No thermoregulatory or ergogenic effect of dietary nitrate among physically inactive males, exercising above gas exchange threshold in hot and dry conditions

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Abstract

The aim of this study was to determine the effect of five days dietary nitrate (NO_3) consumption on exercise tolerance and thermoregulation during cycling in hot, dry conditions. In a double-blind,

randomised crossover design, 11 healthy males participated in an exercise tolerance test (T_{lim}) in the heat (35°C, 28% relative humidity), cycling above the thermoneutral gas exchange threshold, after five days of dietary supplementation, with either NO_3 - -rich beetroot juice (BR; ~ 9.2 mmol NO_{3-}) or placebo (PLA). Changes in plasma [NO_{3-}] and nitrite [NO_{2-}], core and mean skin temperatures, mean local and whole-body sweat rates, heart rate, perceptual ratings and pulmonary gas exchange were measured during exercise, alongside calorimetric estimations of thermal balance. Mean arterial pressures (MAP) were recorded pre-T_{lim}. There were no differences in T_{lim} between conditions (BR = 22.8 ± 8.1 min; Placebo = 20.7 ± 7.9 min) ($P = 0.184$), despite increases in plasma [NO_{3-}] and [NO_{2-}] ($P < 0.001$) and a 3.8% reduction in resting MAP ($P = 0.004$) in the BR condition. There were no other differences in thermoregulatory, cardio-metabolic, perceptual or calorimetric responses to the T_{lim} between conditions ($P > 0.05$). Dietary NO_{3-} supplementation had no effect on exercise tolerance or thermoregulation in hot, dry conditions, despite reductions in resting MAP and increases in plasma [NO_{3-}] and [NO_{2-}]. Healthy, yet physically inactive individuals with no known impairments in vasodilatory and sudomotor function do not appear to require BR for ergogenic or thermolytic effects during exercise in the heat.

Key words:

Beetroot; Hot; Sweating; Cooling; Ergogenic aids

Introduction

During exercise in hot environments, body heat is predominantly gained through internal metabolic heat production, with thermal equilibrium maintained via dry (conductive, convective or radiative) or evaporative heat transfer pathways (Gagge & Gonzalez, 1996). While dry heat transfer (H_{dry}) is sufficient to offset positive heat storage in cooler environments, in the heat (>33 °C), or in response to significant metabolic heat production experienced during exercise, there is a predominant reliance on sweating for latent heat transfer (Armstrong, 2000). Thus, in thermally strained humans exercising in hot laboratory conditions, cutaneous vasodilation and sweating chiefly support heat loss by attenuating the rate of core temperature increases (Sawka & Young, 2006).

Evaporation of sweat from the skin surface is the largest modifiable heat loss pathway for maintenance of thermal balance during exercise in the heat (Gagge & Gonzalez, 1996). The latent heat of vaporisation depends upon the efficiency of the sweating process and the surrounding water vapour pressure (i.e. humidity) (Parsons, 1993) – both of which can be acutely manipulated. For

example, evaporative cooling capacity is greater in hot and dry conditions compared to humid ambient environments (Muhamed et al., 2016), which is explained by the larger vapour pressure gradient between the skin's surface and the ambient air. Thus, the imposition of a dry, hot environment increases the maximal evaporative heat transfer capacity of the environment (E_{max}). However, reliance on evaporative cooling to achieve thermal balance (E_{req}) is partly determined by the interplay of convective, conductive and radiative heat transfer (i.e. H_{dry}), as well as the internal metabolic heat production (H_{prod}) (Gagnon et al., 2013). Therefore, quantification of the individual components of thermal balance (using partitional calorimetry) permits a more accurate assessment of interventions that are intended to acutely enhance evaporative cooling capacity, yet this is seldom performed in empirical research (Cramer & Jay, 2018).

Among many other biological roles, nitric oxide (NO) acts as a signalling molecule for modifiable heat transfer mechanisms; namely, eccrine sweat gland function and cutaneous blood flow (Stapleton et al. 2014; Fujii et al. 2016). On the basis that NO can be produced through the stepwise reduction of inorganic nitrate (NO_{3-}→NO_{2-}→NO), its effects on thermoregulation in healthy populations during exercise in the heat has been investigated (Kuennen et al., 2015; Kent et al., 2017; 2018; McQuillan et al. 2017; Amano et al., 2018). Of these studies, none have reported a benefit of NO_{3-} consumption on exercise performance or reported advantages to thermoregulation. These results were unanticipated since increases in subcutaneous vascular conductance and reductions in blood pressure have been reported following beetroot supplementation in response to locally administered thermal skin stimuli (Keen et al 2015; Levitt et al., 2015), which are hallmarks of the physiological response to increases in NO availability. These apparent null effects may relate to inconsistencies in the adopted research designs, sub-optimal conditions for evaporative cooling or incomplete analyses of the thermoregulatory process. For example, the NO_{3-}→NO_{2-}→NO pathway is potentiated at higher exercise intensities, owing to the lowered O2 tension and resulting muscle pH (Jones et al., 2016). Sustaining exercise intensities above 'moderate' levels will also drive metabolic H_{prod} and E_{req}, thus necessitating heat loss via evaporative cooling mechanisms (Cramer & Jay, 2018). Therefore, the lower (~45-60% $\dot{V}O_{2max}$) continuous exercise intensities utilised in some studies (Kuennen et al., 2015; Kent et al., 2018; Amano et al., 2018) could limit the actions of NO_{3-}→NO_{2-}→NO pathway on heat transfer. In addition, untrained or physically inactive participants are more likely to respond to dietary NO_{3-} supplementation (Porcelli et al., 2015) and have lower skin wettedness (Ravanelli et al., 2017), rendering them more susceptible to thermal stress and specific heat transfer deficiencies that are primed for NO targeting. It is also pertinent to note that most studies have been conducted in relative humidity ranging from 45% - 70% (Kent et al., 2017; 2018; McQuillan et al. 2017; Amano et al., 2018) or have chosen to heavily clothe participants (Kuennen et

