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Dietary nitrate modulates cerebral blood flow parameters and cognitive performance in humans: A double-blind, placebo-controlled, crossover investigation☆,☆☆

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HIGHLIGHTS

- Dietary nitrate is reduced endogenously via nitrite to nitric oxide.
- The effects of nitrate rich beetroot juice on frontal cerebral blood-flow were tested.
- Nitrate modulated the hemodynamic response to task performance in the frontal cortex.
- Performance on one of three tasks (serial 3s subtractions) was improved.
- Plasma nitrite was increased.

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ABSTRACT

Nitrate derived from vegetables is consumed as part of a normal diet and is reduced endogenously via nitrite to nitric oxide. It has been shown to improve endothelial function, reduce blood pressure and the oxygen cost of sub-maximal exercise, and increase regional perfusion in the brain. The current study assessed the effects of dietary nitrate on cognitive performance and prefrontal cortex cerebral blood-flow (CBF) parameters in healthy adults. In this randomised, double-blind, placebo-controlled, parallel-groups study 40 healthy adults received either placebo or 450 ml beetroot juice (~5.5 mmol nitrate). Following a 90 minute drink/absorption period, participants performed a selection of cognitive tasks that activate the frontal cortex for 54 min. Near-Infrared Spectroscopy (NIRS) was used to monitor CBF and hemodynamics, as indexed by concentration changes in oxygenated and deoxygenated-haemoglobin, in the frontal cortex throughout. The bioconversion of nitrate to nitrite was confirmed in plasma by ozone-based chemi-luminescence. Dietary nitrate modulated the hemodynamic response to task performance, with an initial increase in CBF at the start of the task period, followed by consistent reductions during the least demanding of the three tasks utilised. Cognitive performance was improved on the serial 3s subtraction task. These results show that single doses of dietary nitrate can modulate the CBF response to task performance and potentially improve cognitive performance, and suggest one possible mechanism by which vegetable consumption may have beneficial effects on brain function.

1. Introduction

The ubiquitous signalling molecule nitric oxide (NO) plays a modulatory role in a host of key physiological processes, including mitochondrial and platelet function, host defence mechanisms [1,2], neurotransmission, peripheral and cerebral vaso-dilation [3,4], and the neurovascular coupling of neural activity to local cerebral blood-flow (CBF) [5–7]. In most tissues NO is synthesised from L-arginine and is rapidly oxidised to nitrite (NO_2^-) and nitrate (NO_3^-) [8]. However, evidence suggests that circulating

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nitrite can also be reduced back to NO by a wide range of proteins and enzymes in blood and tissue, including deoxygenated haemoglobin, myoglobin, xanthine oxidase, aldehyde oxidase, neuroglobin, cytochrome P 450 and NO synthase [9]. Furthermore, nitrite has also been identified as a cellular signalling molecule, independent of its relationship with NO [10].

Endogenous levels of nitrate, produced as a by-product of the L-arginine/NO pathway, can be augmented by direct sequestration from dietary sources, most notably by eating vegetables high in nitrate; e.g. spinach, lettuce, broccoli and beetroot [11]. Circulating nitrate from both endogenous and dietary sources is actively sequestered and concentrated into saliva before being converted to nitrite by commensal salivary bacteria in the mouth [12]. Entero-salivary recirculation of additional dietary nitrate therefore leads to a sustained increase in circulating nitrite. Following ingestion of nitrate rich vegetable juice, nitrate levels peak or approach their peak in plasma by 60 min post-dose, with nitrite reaching close to peak levels by 60–120 min post-dose, depending on the dose administered [13].

The reduction of nitrite to NO is particularly prevalent in hypoxic conditions [14], but also takes place in normoxic conditions wherein conversion rates can be modulated by the presence of reducing agents, the local oxygen tension and pH levels [8,15].

The ingestion of nitrate, including from dietary sources, is associated with a number of effects consistent with increased levels of endogenous NO synthesis, including reductions in blood pressure [16–20]. This effect has been demonstrated as early as 3 h after a single dose of nitrate rich beetroot juice, with a concomitant protection of forearm endothelial function and in vitro inhibition of platelet aggregation [21]. Dietary nitrate has also been shown to reduce the overall oxygen cost of submaximal exercise 2.5 h after ingestion [22] and after three or more days of administration [17,22–24]. Similarly, an increase in peak power and work-rate [22], a speeding of VO_2 mean response time in healthy 60–70 year olds [19] and delayed time to task failure during severe exercise [23,24] have also been reported following the consumption of nitrate rich beetroot juice consumed daily for 4 to 15 days. Nitrate related reductions have also been demonstrated with regard to the rate of adenosine-5′-triphosphate (ATP) turnover using magnetic resonance spectroscopy [23], whilst improved oxygenation [24] has been confirmed directly in the muscle during exercise using Near-Infrared Spectroscopy (NIRS).

