Respiratory Physiology & Neurobiology 193 (2014) 11–20

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Influence of dietary nitrate supplementation on physiological and cognitive responses to incremental cycle exercise

Kevin G. Thompson[a,*], Louise Turner[b], Jonathon Prichard[b], Fiona Dodd[b], David O. Kennedy[b], Crystal Haskell[b], James R. Blackwell[c], Andrew M. Jones[c]

[a] University of Canberra, Canberra, ACT 2601, Australia
[b] Northumbria University, Newcastle NE18ST, UK
[c] University of Exeter, Exeter EX12LU, UK

ARTICLE INFO

Article history:
Accepted 24 December 2013

Keywords:
Dietary nitrate
Exercise tolerance
Cognitive function

ABSTRACT

Dietary inorganic nitrate supplementation causes physiological effects which may enhance exercise tolerance. However it is not known whether nitrate might alter cognitive function during exercise. In a double-blind, cross-over study, sixteen subjects ingested either nitrate-rich beetroot juice or a placebo and completed a continuous cycle exercise test involving 20 min stages at 50% and 70% $\dot{V}O_{2peak}$ and a final stage at 90% $\dot{V}O_{2peak}$ until volitional exhaustion. Cognitive tasks were completed before, during and after exercise. In the dietary nitrate condition: plasma [nitrite] increased ($p < 0.01$), systolic blood pressure decreased ($p < 0.05$) and there was a trend for a reduced oxygen uptake at 50% $\dot{V}O_{2peak}$. Tissue oxygenation improved across exercise intensities and exercise tolerance was greater at 90% $\dot{V}O_{2peak}$ ($p < 0.05$). Rating of perceived exertion, energy levels and cognitive performance were similar between conditions with mental fatigue being evident from 70% $\dot{V}O_{2peak}$ onwards ($p < 0.05$). Dietary nitrate supplementation enhanced short-term endurance exercise performance with concomitant mental fatigue but did not improve cognitive performance post-fatigue.

1. Introduction

The determinants of human exercise tolerance remain obscure but, depending on the subject population and the exercise modality, intensity and duration, may be related to both central and peripheral factors and to the balance between oxidative and non-oxidative contributions to energy turnover (Knicker et al., 2011). For endurance type exercise, it has been proposed that the interaction between exercise efficiency or economy and the peak oxygen uptake ($\dot{V}O_{2peak}$) may be a critical determinant of performance (Coyle, 1995). Therefore, interventions which enhance exercise efficiency or $\dot{V}O_{2peak}$ are of considerable interest to athletes and coaches.

The increase in $\dot{V}O_2$ per unit increase in work rate (i.e., the response 'gain') is often used to characterise muscle efficiency during exercise (Whipp et al., 1981). For constant-work-rate cycle exercise performed below the lactate threshold (LT), the gain approximates 10 ml/min/W and has been considered to be essentially unaffected by differences in the age, health or fitness status of the subjects tested or by interventions such as training, prior exercise, hypoxia or hyperoxia (Poole and Richardson, 1997). It is of significant interest, therefore, that several recent studies have reported that dietary nitrate supplementation either with nitrate salts or nitrate-rich beetroot juice consumption, enhances muscle efficiency (Bailey et al., 2009, 2010; Larsen et al., 2007; Vanhatalo et al., 2010a,b). Dietary nitrate intake results in a significant elevation of plasma [nitrite] which may be reduced to nitric oxide (NO) and other reactive nitrogen intermediates under appropriate physiological conditions (Lundberg et al., 2008). This NO production pathway is believed to supplement NO production from the oxidation of L-arginine catalysed by nitric oxide synthase (NOS), and may be particularly important in conditions of lowered tissue PO_2 or pH (Cosby et al., 2003; Shiva et al., 2007) such as would be extant in skeletal muscle during exercise. It has been reported that during <LT exercise, the steady-state $\dot{V}O_2$ is reduced by 3–5% following nitrate supplementation (Bailey et al., 2009; Larsen et al., 2007; Vanhatalo et al., 2010a), and that during >LT exercise, the $\dot{V}O_2$ slow component is attenuated and T_{lim} is extended (Bailey et al., 2009; Lansley et al., 2011). The mechanistic bases for these effects on muscle efficiency remain unclear but may be linked to an NO-mediated reduction in the ATP cost of muscle force production (Bailey et al., 2009), altered calcium handling and force development in type II fibres