al., 2015), limiting evaporative cooling potential and, thus, the capacity for NO-induced increases in latent heat transfer.

The aim of the current study was to determine the effect of five days dietary NO_{3-} consumption on exercise tolerance and thermoregulation during cycling above the thermoneutral gas exchange threshold in hot (35 °C), dry (28% RH) conditions. We hypothesised that the dry, hot conditions, coupled with the higher exercise intensity, would reveal the beneficial effects of NO_{3-} supplementation on evaporative cooling, dry heat losses and metabolic cost of untrained participants.

Methods

Participants

Eleven non-heat acclimated healthy males volunteered for this study (age 25 ± 5 years, stature 182.0 ± 4.6 cm, body mass 78.7 ± 7.5 kg, maximal oxygen uptake ($\dot{V}O_{2max}$) 41.1 ± 3.6 ml·min^{-1}·kg^{-1}). Participants were asked to refrain from consuming alcohol or any other dietary supplements for 24-h prior to the initial testing session or during the study period. None of the participants trained for endurance exercise on a regular basis and were deemed to be physically inactive based on exercising < 30 min of moderate exercise per week (Department for Health & Social Care, 2019). Trials were conducted between December and March in the UK, thereby avoiding additional external heat exposure during the period of the study. None of the participants had visited hot climates in the five months prior to the trial and were deemed to be nonacclimated. All participants provided written informed consent to take part in the study. Ethical approval was provided by the institutional ethics committee.

Design

The study adopted a double-blind, placebo-controlled, randomised crossover design. All participants reported to the laboratory on three separate occasions, across a 20- day period. The first visit comprised preliminary testing and familiarisation, after which a five-day supplementation period commenced. Following supplementation, the participants completed an exercise test to the limit of tolerance (T_{lim}) (visit two). A seven-day washout period was provided prior to the second, five-day supplementation period. A final T_{lim} (visit three) was conducted following the counterbalanced supplementation period. Randomisation was conducted by generating random 6 numbers for all participants, across each condition using online software (http://www.randomizer.org/). Each laboratory visit was conducted at the same time of day.

Preliminary testing

During visit one, participants undertook an incremental exercise test to volitional exhaustion on a mechanically-braked cycle ergometer (Monark Exercise AB, Ergomedic 874E, Varberg, Sweden) in thermoneutral conditions (19.5 ± 1.1 °C) to determine $\dot{V}O_{2max}$ and the power output at the gas exchange threshold. The test started at a workload of 120 W and increased 24 $W \cdot min^{-1}$ at a fixed cadence of 80 $rev \cdot min^{-1}$ until volitional exhaustion or when cadence dropped below 70 $rev \cdot min^{-1}$ for more than 10-s. The rate of oxygen uptake ($\dot{V}O_2$) was measured using breath-by-breath expired air analysis (Vyntus CPX, Hoechberg, Germany). The gas analyser was calibrated before every trial with gases of known concentration (15.95% O2, 4.97% CO_2, BAL. N_2) and the turbine volume transducer was calibrated automatically by the system at flow values of 2 $L \cdot s^{-1}$ and 0.2 $L \cdot s^{-1}$. Heart rate was recorded throughout the trial (Polar Heart Rate Monitor M400, Warwick, UK). $\dot{V}O_{2max}$ was calculated as the highest 30-s average $\dot{V}O_2$. Breath-by-breath $\dot{V}O_2$ and $\dot{V}CO_2$ data from the incremental cycling test were used to determine the gas exchange threshold, using the simplified v-slope method (Scheider, Phillips & Stoffolano, 1993). The mean power output at thermoneutral gas exchange threshold was 173 ± 32 W, which was fixed for all experimental trials. This threshold was selected as it was deemed appropriate to evaluate endurance capacity at a repeatable, fixed intensity, whilst increasing the rate of metabolic heat production sufficiently to induce thermoregulatory responses. After 20-min of rest, participants conducted a familiarisation trial, comprising constant load exercise at the power output associated with their thermoneutral gas exchange threshold in an environmental chamber (Sporting Edge UK, Basingstoke, UK) set to experimental conditions (35 ± 0.3 °C, 28 ± 1.9 % RH).

