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Effects of dietary nitrate supplementation on the response to extremity cooling and endothelial function in individuals with cold sensitivity. A double blind, placebo controlled, crossover, randomised control trial

Clare M. Eglin [a], Joseph T. Costello [a], Stephen J. Bailey [b], Mark Gilchrist [c], Heather Massey [a], Anthony I. Shepherd [a, *]

[a] Department of Sport and Exercise Science, University of Portsmouth, UK
[b] School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK
[c] University of Exeter Medical School, NIHR Exeter Clinical Research Facility, Royal Devon and Exeter Hospital, Exeter, Devon, UK

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ABSTRACT

Individuals with cold sensitivity have low peripheral skin blood flow and skin temperature possibly due to reduced nitric oxide (NO•) bioavailability. Beetroot has a high concentration of inorganic nitrate and may increase NO-mediated vasodilation. Using a placebo-controlled, double blind, randomised, crossover design, this study tested the hypotheses that acute beetroot supplementation would increase the rate of cutaneous rewarming following a local cold challenge and augment endothelium-dependent vasodilation in cold sensitive individuals.

Thirteen cold sensitive participants completed foot and hand cooling (separately, in 15 °C water for 2 min) with spontaneous rewarming in 30 °C air whilst skin temperature and cutaneous vascular conductance (CVC) were measured (Baseline). On two further separate visits, participants consumed 140 ml of either concentrated beetroot juice (nitrate supplementation) or nitrate-depleted beetroot juice (Placebo) 90 min before resting seated blood pressure was measured. Endothelial function was assessed by measuring CVC at the forearm, finger and foot during iontophoresis of 1% w/v acetylcholine followed by foot and hand cooling as for Baseline.

Plasma nitrite concentrations significantly increased in nitrate supplementation compared to Placebo and Baseline (502 ± 246 nmol L^{-1}; 73 ± 45 nmol L^{-1}; 74 ± 49 nmol L^{-1} respectively; $n = 11$; $P < 0.001$). Resting blood pressure and the response to foot and hand cooling did not differ between conditions (all $P > 0.05$). Nitrate supplementation did not alter endothelial function in the forearm, finger or foot (all $P > 0.05$) compared to Placebo.

Despite a physiologically meaningful rise in plasma nitrite concentrations, acute nitrate supplementation does not alter extremity rewarming, endothelial function or blood pressure in individuals with cold sensitivity.

1. Introduction

Non-freezing cold injury (NFCI) is caused by prolonged exposure to cold, and often cold and wet, conditions. NFCI most commonly affects the feet, although the hands can also be affected [16]. Chronic NFCI, which may last for many years, is characterised (in variable combination and severity) by cold sensitivity, numbness, hyperhidrosis and persistent pain which can significantly affect an individual's quality of life [26]. NFCI has been reported in individuals following exposure to cold environments such as: mountaineering and hill walking [32], diving [40], cycling [19], in homeless individuals [68] and the elderly [67] as well as in individuals working in cold environments, [9,26,49].

Although severe NFCI can be debilitating, its pathophysiology is not fully understood and therefore a definitive diagnostic tool is not

* Corresponding author. Department of Sport and Exercise Science, Spinnaker Building, Cambridge Road, Portsmouth, PO1 2ER, UK.
E-mail address: ant.shepherd@port.ac.uk (A.I. Shepherd).

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Abbreviations

ACh	acetylcholine
CVC	cutaneous vascular conductance
MAP	mean arterial pressure
NFCI	non-freezing cold injury
NO•	nitric oxide
RSNO	*S*-Nitrosothiols
eNOS	endothelial nitric oxide synthase

available [16]. Sub-clinical forms of NFCI have also been characterised in individuals frequently exposed to cold conditions for short durations during recreational activities such as windsurfing, surfing and open water swimming. These individuals are cold sensitive (their hands and feet are cooler than "normal" individuals and they take longer to rewarm following cold exposure) but are not considered to have a cold injury [15]. As with NFCI and primary Raynaud's [20,59], this cold sensitivity is associated with a reduced basal skin blood flow and a smaller increase in skin blood flow upon rewarming [12,29]. The resultant cooler peripheral skin temperature will result in reduced skin oxygen tension [51,60] and may put these individuals at greater risk of cold injury on subsequent cold exposure [9].