NO plays a pivotal role in cerebral vasodilation and the neurovascular coupling of local neural activity and blood-flow [25] and enhanced cerebral blood perfusion has been observed in the prefrontal cortex in response to increased circulating levels of dietary nitrate [11]. Several studies have probed the effects of dietary nitrate derived from beetroot or spinach on brain function, including three studies that have included some form of cognitive testing either as an additional measure [19,20], or as the primary focus of the project [26]. Whilst these studies demonstrated modulation of a number of physiological parameters they did not provide evidence of cognitive improvements, possibly due to comparatively small sample sizes and other methodological factors. Two studies have also investigated the effects of dietary nitrate on cerebral blood-flow parameters. In the first of these, Presley et al. [11] demonstrated, using arterial spin labelling magnetic resonance imaging (MRI), that a diet high in nitrate consumed for 24 h increased regional white matter perfusion in elderly humans, but with this effect restricted to areas of the frontal cortex. More recently, Aamand et al. [27], investigated the effects of 3 days of administration of dietary nitrate (sodium nitrate) on the haemodynamic response in the visual cortex elicited by visual stimuli, as assessed by functional MRI (fMRI). They demonstrated a faster, smaller and less variable blood-oxygen-level dependent (BOLD) response following nitrate, which they interpreted as indicating an enhanced neurovascular coupling of local CBF to neuronal activity. As the BOLD response simply reflects the contrasting magnetic signals of oxygenated and deoxygenated haemoglobin (with increased activity imputed from an assumed relative decrease in deoxyhemoglobin as local activation engenders a greater influx of blood borne oxygenated -Hb), it cannot disentangle the contributions of changes in blood-flow and changes in oxygen consumption to the overall signal. The current study therefore utilised Near-Infrared Spectroscopy (NIRS), a brain imaging technique that has the advantage over fMRI BOLD in that it measures both concentration changes in deoxy-Hb and overall local CBF (changes in oxy-Hb and deoxy-Hb combined).

The current double-blind, placebo controlled, parallel groups study investigated the effects of a single dose of dietary nitrate on cognitive performance and the CBF haemodynamic response in the prefrontal cortex during tasks that activate this brain region.

2. Materials and methods

2.1. Participants

40 healthy adults (mean age: 21.28 years, range: 18–27 years) took part in the study. Prior to attending the laboratory all participants refrained from eating for 12 h, and consumed no vegetables for 36 h prior to testing. Participants were allowed their usual morning caffeinated beverages, but consumed no caffeine for a minimum of 2 h prior to the assessment. Following arrival they were not permitted any food or drink, other than the study treatments, until the end of the assessment session. The age and physical characteristics of the two groups are shown in Table 1.

All participants reported themselves to be in good health and free from illicit drugs, alcohol, prescription medication and herbal extracts/food supplements. Participants who had suffered a neurological disorder or neuro-developmental disorder were excluded from participation, as were those who had any relevant food allergies or intolerances, smoked tobacco, drank excessive amounts of caffeine (more than 6 cups of coffee per day) or took illicit social drugs.

The study received ethical approval from the Northumbria University Department of Psychology and Sport Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent prior to their inclusion in the study. Prior to data collection this study was registered on the clinicaltrials.gov website with the following reference number: NCT01169662.

2.2. Treatments

Participants were randomly assigned to receive either:

a) 450 ml organic beetroot juice (including 10% apple juice — Beet It, James White Drinks, Ipswich, UK) containing 5.5 mmol nitrate [24]

Table 1
Age and physical characteristics of participants.

		Placebo, n = 20		Beetroot, n = 20	
Age (years)		21.40	0.73	21.15	0.48
Male/female		7/13		5/15	
Height (m)		1.71	0.02	1.70	0.02
Weight (kg)		74.93	3.43	68.24	3.12
BMI		25.39	0.80	23.34	0.72
Heart rate (bpm)	Pre	64.3	2.05	66.85	2.24
	Post	59.4	1.54	67.15	2.38*
Systolic BP	Pre	115	2.3	114.6	3.16
	Post	116.8	2.26	115.7	2.48
Diastolic BP	Pre	74.2	1.86	73.15	1.61
	Post	79.05	1.91	76.35	1.59
Nitrite (nM)	Pre	228	14.8	226	23.2
	Post	246	28.2	598	78.3*

Physical characteristic data (means plus SEMs) from the placebo and dietary nitrate conditions (n = 20 per group) including pre- and post-treatment heart rate, blood pressure and plasma nitrite measurements. Analysis on the latter measures was by two-way ANOVA with Bonferroni adjusted post-hoc comparisons (*$p < 0.05$, placebo versus dietary nitrate at that time point).

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