

An isotonic beetroot drink improves muscle oxygenation and cycling time-trial performance in physically active individuals: a randomised crossover trial

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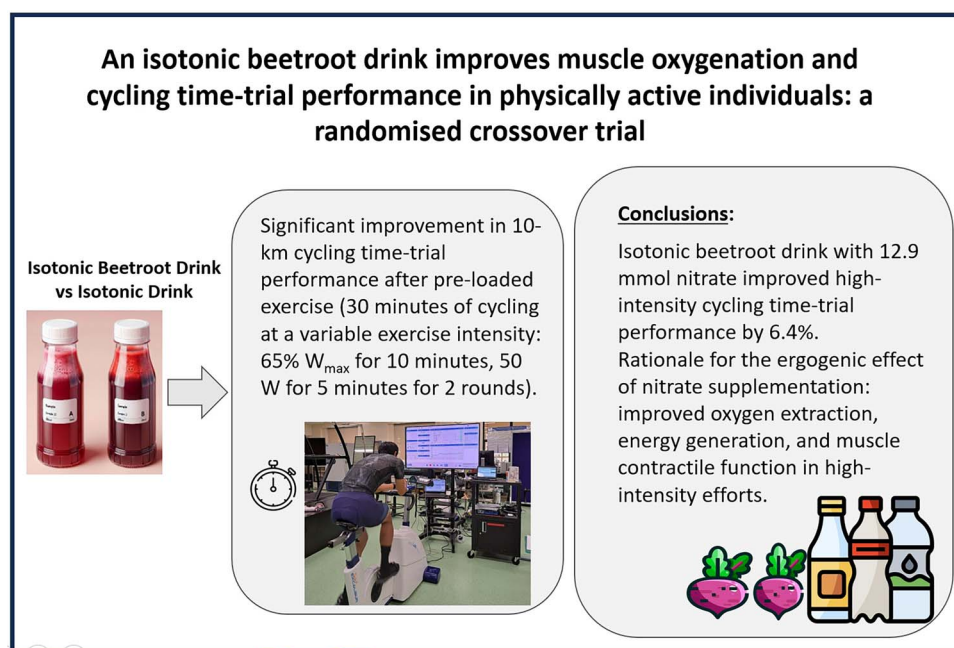
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Abstract

Dietary nitrate supplementation can improve exercise performance. This study investigated whether nitrate (beetroot) added to an isotonic drink could improve high-intensity cycling time-trial performance and muscle oxygenation in active individuals. Twenty-four (17 male) physically active participants [$M \pm SD$; age: 28 ± 5 years; body mass index (BMI): 22.6 ± 1.9 kg/m²] completed a double-blind, randomised, crossover study, where they consumed 570 ml of either isotonic sports drink (ISO-C) or isotonic beetroot drink (ISO-BR) 1 hr before performing preloaded exercise, followed by a 10-km cycling time-trial on a cycle ergometer. Both drinks had equal osmolality (290 mOsm/kg), but differed in nutrients from beetroot extract, particularly nitrate content (ISO-C: 0 mmol, ISO-BR: 12.9 mmol). Salivary total nitrate and nitrite (NO_x) 1-hr post-ingestion were significantly higher after consuming ISO-BR than ISO-C ($p < .001$). ISO-BR significantly improved time-trial performance compared to ISO-C by 6.4% (ISO-BR: 1068.4 ± 333.3 s versus ISO-C: 1141.0 ± 381.8 s, $p < .001$), cadence by 3.8% (ISO-BR: 83 ± 6 rpm versus ISO-C: 80 ± 7 rpm, $p < .001$) and average load during the time trial by 5.3% (ISO-BR: 153.9 ± 47.7 W versus ISO-C: 146.2 ± 48.4 W, $p < .001$). Oxygenated blood (ΔO_2Hb) was significantly higher ($p = .03$, partial $\eta^2 = .015$), and deoxygenated blood was substantially lower ($p = .01$, partial $\eta^2 = .021$) over the trials after ISO-BR supplementation than ISO-C. An ISO-BR drink provided pre-exercise can improve cycling time-trial performance over an ISO-C drink, potentially via increases in NO_x.

Keywords: beetroot, nitrate, cycling, time-trial, high-intensity, muscle oxygenation, functional drink

Graphical abstract



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Introduction

Nitrate-containing foods or juices can improve exercise performance (Bailey et al., 2009; Bailey et al., 2015; Hoon et al., 2014; Maughan et al., 2018; Thompson et al., 2016; Wylie et al., 2016). Beetroot juice is often utilised as a source of dietary nitrate to enhance sports performance. Polyphenols and ascorbic acid in beetroot juice play specific roles in nitrate reduction and metabolism to nitric oxide (NO) (Flueck et al., 2016)—a signalling molecule that contributes to numerous physiological functions essential for exercise metabolism (Cuenca et al., 2018; Jodra et al., 2020; Jones et al., 2021; Stamler & Meissner, 2001).