* Corresponding author at: University of Canberra, National Institute of Sport Studies (UCNISS), Canberra, ACT 2601, Australia. Tel.: +61 02 6201 5943; fax: +61 02 6201 5615.
E-mail address: kevin.thompson@canberra.edu.au (K.G. Thompson).

1569-9048/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2013.12.015

(Hernández et al., 2012), and/or improved mitochondrial efficiency (i.e., enhanced P/O ratio; Larsen et al., 2011).

In addition to the direct effects of NO or nitrite on muscle efficiency, it is possible that the increased NO bioavailability following nitrate supplementation may enhance bulk muscle blood flow, improve blood-muscle O_2 driving pressure or result in a better matching of local O_2 supply to metabolic rate (Ferguson et al., 2012, 2013) factors which might also predispose to improved exercise tolerance. Alterations in vascular tone following nitrate supplementation are reflected in significant reductions in resting blood pressure (Webb et al., 2008) and in greater muscle oxygenation at rest and during moderate exercise, as demonstrated by changes in total haemoglobin, oxyhemoglobin or deoxyhemoglobin concentrations in the area of interrogation, as measured with near infra-red spectroscopy (NIRS), (Bailey et al., 2009; Kenjale et al., 2011; Masschelein et al., 2012).

Given recent suggestions that changes in cerebral blood flow during high-intensity exercise might be related to the fatigue process (Nybo, 2008; Rooks et al., 2010; Rupp and Perrey, 2008) it is pertinent to note that dietary nitrate supplementation has been reported to enhance cerebral perfusion in brain areas associated with executive functioning in older adults at rest (Presley et al., 2011). Specifically, a nitrate-rich diet increased regional cerebral perfusion in frontal lobe white matter, particularly between the anterior cingulated cortex (ACC) and dorso-lateral prefrontal cortex (Presley et al., 2011). The ACC is purported to be affected by mental fatigue (Cook et al., 2007) and its activity has been shown to be related to the perception of effort during exercise (Williamson et al., 2001, 2002, 2006). The ACC and pre-frontal cortex have also been associated with processing fatigue-related feedback, emotion, arousal states and decision making (Senn, 2002; Walton et al., 2003, 2006) and in adjusting descending command, including motor control, during an ongoing task (Liu et al., 2003). Therefore an enhancement of cerebral blood flow during exercise, through dietary nitrate supplementation, might better maintain these functions, reducing mental fatigue and effort perception, and aiding cognition. This might, in turn, contribute to improved exercise tolerance given that a raised RPE due to mental fatigue has been suggested to lead to an earlier withdrawal from exercise (Marcora et al., 2009).

The purpose of the present study, therefore, was to investigate the influence of dietary nitrate supplementation on cerebral and skeletal muscle oxygenation, exercise efficiency, effort perception, mental fatigue and cognitive function at rest and over a range of exercise intensities (50%, 70% and 90% $\dot{V}O_{2peak}$). We employed this comprehensive approach to enable simultaneous assessment of the central and peripheral effects of nitrate supplementation on brain and muscle function. We hypothesised that, relative to a placebo condition, dietary nitrate supplementation would: (1) elevate plasma [nitrite] and reduce blood pressure at rest; (2) improve cerebral and muscle oxygenation at rest and during exercise; (3) reduce cerebral and muscle fractional O_2 extraction and lower pulmonary $\dot{V}O_2$ during exercise; (4) improve performance on a battery of cognitive tests at rest and during exercise; (5) attenuate mental fatigue and effort perception; and (6) enhance exercise tolerance.