Supplementation

The participants received five days of dietary supplementation, with either NO_{3-} -rich beetroot juice (BR) (~ 9.2 mmol NO_{3-} ; Beet It, James White Drinks, Ipswich, UK) or NO_{3-} -depleted BR as a placebo (PLA; 0.0034 mmol NO_{3-} ; Beet It, James White Drinks, Ipswich, UK). This BR dose was based on the established dose-response profile (Breese et al. 2017). The participants consumed either the BR or PLA (95 ml divided into equal morning and afternoon doses) on days one to four of the supplementation period. On day five of supplementation, the participants consumed all of the beverages 2-h prior to the start of the exercise test. A seven-day washout period separated each supplementation period, where participants returned to their normal diet, as well as exercising freely up to 24-h testing. Throughout the study, participants completed a food diary, which was replicated for the alternative condition. The participants were also provided with a list of NO_{3-}

dietary sources and asked to refrain from their consumption, as well as avoiding using mouthwash products, for the duration of each supplementation period.

Experimental trials

All subsequent tests were conducted in the heat (35 ± 0.3 °C, 28 ± 1.9 % RH), with participants wearing socks, trainers and cycling shorts. Upon arrival at the laboratory, the participants lay supine for 10-min, after which their resting blood pressure (BP) was measured from their left upper arm, with the last of three mean arterial pressures (MAP) recorded (Omron M7 BP Monitor, OMRON Healthcare Europe, Hoofddrop, Netherlands). Subsequently, venous blood was drawn from the participants' right arm at the antecubital fossa, using a hypodermic needle and a lithium-heparinized vacutainer (4 mL). The whole blood was then centrifuged for 15-min at 1000 *g* and immediately stored at -20 °C. Blood plasma was later thawed and analysed for NO_{3-} and NO_{2-} using colorimetric assays (R&D Systems, Parameter Nitric Oxide Kit), with an intra-assay and inter-assay coefficient of variation of 2.1% and 4.2%, respectively.

Prior to exercise testing, participants were instructed to insert a rectal probe 10 cm past the anal sphincter to measure core temperature (T_{core}). T_{core} was recorded every 1-min via a data logger (SQ2010, Grant Instruments Ltd., Cambridge, UK). A urine sample was also provided to determine hydration status using a refractometer (Pocket 8 Osmochek, Vitech Scientific Ltd, West Sussex, UK). A reading of >600 $mOsm \cdot kg^{1} \cdot H2O^{-1}$ indicated hypohydration, in which case the participant consumed 500 ml of water and waited 30-min before being re-tested and beginning the trial. The participants' nude body mass was recorded (MPMS-230, Marsden Weighing Group, Oxfordshire, UK), with the mass of the probe subtracted, after which no further drinking fluids were consumed until after the test. Skin thermistors (Grant Instruments Ltd., Cambridge, UK) were then attached to four sites on the participants' right side: upper chest, mid-ventral forearm, mid-calf and mid-thigh. Skin temperature was recorded continuously (SQ2010, Grant Instruments Ltd., Cambridge, UK) and reported every 30-s. Mean skin temperature (T_{skin}) was calculated based on the four measured sites (Ramanathan, 1964). Prior to fitting skin thermistors, the skin was cleaned with soap and water and dry shaved, before being thoroughly dried. Local sweat rate was measured using a Q-Sweat system (WR Medical Electronics Co., Stillwater, MN). Ventilated capsules were fixed proximal to the skin thermistors on the right-side of the body. Sweat rate was calculated using standard vapour pressure equations and expressed in $nL \cdot min^{-1}$. Mean sweat rate was calculated by averaging the four sweat sites and reported every 1-min.

Participants then entered the environmental chamber and were instructed to maintain a pedal cadence of 70 rev·min^{-1} at an intensity equivalent to thermoneutral gas exchange threshold until complete exhaustion. Given the effect of the hot environment on sub-maximal endurance thresholds (Aleksander et al., 2010), the intensity was deemed to be above the thermoneutral gas exchange threshold. Exhaustion was defined as voluntary withdrawal or when pedal cadence dropped below 70 rev·min^{-1} for more than 10-s. The coefficient of variation for power output in this test in our laboratory is 3.8% while cycling in the heat. Ratings of perceived exertion (RPE) were recorded on a 6 to 20-point Borg scale. Thermal sensation (TS) was recorded on a 9- point scale where -4 = "very cold", 0 = "neutral", and 4 = "very hot". RPE and TS were recorded every 2-min but are reported at the start, mid-point and at completion of the T_{lim}. Skin thermistors and sweat capsules were also removed post-exercise, before being towel-dried and re-weighed to indicate fluid losses during exercise.

Partitional calorimetry

Using standard partitional calorimetry equations, elements of heat production and heat dissipation were estimated (see supplementary material).

