 ± 87*	444 ± 127*
CMR	46 ± 33	181 ± 102*	288 ± 141*	288 ± 77*	456 ± 116*
CAFF + CMR	75 ± 81	216 ± 70*	360 ± 109*	337 ± 96*	541 ± 171*
NEFA (mmol.L^{-1})					
PLACEBO	0.81 ± 0.31	1.00 ± 0.28	1.48 ± 0.41*	1.98 ± 0.38*	2.42 ± 0.48*
CMR	0.80 ± 0.25	1.14 ± 0.47	1.32 ± 0.53*	1.49 ± 0.32*	2.43 ± 0.53*
CAFF + CMR	0.80 ± 0.23	1.00 ± 0.35	1.63 ± 0.38*	1.67 ± 0.57*	2.69 ± 0.91*

*denotes significant difference from pre-exercise, $P < 0.05$.

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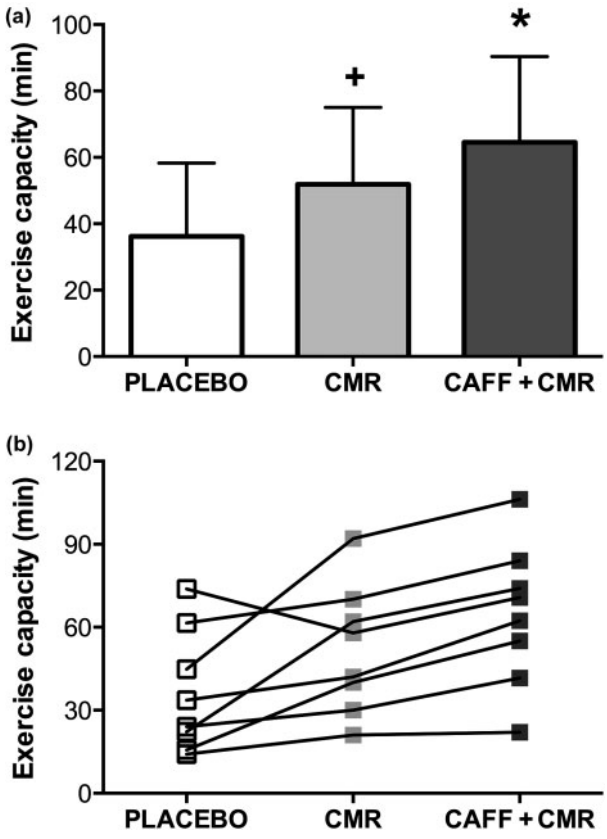

Figure 2. (a) Exercise capacity during the HIT capacity test (undertaken immediately after completion of the 45-min SS exercise protocol) in the PLACEBO, CMR and CAFF + CMR experimental trials. Data are expressed as groups means where + denotes difference from PLACEBO (P = 0.06) and * denotes significant difference from both PLACEBO and CMR (P < 0.01). (b) Individual subject's exercise capacity during the HIT capacity test in the PLACEBO, CMR and CAFF + CMR experimental trials.

per se. Given that the capacity to perform HIT exercise was enhanced with this feeding protocol, we therefore consider our data to have obvious practical implications for those athletes who deliberately train in carbohydrate restricted states in an attempt to strategically enhance mitochondrial related adaptations.

To achieve our model of carbohydrate restriction, we employed a 'sleep-low, train-low' dietary and exercise protocol recently studied in our laboratory, whereby subjects perform a morning training session after refraining from carbohydrate intake and having also completed a glycogen depletion training protocol in the evening prior (Bartlett et al., 2013). Although we did not directly quantify muscle glycogen levels, our observation of capillary metabolite data (e.g. glucose, lactate, NEFA and glycerol) are consistent with that previously observed in our laboratory when exercising in conditions of severe carbohydrate restriction (Bartlett et al., 2013; Taylor et al., 2013). In an attempt to prevent excessive protein breakdown and promote muscle protein synthesis, we also fed 25 g of protein prior to sleep (Res et al., 2012) and at 45 min prior to commencing the SS exercise bout (Coffey et al., 2011). We have recently reported that this approach is associated with positive modulation of those molecular pathways purported to regulate both mitochondrial biogenesis (e.g. AMPK, p53 activation) and protein synthesis (eEF2 activation) (Bartlett et al., 2013; Taylor et al., 2013). As such, this protocol represents a model of exercising with reduced carbohydrate but high protein availability and is a nutritional approach to fuelling training sessions recently reported to be adopted by elite endurance athletes (Walsh, 2014).

Despite the theoretical rationale for adopting this dietary approach to training, its practical application is often limited by the inability to maintain the required training intensity and/or duration. To this end, we observed that rinsing a 10% carbohydrate solution for 10-second periods at 4-min intervals during exercise significantly improved HIT capacity when compared with rinsing a placebo solution. These data therefore extend a growing body of literature demonstrating the carbohydrate mouth rinsing improves endurance performance during both cycling (Carter, Jeukendrup, & Jones, 2004a; Carter, Jeukendrup, Mann, & Jones, 2004b) and running exercise (Rollo, Cole, Miller, & Williams, 2010), an effect likely mediated via direct effects on the CNS (Chambers, Bridge, & Jones, 2009). Indeed, using functional magnetic resonance imaging, the latter authors observed that oral taste receptors in the mouth appear receptive to carbohydrate (independent of sweetness) that, in turn, activates a multitude of brain regions including the anterior cingulate cortex and ventral striatum thereby inducing emotional, cognitive and behavioural responses (Rolls, 2007). Importantly, this is also the first report to demonstrate a positive effect of carbohydrate mouth rinsing when simultaneously exercising in both a glycogen reduced and overnight fasted state as opposed to the latter *per se*. Indeed, seven of our eight subjects displayed substantial improvements in exercise capacity when compared with placebo rinsing (95% CI for differences of −2 to +33 min), a magnitude of improvement which was typically larger than when subjects commenced an exercise capacity test in the fed state (Fares & Kayser, 2011). As such, these data appear consistent with emerging data demonstrating that the ergogenic effects of carbohydrate mouth rinsing are more prominent when the duration of the pre-exercise fasting period increases (Beelen et al., 2009; Lane et al., 2013a). Support for this hypothesis was provided by Haase, Cerf-Ducastel, and Murphy (2009) who reported that greater brain activity was observed in the ventral striatum, amygdala and hypothalamus when sucrose was ingested in conditions of hunger