2. Methods

2.1. Subjects

Sixteen healthy, recreationally active males (mean ± SD; age 24 ± 4 yr, height 1.77 ± 0.07 m, weight 75.6 ± 9.2 kg, $\dot{V}O_{2\,max}$ 47.3 ± 6.3 ml kg^{-1} min^{-1}) volunteered to participate in this study. All subjects reported to be non-smokers, who were not using any prescription medications, illicit social drugs or dietary supplements. The study received ethical approval from the Northumbria University School of Psychology and Sport Sciences Ethics Committee and adhered to the principles of the Declaration of Helsinki. All subjects gave their written, informed consent prior to commencement of the study. The subjects were instructed to arrive at the laboratory at 8 am on experimental days, fully hydrated, following an overnight fast, and to avoid strenuous exercise 24-h prior to each visit. Subjects were also asked to refrain from alcohol and caffeine consumption for at least 24-h and 6-h prior to each exercise session, respectively. Subjects were provided with a list of foods rich in nitrate and were asked to abstain from these foods 36-h prior to the experiment beginning and thereafter 36-h prior to each testing session.

2.2. Procedures

The subjects were required to visit the laboratory on three separate occasions to complete exercise tests on an electronically-braked cycle ergometer (Velotron, Dynafit Pro, RacerMate Inc., Seattle, USA). During visit one, subjects completed an incremental exercise test to determine $\dot{V}O_{2peak}$. Following a 5-min rest period, subjects completed 3-min of 'unloaded' (20 W) cycling, after which the work rate was increased by 30 W/min until volitional exhaustion. $\dot{V}O_{2peak}$ was determined as the highest 30-s mean $\dot{V}O_2$ value achieved prior to exercise termination. The configuration of the saddle and handlebar position was measured and recorded, and repeated for all subsequent exercise tests.

During visits 2 and 3 (Fig. 1), the subjects received two single-dose dietary supplements in a double-blind, cross-over design which was randomly allocated in a counterbalanced order (Latin square). Subjects consumed 0.5 litres of either a nitrate supplement (450 ml of organic beetroot juice containing 5 mmol nitrate; Beet It, James White Drinks, Ipswich, UK and 50 ml of low calorie blackcurrant cordial) or a placebo (50 ml low calorie blackcurrant cordial, 45 ml pressed apple juice, 405 ml H_2O, containing negligible nitrate). No adverse effects were reported. At the time of the experiment the Beet It beetroot juice and shot placebo products were not available (Lansley et al., 2011). Experimental exercise sessions were separated by at least 7 days.

Prior to the exercise test, baseline measures of plasma nitrite concentration, blood pressure and the cognitive tasks [Rapid visual information processing (RVIP) then Stroop tasks] were taken pre- and post-supplementation. Resting cerebral oxygenation (COX) was established prior to beverage ingestion and then monitored throughout the experimental session. Subjects consumed the beverage (over ~10-min) and sat quietly, watching non-arousing DVDs during a 90-min absorption period. Resting measurements of muscle oxygenation (MOX) for the right m. vastus lateralis, pulmonary gas exchange, blood lactate concentration [Lac$^-$], mental fatigue rating, energy level, mood (BRUMS) scores and rating of perceived exertion (RPE, Borg, 1998) were taken after beverage ingestion and before the exercise test began.

The exercise test consisted of 3-min of 'unloaded' (20 W) cycling during which baseline EMG of the m. vastus lateralis was established, followed by two, 20-min incremental stages at work rates required to elicit ~50% and ~70% $\dot{V}O_{2peak}$, and a final work rate corresponding to 90% $\dot{V}O_{2peak}$, which was continued until task failure (defined as a fall in pedal rate of >10 rpm below self-selected cadence). These intensities were selected to be positioned within the moderate, heavy, and severe exercise domains, respectively (Poole and Jones, 2012). During the 50% and 70% $\dot{V}O_{2peak}$ stages, the subjects completed the cognitive tasks while cycling. Heart rate, pulmonary gas exchange, EMG, COX and MOX were measured continuously throughout each