Statistical analysis

Two-way repeated measures analyses of variance were conducted to test for effects of condition (BR or PLA), time (10% epochs across the trials) and their interactions on dependent variables measured across the exercise trials. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. Where time or interaction effects were found, *post-hoc* analyses were performed with Bonferroni tests to identify pairwise differences. Paired samples *t*-tests were used to assess differences between the performance trials (T_{lim}), plasma [NO_{3-}] and [NO_{2-}], body mass changes (Δ%BM) during the T_{lim} test and pre-test urine osmolality during PLA and BR conditions. A paired t-test was also used to assess the trial-order effects. A one-way ANOVA was performed on resting blood pressure (mean arterial pressure; MAP) during preliminary testing and pre-test for PLA and BR conditions. Statistical significance was accepted at $P \leq 0.05$ and all analyses were performed on IBM SPSS Statistics (Version 21, IBM Corp., Armonk, NY, USA).

Results

Limit of exercise tolerance (T_{lim})

There were no differences ($t_{(10)}$ = 1.4, P = 0.184) in T_{lim} between the BR and PLA conditions, despite seven out of the eleven participants extending their performance after BR supplementation (BR = 22.8 ± 8.1-min; Placebo = 20.7 ± 7.9-min). There were also no trial order effects on the time to exhaustion (P = 0.168).

Plasma nitrate ($[NO_{3-}]$) and nitrite ($[NO_{2-}]$) concentrations

There were condition effects on plasma $[NO_{3-}]$ ($t_{(10)}$ = 12.3, P < 0.001) and plasma $[NO_{2-}]$ ($t_{(10)}$ = 6.4, P < 0.001). Specifically, the values for $[NO_{3-}]$ in the PLA and BR conditions 10 were 22.9 ± 5.5 μM and 604 ± 158 μM, respectively, whilst those for $[NO_{2-}]$ were 99.5 ± 30.2 nM and 430.7 ± 149.8 nM, respectively.

Core (T_{core}) and skin (T_{skin}) temperature

T_{core} increased with time ($F_{(9,90)}$ = 89.598, p < 0.001), with no main effect of condition ($F_{(1,10)}$ = 0.248, p = 0.629) or interaction with condition ($F_{(9,90)}$ = 1.873, p = 0.066) (Figure 1A). The highest core temperatures reached were 37.9 ± 0.2 °C and 38.0 ± 0.4 °C in the BR and PLA conditions, respectively. T*skin* increased with time ($F_{(9,90)}$ = 7.179, P < 0.001), without condition effects ($F_{(1,10)}$ = 0.796, P = 0.096) or an interaction with time ($F_{(9,90)}$ = 0.122, P = 0.099) (Figure 1B).

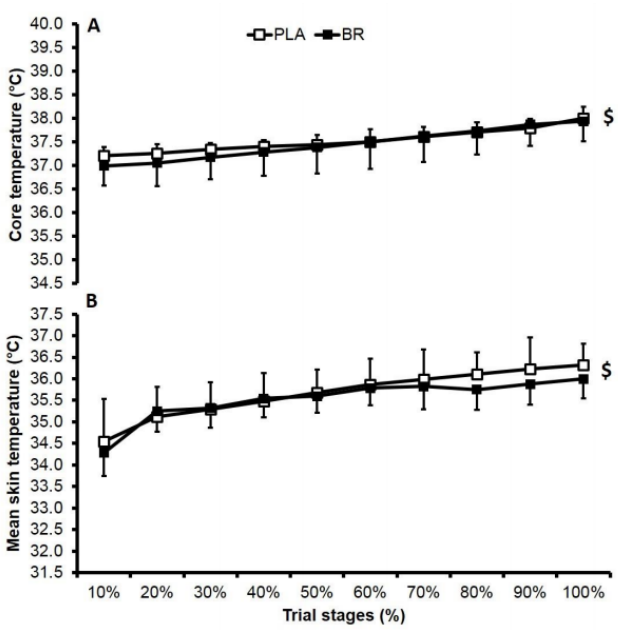

Figure 1 Core (A) and skin (B) temperature during exercise at the power output associated with gas exchange threshold following beetroot (black squares) supplementation or placebo (white squares) in a hot, dry environment. Data are expressed as a proportion of the exercise trial (n = 11). $ = main effect of time (P < 0.05).

Mean local sweat rate and heart rate

Mean local sweat rate increased with time ($F_{(9,90)}$ = 102.491, P < 0.001) but there were no effects of condition ($F_{(1,10)}$ = 0.047, P = 0.832) or condition × time interactions ($F_{(9,90)}$ = 0.101, P = 1.000) (Figure 2A). Heart rate increased with time ($F_{(9,90)}$ = 214.520, P < 0.001); however, there were no main effects for condition ($F_{(1,10)}$ = 0.175, P = 0.685), and no significant interaction ($F_{(9,90)}$ = 1.178, P = 0.319) (Figure 2B).

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$)

Both $\dot{V}O2$ ($F_{(9,90)}$ = 12.240, P < 0.001) and $\dot{V}CO2$ ($F_{(9,90)}$ = 6.379, P < 0.001) changed across time. Neither $\dot{V}O2$ ($F_{(1,10)}$ = 0.172, P = 0.687) nor $\dot{V}CO2$ ($F_{(1,10)}$ = 0.010, P = 0.922) were different between conditions and no interactions were observed (P > 0.05) (Figure 2C & 2D, respectively).