Animal models of NFCI have shown reduced levels of oxygen in the cooled tissues [52] and that NFCI may be associated with a pro oxidant state [21]. Local cooling has been shown to inhibit endothelial nitric oxide synthase (eNOS) as well as increase noradrenaline release [28]. In addition, eNOS activity has been shown to be positively associated with temperature [36]. Nitric oxide (NO•) is a known vasodilator and plays a fundamental role in the control of skin blood flow [28,50]. Moreover, NO• released from *S*-nitrosohemoglobin [64] in hypoxic environments plays a key role in regulating the physiological oxygen gradient. We have previously shown that glyceryl trinitrate, a NO• donor, increases the rate of rewarming following foot cooling in individuals with cold sensitivity [29]. However, individuals develop a tolerance to GTN and show diminishing vasodilatory effects with chronic treatment [55]. In addition, the deleterious side effects such as headaches [31] suggests that organic nitrates are not optimal long-term therapies for individuals with cold sensitivity.

Leafy green vegetables and particularly beetroot have a high concentration of inorganic nitrate [7]. These vegetables are thought to be beneficial to cardiovascular health due to their vasodilatory effects [23] with recent reports suggesting that tolerance to inorganic nitrate does not occur (as inferred by blood pressure responses) for at least 28 days [65]. Inorganic nitrate can act as a source of systemic NO• generation [44]. Briefly, inorganic nitrate is converted to nitrite by facultative anaerobic bacteria on the dorsum of the tongue [14] with small quantities of this nitrite being converted to NO• and other nitrogen oxides such as *S*-Nitrosothiols (RSNO) by the acidic environment of the stomach [4]. The remaining nitrite and RSNO are then absorbed into the circulation where they act as a storage pool for subsequent NO• production [45], which is expedited in hypoxaemia [10], such as that observed in cold sensitivity [12,29,51,60]. This enterosalivary pathway and its purported therapeutic effects have been reviewed elsewhere [46]. Inorganic nitrate, in the form of beetroot juice, improves skin blood flow [43], microvascular function [35] and lowers blood pressure (BP) in healthy individuals [66] and in individuals with hypertension [34] and peripheral arterial disease [37]. In contrast, some studies have shown no effect of nitrate supplementation on vascular health markers despite increases in circulating NO• intermediates in healthy [2,63] and clinical populations [24]. However, the potential for beetroot juice to offer an inexpensive, safe and potentially effective intervention to improve peripheral circulation in individuals with cold sensitivity has not been studied. Recently, nitrate supplementation has also been shown to lower sympathetic nerve activity [57] and nitrate/nitrite has been shown to restore vascular function when NOS is inhibited [8,17]. Therefore, as cold sensitive individuals exhibit impaired vascular function possibly due to lower eNOS activity and increased sympathetic drive, nitrate supplementation, and the associated increase in the circulating NO• pool, might help alleviate the associated detrimental effects as shown with organic nitrates [1,29].

We hypothesised that compared to baseline and placebo, nitrate supplementation would increase plasma nitrite concentration, the rate of cutaneous rewarming following a local cold challenge and augment endothelium-dependant vasodilation in individuals with cold sensitivity.

2. Methods

All procedures for this randomised placebo-controlled, double-blind, cross-over designed trial were approved by the University of Portsmouth Science Faculty Research Ethics committee (2016-107A) and all volunteers provided written informed consent prior to participation. All testing took place at the Department of Sport and Exercise Science, University of Portsmouth between January and March 2017 when the outdoor air temperature averaged 5.8 ± 2.9 °C at the time of testing (range 0 °C–10 °C).

2.1. Participants

Participants were recruited based on their self-reported frequent exposure to cold environments (e.g. winter sea swimming, sailing etc.) or often having cold hands and feet. A baseline cold sensitivity test (described below) was conducted to determine whether the participants had cold sensitive feet or hands. This was defined as a toe or finger skin temperature less than 32 °C prior to the cold water immersion and after 5 min of rewarming in 30 °C air [16,30]. Exclusion criteria included; diagnosis of a prior freezing injury, peripheral vascular disease, thalassaemias affecting haemoglobin and/or hepatitis B.

All participants were non-smokers (for at least 1 year). Participants refrained from consuming food high in nitrate the day before testing and were asked to keep a food diary for the 24 h before their first visit to the laboratory and to replicate this prior to each visit. Participants abstained from alcohol for 24 h and caffeine for 3 h prior to testing. The participants also refrained from using any antibacterial mouth wash for 7 days prior to each test as this has been shown to reduce the concentration of oral bacterial that are responsible for the reduction of nitrate to nitrite [27]. Female volunteers were asked about their menstrual cycle to determine whether they were in the follicular or luteal phase or whether they were peri- or post-menopausal. However, the phase of the menstrual cycle was not controlled for since reproductive hormone status does not affect the responses to local cooling [47], thermal perception [47] or iontophoresis of acetylcholine [39].

2.2. Protocol

The participants attended the laboratory on three separate occasions at the same time of day to reduce any circadian effects. On arrival at the laboratory, resting seated blood pressure was measured using an automated blood pressure monitor (Omron HEM-705C, Omron, Milton Keynes, UK) with the average of the final