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(i.e. after a 12 h fast) versus conditions of satiety (i.e. the post-prandial state).

In an attempt to further augment HIT exercise capacity, we also conducted an experimental trial in which subjects performed the carbohydrate mouth rinsing protocol but also consumed 200 mg of caffeine immediately prior to SS and a further 200 mg immediately prior to the HIT test. We deliberately chose to administer a standardised dose of caffeine (i.e. 2 × 200 mg) based on feedback from athletes that tend to adopt a standardised caffeine feeding protocol (as based on the dose inherent to commercially available products) as opposed to prescribing to body mass. Nevertheless, in relation to the body mass of the current subjects, this dose corresponds to 5–6 mg/kg body mass and is therefore consistent with the upper range of the dose that is now well documented to be ergogenic to performance (Burke, Desbrow, & Spriet, 2013). Accordingly, we observed that co-ingestion of caffeine improved HIT capacity when compared with carbohydrate mouth rinsing per se, an effect that was apparent in all 8 subjects (95% CI for differences of +8 to +17 min).

It should be noted, however, that the ergogenic proprieties of caffeine observed here are likely to have been observed with less aggressive forms of caffeine supplementation. For example, although we chose a 24–48 h withdrawal period from habitual caffeine intake prior to the main experimental trial, caffeine has also been shown to enhance endurance performance in the absence of a withdrawal period, an effect that is evident in both moderate (240 ± 162 $mg.day^{-1}$) and high (761 ± 12 $mg.day^{-1}$) habitual users (Irwin et al., 2011; Van Soeren & Graham, 1998). Furthermore, a much smaller absolute dose of caffeine (3 $mg.kg^{-1}$) ingested 60–90 min before an acute exercise protocol undertaken in conditions of both high (Irwin et al., 2011) and low carbohydrate availability (Lane et al., 2013a) has also proven ergogenic. When taken together, it is therefore apparent that a dosing strategy prescribed without prior withdrawal and according to body mass (and not on the basis of the absolute caffeine dose present in commercial products) may be a more practically applicable protocol. This is especially relevant for those athletes who are smaller in stature (e.g. <60 kg), given that negative side effects may actually be observed with the high absolute dose adopted here (Graham & Spriet, 1995).

We also acknowledge that our data are limited in that we did not also study caffeine only trial and hence, our observed performance effects may be due to the effects of caffeine *per se* as opposed to additive effects when combined with carbohydrate mouth rinsing. Nevertheless, we extend recent data from cycling based training sessions commenced in glycogen depleted states (Lane et al., 2013b) and demonstrate that caffeine ingestion is also a highly practical ingestion to improve HIT capacity when running is the exercise modality and exercise is also completed in conditions of reduced carbohydrate availability. Given that we observed no differences in plasma NEFA or glycerol availability, the observed ergogenic effect is likely to be due to the now well-documented effects of caffeine on the CNS (Meeusen, 2014) as opposed to alterations in substrate utilisation. Indeed, caffeine is readily transported across the blood-brain barrier and can act as an adenosine antagonist thereby opposing the action of adenosine. As such, caffeine can increase concentrations of important neurotransmitters such as dopamine (Fredholm, 1995), the result of which manifests itself as increased motivation (Maridakis, Herring, & O' Connor, 2009) and motor drive (Davis et al., 2003). Alternatively, our observed performance effect may also be due, in part, to an additional mechanism related to maintenance of muscle membrane excitability (Mohr, Nielsen, & Bangsbo, 2011). For example, the latter authors observed improved performance on Yo-Yo Intermittent Recovery Test 2 following caffeine supplementation (using a 6 mg/kg dose similar to that employed here) that was associated with reduced muscle interstitial accumulation of potassium (K^+) during intense intermittent exercise. The latter observation is consistent with the notion that extra-cellular accumulation of potassium is a contributing cause of fatigue during very high-intensity exercise.

In summary, we provide novel data by demonstrating that both pre-exercise caffeine ingestion and carbohydrate mouth rinsing during exercise augments HIT running capacity in conditions of carbohydrate restriction, when compared with either mouth-rinse or placebo conditions. As such, we consider our data to have practical applications for those athletes who deliberately incorporate periods of carbohydrate restriction into their training programmes in an attempt to strategically enhance mitochondrial related adaptations of skeletal muscle. Future studies should now test whether the improved physical performance observed during acute training sessions translates to improvements in training adaptations and endurance performance when performed chronically as part of a periodised endurance training programme.

Disclosure statement

No potential conflict of interest was reported by the authors.

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