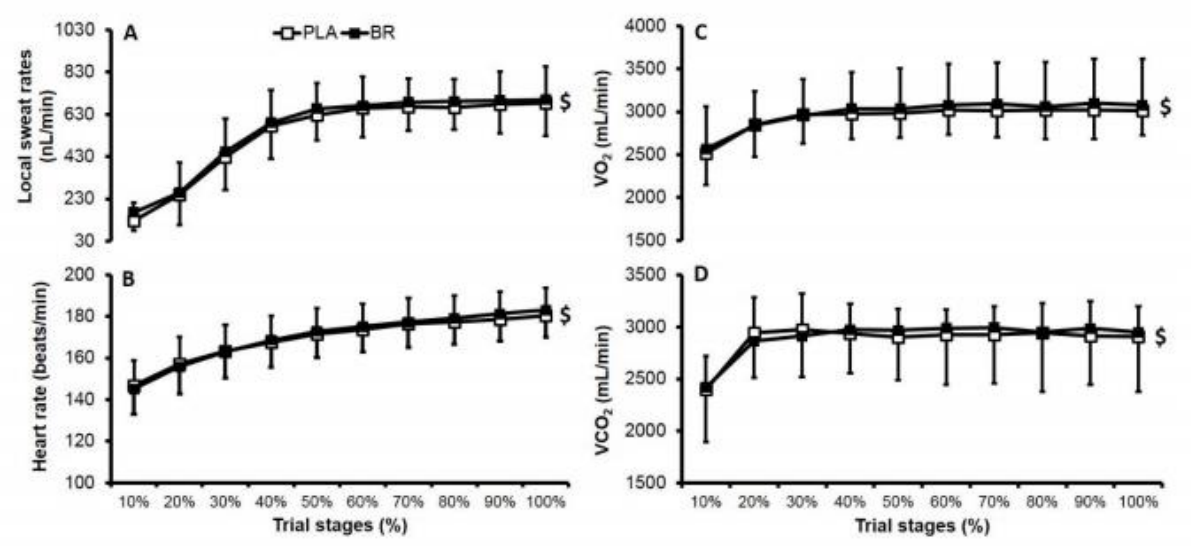

Figure 2 Mean local sweat rate (A). heart rate (B), oxygen consumption (VO2; C) and carbon dioxide production (VCO2; D) during exercise at the power output associated with gas exchange threshold following beetroot (black squares) supplementation or placebo (white squares) in a hot environment. Data are expressed as a proportion of the exercise trial (n = 11). $ = main effect of time (P < 0.05).

Thermal sensation (TS) and rating of perceived exertion (RPE)

Both TS ($F_{(9,90)}$ = 30.507, P < 0.001) and RPE ($F_{(9,90)}$ = 64.895, P < 0.001) changed across time. Neither TS ($F_{(1,10)}$ = 0.034, P = 0.858) nor RPE ($F_{(1,10)}$ = 0.239, P = 0.635) were different between conditions and no interactions were observed (P > 0.05).

Hydration, whole-body sweat rate (Δ%BM) and resting mean arterial pressure

As presented in Table 1, there was a significant main effect of condition on MAP ($F_{(2,20)}$ = 7.772, P = 0.003), with *post-hoc* tests demonstrating higher (P = 0.004) values in the PLA compared to the BR condition. There were also differences between PLA (P = 0.050) and preliminary measures but not between preliminary and BR (P = 1.000). There were no changes in osmolality ($t_{(10)}$ = 0.595, P = 0.565) or Δ%BM ($t_{(10)}$ = 1.473, P = 0.172) between conditions.

Table 1 Urine osmolality, pre-to-post exercise body mass changes, and resting pre-exercise mean arterial pressure during preliminary testing or following 5-days of placebo or beetroot supplementation.

	Pre-testing	Placebo	Beetroot
Urine osmolality ($mOsm \cdot kg^{-1} \cdot H_2O^{-1}$)	-	292 ± 166	262 ± 115
Body mass change (%)	-	0.71 ± 0.18	0.85 ± 0.36
Mean arterial pressure (mmHg)	98.9 ± 5.1*	99.8 ± 6.7*	96.1 ± 5.1

Note: * = sig. different from beetroot condition (P ≤ 0.05).

Partitional calorimetry

SkBF requirements did not change between the PLA (7.7 ± 2.4 $L \cdot min^{-1}$) and BR (7.5 ± 4.4 $L \cdot min^{-1}$) conditions ($t_{(10)}$ = 0.207, P = 0.840). There were no differences between PLA and BR for H_{prod} ($t_{(10)}$ = 0.103, P = 0.920), H_{dry} ($t_{(10)}$ = 1.913, P = 0.085), E_{req} ($t_{(10)}$ = 0.789, P = 0.448), heat storage ($t_{(10)}$ = 0.941, P = 0.369), E_{max} ($t_{(10)}$ = 1.919, P = 0.084) or W ($t_{(10)}$ = 0.101, P = 0.337) (Table 2).

Table 2 Partitional calorimetry during fixed-intensity exercise trials in a hot, dry environment following 5-days of placebo or beetroot supplementation (n=11).

	Placebo	Beetroot
H_{prod} ($W \cdot m^{-2}$)	428.4 ± 41.4	426.9 ± 54.8
H_{dry} ($W \cdot m^{-2}$)	7.7 ± 3.6	5.8 ± 2.4
E_{req} ($W \cdot m^{-2}$)	276.7 ± 60.6	299.5 ± 55.9
Heat storage ($W \cdot m^{-2}$)	142.1 ± 64.5	121.2 ± 35.3
E_{max} ($W \cdot m^{-2}$)	316.2 ± 8.6	314.2 ± 9.2
W (E_{req}:E_{max})	0.89 ± 0.15	0.95 ± 0.14

Note: H_{prod} = Heat production; E_{req} = required evaporative heat transfer; E_{max} = maximal evaporative cooling capacity of the environment; H_{dry} = dry heat transfer.

Discussion

We investigated the effects of five days dietary NO_{3-} supplementation on exercise tolerance and thermoregulation in hot, dry conditions among physically inactive males. Contrary to our hypothesis, there were no effects of the supplement on core or shell temperatures, nor were there any physiological changes during cycling exercise, despite participants in the BR condition descriptively increasing their T_{lim} by 2.1-min and demonstrating significant reductions in MAP compared to PLA. Further investigation of thermal balance using partitional calorimetry revealed no change in dry or latent heat exchange between conditions during exercise. Thus, in accordance with the findings of others (Kuennen et al., 2015; Kent et al., 2017; 2018; McQuillan et al. 2017; Amano et al., 2018), there appears to be no thermoregulatory or performance benefits associated with the consumption of NO_{3-} -rich beetroot juice prior to exercise in the heat.

Although the null effects of NO_{3-} supplementation on exercise tolerance are in contrast to our hypothesis, these results were not completely unanticipated, given the equivocal findings reported on exercise performance to date (McMahon et al., 2017). While a noteworthy mean increase in T_{lim} of 2.1-min (9.4%) was observed in the BR condition, the variability in response among individuals precluded any clear ergogenic effect, thus highlighting the potential for individual responders. Training status has been proposed to account for the variability in thermoneutral performance responses to NO_{3-}, (Porcelli et al., 2015). On this basis, coupled with the likelihood of inferior heat tolerance (Ravanelli et al., 2017), we intentionally recruited those of lower fitness levels. The lower $\dot{V}O_{2max}$ and reported training frequency, alongside *W* values (i.e. E_{req}/E_{max}) provide evidence to support the physically inactive and non-acclimated status, respectively. However, based on the null and variable findings, it is likely that other phenotypic factors explain variance in the response to NO_{3-} supplementation during hot exercise. Further research is required in this regard but it is likely that individual responses are related to factors that directly limit heat tolerance, such as baseline deficiencies in peripheral avenues of heat dissipation or variable responses to the BR supplement. Indeed, despite no whole-body sweat rate changes, three participants lost > 1% of their body mass during the T_{lim} following BR, compared to ~ 0.5% following PLA. Given that sweat production and subcutaneous vasomotor function can be modified by serial thermal exposures among both healthy (Lorenzo & Minson, 2010) and some clinical populations with peripheral impairments (Kenny et al., 2016), these factors provide a logical basis for individual variation in response to BR during exercise in the heat and require further investigation.

The hot, dry environment of the current study was conducive to evaporative cooling and the untrained status of the male participants, exercising at intensities above gas exchange threshold was hypothesised to potentiate the effects of the $NO_{3-} \rightarrow NO_{2-} \rightarrow NO$ pathway on exercise capacity and

heat transfer. Given the capacity of this pathway to facilitate NO availability, it was assumed that an effect would be identified in the sweat gland or sub-cutaneous vasculature, capable of assisting with heat dissipation during exhaustive exercise. Indeed, increasing circulating plasma NO_{2^-} might enhance its delivery to the skin microvasculature (Fujii et al. 2016; McNamara et al. 2014) or sweat gland (Weller, 1996), where reduction of NO_{2^-} to NO can facilitate cutaneous vasodilation and/or sweat production. Furthermore, the prescribed exercise intensity was intended to enhance reliance upon NOS-independent pathways (Fujii et al., 2014), thus potentiating the effect of $NO_{3^-} \rightarrow NO_{2^-} \rightarrow NO$ cascade on cutaneous vasculature. However, despite these conditions, the similarity in local sweat rates, skin temperatures, SkBF requirements and E_{req} reported herein question the thermoregulatory advantages of dietary NO_{3^-} among healthy participants exercising at higher intensities in the heat.

Nitric oxide is a primary regulator of arterial pressure, acting as a signalling molecule for smooth muscle endothelia (Gilchrist et al. 2011). These mechanisms facilitate vasodilation of blood vessels and blood flow to the working musculature in thermoneutral environments (Ferguson et al. 2013). Accordingly, reductions in resting (Bailey et al. 2009; Bond et al. 2014; Fujji et al., 2015; Levitt et al., 2015; Kent et al., 2018) and exercising (Bond et al. 2014; Amano et al., 2018) blood pressure have been frequently reported after consumption of NO_{3^-}. These reports support our findings, where resting MAP was reduced by 3.8% in the BR condition compared to PLA. Whilst the effects of dietary NO_{3^-} on blood pressure are consistently reported and ascribed to NO-mediated actions on the macro-vasculature (Gilchrist et al., 2011), the effects on the subcutaneous vessels are less clear. For example, increases in resting 14 subcutaneous vascular conductance have been demonstrated in response to whole-body and local limb heating (43 °C), following 3-days of NO_{3^-} supplementation (Levitt, et al. 2015; Keen et al., 2015). However, a subsequent study did not report any changes in subcutaneous vascular conductance or local sweat rates after 3-days of dietary NO_{3^-} supplementation among healthy participants, despite similar reductions in exercising blood pressure (Amano et al., 2018). Unlike previous investigations, we and others (Amano et al., 2018) increased thermal load in a more ecologically valid manner, via heating of ambient room air and controlling exercise intensity, rather than water suit perfusion and ventral forearm heating (Levitt et al., 2015). These contrasting approaches provide markedly different afferent signals to the thermoregulatory centre, with the controlled water-suit and ventral forearm heating methods preventing typical dry cooling mechanisms and delivering a more focussed artificial stimulus, respectively (Romanovsky, 2018). The different heating methods, therefore, most likely explain the discrepancies between studies. Thus, the typical thermal stresses posed by exercise in a hot room environment appear to be insufficient to require additional NO availability (i.e. via $NO_{3^-} \rightarrow NO_{2^-} \rightarrow NO$ pathway) for heat loss mechanisms that rely on sudomotor and subcutaneous microvascular function.

The current participants were recruited without any known impairment in sudomotor function or vasodilatory capacity that would limit the autonomic response to whole-body heating and exercise. Consistent with our aforementioned argument regarding the severity of the imposed thermal signal, it is also feasible that impairments in sweating or microvascular function are necessary to observe changes in thermoregulation during hot exercise, since this would effectively lower the participant's individual threshold of thermal tolerance. Indeed, there are age-related impairments in NOS-dependent sweating during exercise (Stapleton et al., 2014; Fujji et al., 2015), such that older adults may experience thermoregulatory deficits in response to heat exposures. Therefore, future research could consider the effects of NO_{3-} supplementation among elderly populations and, perhaps, other clinical conditions known to impair sweating or skin microvascular function in response to thermal challenge. For example, it was recently reported that dietary NO_{3-} supplementation can improve peripheral blood flow following cold exposure among those with Raynaud's phenomenon (Shepherd et al., 2019) and it is known that both type 1 and 2 diabetics demonstrate irregular peripheral responses to hot and cold exposures (Kenny et al., 2016), which could be modified by supplements with vasoactive properties.

The absolute final core temperatures reached in the current study were not as high as anticipated, given the high exercise intensity and thermal environment, and are a possible limitation. However, the rate of change in core and skin temperature, as well as the substantial local sweat response and fluid losses (0.71 to 0.85% body mass loss in ~ 20 min) are evidence of a notable thermoregulatory response to the heat. Thus, the intensity of the exercise, in combination with the ambient temperature, appear to have induced notable physiological adjustments to the heat, sufficient to prevent the continuation of exercise. The participants' perceptions of this hot environment also confirm this. This outcome appears to be a consequence of the deliberately chosen higher intensities. Finally, while there have been inconsistent findings to date (Balsalobre-Fernández et al., 2018), it was feasible that BR supplement would lower $\dot{V}O_2$ during fixed-intensity exercise (Larsen et al., 2011). Indeed, it was possible that an improved exercise efficiency would be useful for thermal balance via reductions in metabolic heat gain. There are a number of reasons for the null effects reported herein, such as the increased energy demands (such as myocardial oxidative demand; Nielsen et al. 1990) or reduced mitochondrial efficiency (Willis & Jackman 1994) induced by exercising in the heat – all of which might have offset the reported metabolic advantages conferred by BR supplements in thermoneutral environments.

Conclusion

Five days of dietary NO_{3-} supplementation had no effect on exercise tolerance or thermoregulation in hot, dry conditions among physically inactive male participants, despite the typically observed reductions in pre-exercise resting MAP and increases in plasma [NO_{3-}] and [NO_{2-}]. To enhance our understanding of dietary NO_{3-} on thermal tolerance, future research should consider populations with impaired vasodilatory or sudomotor function in response to thermal challenge or consider increasing the severity of the whole-body temperature changes.

Declaration of interest statement

The authors have no conflicts of interest to declare.

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Supplementary material (partitional calorimetry equations):

Measured $\dot{V}O2$ and $\dot{V}CO_2$ were used to determine metabolic energy expenditure ($\dot{M}$) estimated by equation 1. H_{prod} was calculated by subtracting the rate of mechanical work (Wk) from M (equation 2):

$$M = \frac{VO_2\left(\frac{RER-0.7}{0.3}\right)e_c + \left(\frac{1.0-RER}{0.3}\right)e_f}{60(\mathrm{BSA})}\ (1{,}000)[W \cdot \mathrm{m}^{-2}] \qquad \text{equation 1.}$$

$$\mathrm{H_{prod}} = \mathrm{M} - \mathrm{Wk}\,(\mathrm{W} \cdot \mathrm{m}^{-2}) \qquad \text{equation 2.}$$

where RER is the respiratory exchange ratio, e_c and e_f are the caloric equivalents per L of O_2 for oxidation of carbohydrates (21.13 kJ) and fats (19.62 kJ), respectively (Cramer & Jay, 2018).

Convective heat transfer was determined by equation 3 (Parsons, 1993):

$$C = h_c(\mathrm{T_{skin}} - \mathrm{T_a})[\,\mathrm{W} \cdot \mathrm{m}^{-2}] \qquad \text{equation 3.}$$

$$h_c = 8.3\mathrm{v}^{0.6}[\mathrm{W} \cdot \mathrm{m}^{-2}] \qquad \text{equation 4.}$$

where hc is the convective heat transfer coefficient (Kerslake, 1972; equation 4), T_{skin} is the weighted mean temperature of the skin (°C), T_a is the temperature of the ambient air (°C), and v is the air velocity of 0.5 $m \cdot s^{-1}$ in the chamber. Radiant heat transfer was determined by equation 5 (Parsons, 1993):

$$R = h_r(\mathrm{T}_{skin} - \mathrm{T}_r)[\mathrm{W} \cdot \mathrm{m}^{-2}] \qquad \text{equation 5.}$$

where T_r refers to the mean radiant temperature of the environment (°C), which was assumed to be equivalent to T_a, h_r refers to the radiative heat transfer coefficient (Parsons, 1993) (equation 6).

$$h_r = \varepsilon 4\sigma(A_r/A_D)\left(\frac{\mathrm{T}_{skin}+\mathrm{T}_r}{2}\right)+273.15)^3[\mathrm{W}\cdot\ \mathrm{m}^{-2}\cdot\mathrm{K}^{-1}] \qquad \text{equation 6.}$$

where ε is the weighted area emissivity of the skin (0.98; Gonzalez, 1995) σ is the Stefan-Boltzmann constant ($5.67 \cdot 10^{-8}$ W $\cdot$ m^{-2} $\cdot$ K^{-4}), and A_r/A_D is the effective radiative area of the body, assumed to be 0.70 for ergometer sitting (Tanabe et al. 2000).

Conductive heat transfer and respiratory losses were deemed to be negligible and not included in the calculations. Thus, dry heat exchange (H_{dry}) at the skin surface was calculated as (Havenith, 2016):

$$\mathrm{H}_{\mathrm{dry}} = \mathrm{C} + \mathrm{R} \qquad \text{equation 7.}$$

E_{req} was calculated as (Havenith, 2016):

$$\mathrm{E}_{\mathrm{req}} = \mathrm{H}_{\mathrm{prod}} - (\mathrm{C}+\mathrm{R}+\mathrm{S})(\mathrm{W}\cdot\ \mathrm{m}^{-2}) \qquad \text{equation 8.}$$

E_{max} and *W* were determined according to equations 9 and 10, respectively (McIntyre, 1980):

$$E_{max} = f_{pcl}\ \cdot\ h_e\ \cdot(P_s - P_a)(\mathrm{W}\cdot\ \mathrm{m}^{-2}) \qquad \text{equation 9.}$$

$$W = \frac{\mathrm{E}_{\mathrm{req}}}{\mathrm{E}_{\mathrm{max}}} \qquad \text{equation 10.}$$

where f_{pcl} = permeation efficiency factor of clothing (fixed at 0.85 based on presumed clothing insulation of 0.0295 m^2 ·K·W^{-1} while wearing shoes, socks and cycling shorts, h_e = evaporative heat transfer coefficient (assumed to be 77.19 W·m^{-2}·kPa^{-1} based on 0.5 m·s^{-1} air velocity), *Ps* = partial water vapor pressure at the skin surface (kPa) and P_a = partial water vapor pressure of ambient air (kPa).

Heat storage was therefore determined as (Parsons, 1993):

$$\mathrm{S}\ (\mathrm{W}\cdot\ \mathrm{m}^2) = ((3473\ \cdot \mathrm{wt}\ \cdot (\mathrm{T_b}\ final - \mathrm{T_b}\ initial))/t\ /A_D \quad \text{equation 11.}$$

where 3474 = average specific heat of body tissue ($J \cdot kg^{-1} \cdot °C^{-1}$), wt = body mass (kg), T_b = mean body temperature (°C) (according to Kerslake, 1972), t = exercise time (s) and A_D = body surface area (m^2).

On the assumption that blood entering and leaving the cutaneous circulation was equal to core and skin temperatures, respectively, maximum skin blood flow (SkBF) was determined as (Sawka & Young, 2006):

$$\mathrm{SkBF} = \frac{(\frac{1}{\mathrm{SH}} \cdot \mathrm{H_{prod}})}{(T_{core} - T_{skin})} \quad \text{equation 12.}$$

where SH = specific heat of the blood (~1 $kcal \cdot °C^{-1}$) and H_{prod} is expressed in $kcal \cdot min^{-1}$. T_{core} and T_{skin} where taken as the final measurements of the T_{lim} to provide a maximal SkBF measure.