Systematic reviews and meta-analyses (Wong et al., 2022) suggest that dietary nitrate ingestion can provide a significant effect on time-to-exhaustion tests (Bailey et al., 2009; Hoon et al., 2013; Lansley et al., 2011) and total distance travelled (Nyakayiru et al., 2017; Thompson et al., 2016; Wylie et al., 2013) during endurance exercise. Findings from our systematic review and meta-analysis suggest that chronic nitrate supplementation may improve time trial performance ranging from 5 to 30 min in duration, particularly when nitrate from a beetroot source is supplemented in high doses in a chronic manner (Wong et al., 2022). Another similar systematic review and meta-analysis suggested that the benefits of nitrate on performance are confined to distances completed between 3 and 10 min in length (Silva et al., 2022). According to the current understanding of nitrate metabolism, there is potential for improved endurance performance in time trials of higher intensity and shorter duration (Lorenzo Calvo et al., 2020; Shannon et al., 2017; Wong et al., 2022), because NO production is facilitated in low pH and low oxygen environments. In animal models, nitrate supplementation can affect the contractile function and blood flow to type II muscle fibres recruited during high-intensity work (Andrade et al., 1998; Ferguson et al., 2015; Jones et al., 2021). In addition, nitrate and nitrite can be stored in muscle tissue, potentially contributing to performance improvements in power observed during high-intensity exercise (Jodra et al., 2020; Kadach et al., 2022). However, the impact of nitrate supplementation during endurance time trials has not been observed when subjected to meta-analysis (Lorenzo Calvo et al., 2020; Shannon et al., 2017; Wong et al., 2022). Indeed, a recent umbrella review of 20 systematic reviews and meta-analyses suggests that the endurance performance effects are confined to open-ended tasks rather than time trials (Poon et al., 2025).

There may be several reasons for this discrepancy between endurance capacity and endurance performance. One consideration is that the fatigue mechanism may differ depending on the length of a time trial. Peripheral fatigue has been considered to constrain shorter high-intensity time trials while central fatigue may predominate with longer, lower-intensity time trials (Thomas et al., 2015). As exercise intensity and peripheral oxygen demand in maximal efforts are directly associated with the duration of any event, it is possible that nitrate supplementation may still be effective in shorter, high-intensity endurance time-trial events (Lorenzo Calvo et al., 2020; Shannon et al., 2017).

Enhancing the functionality of the oral microbiome presents an exciting opportunity for boosting NO levels in the bloodstream (Blot, 2021; Duncan et al., 1995; Jones et al., 2021). In this light, incorporating nitrate into an isotonic beverage is a compelling innovation. Isotonic drinks are recognised for their ability to optimise gastric emptying and support the absorption of carbohydrates, sodium, and fluids through the intestinal wall (Leiper, 2015). Notably, when combined with ascorbic acid in an

isotonic drink, nitrate may further promote NO production in the stomach's acidic environment (Peri et al., 2005). Additionally, isotonic beverages have been shown to increase salivary flow rates (Bishop et al., 2000; Siow et al., 2017), thereby potentially enhancing the movement of nitrite from the mouth into the systemic circulation. This strategy could represent a significant advancement in performance enhancement for athletes, and we recently demonstrated that an isotonic beetroot drink can elevate both salivary and plasma nitrate/nitrite and that peak power output during a single sprint exercise is greater than when compared to non-isotonic beetroot juice drink with the same nitrate content (Wong et al., 2024).

Nitrate supplementation is also suggested to impact muscle blood flow, oxygen extraction, mitochondrial respiration, and, indirectly, NO production in a low-oxygen environment. Muscle deoxygenation rate has been suggested as a non-invasive indicator for estimating fractional muscle oxygen extraction during exercise (Rowland et al., 2023). Non-invasive near-infrared spectroscopy (NIRS) is one method to obtain a real-time measurement of muscle oxygenation/deoxygenation and blood flow in muscle tissue during high-intensity time-trial exercise. This measurement would help support the efficacy of an isotonic beetroot juice drink in time-trials of shorter duration.

Thus, the primary aim of this study was to determine whether the provision of dietary nitrate in an isotonic drink (ISO-BR) can improve high-intensity cycling time-trial performance (<30 min in length) in active individuals compared with an isotonic drink (ISO-C) with similar osmolality. Beetroot juice was selected as the nitrate source due to its high nitrate concentration, presence of polyphenols and ascorbic acid that facilitate nitrate-to-nitrite conversion, and its widespread use in previous ergogenic studies (Bailey et al., 2009; Jodra et al., 2020). It was hypothesised that an ISO-BR drink would improve high-intensity cycling time-trial performance via better muscle oxygenation brought about by the increased nitrate-nitrite availability. Recreationally active individuals were chosen as evidence suggests that trained athletes may be less responsive to the ergogenic effects of nitrate supplementation because they have higher endogenous NO bioavailability (Porcelli et al., 2015; Wong et al., 2021).

Materials and methods

Participants

The study findings were reported using as per CONSORT criteria. This study was approved by the Institutional Review Board of Nanyang Technological University, Singapore (IRB-2022-880) and prospectively registered at ClinicalTrials.gov (ID: NCT05841407). A priori sample size calculation using G*Power (version 3.1, Universität Kiel, Kiel, Germany) was determined from a previous study examining the effect of beetroot juice on cycling time-trial performance (Lansley et al., 2011). It was determined that 20 participants would be sufficient to achieve a statistical power of 0.95 using a two-tailed paired t-test design with an effect size (Cohen's *d*) of 0.87 calculated from previously published data ($M \pm SD$: placebo 27.7 ± 2.1 min versus beetroot juice intervention: 26.9 ± 1.8 min) with a correlation between groups of 0.9 (based on power output measures using an isotonic beetroot juice drink) (Wong et al., 2024). Twenty-eight physically healthy and active participants volunteered and provided written informed consent to participate. Four participants failed to complete the study because of injury (not related to this study) or illness ($n = 2$) and

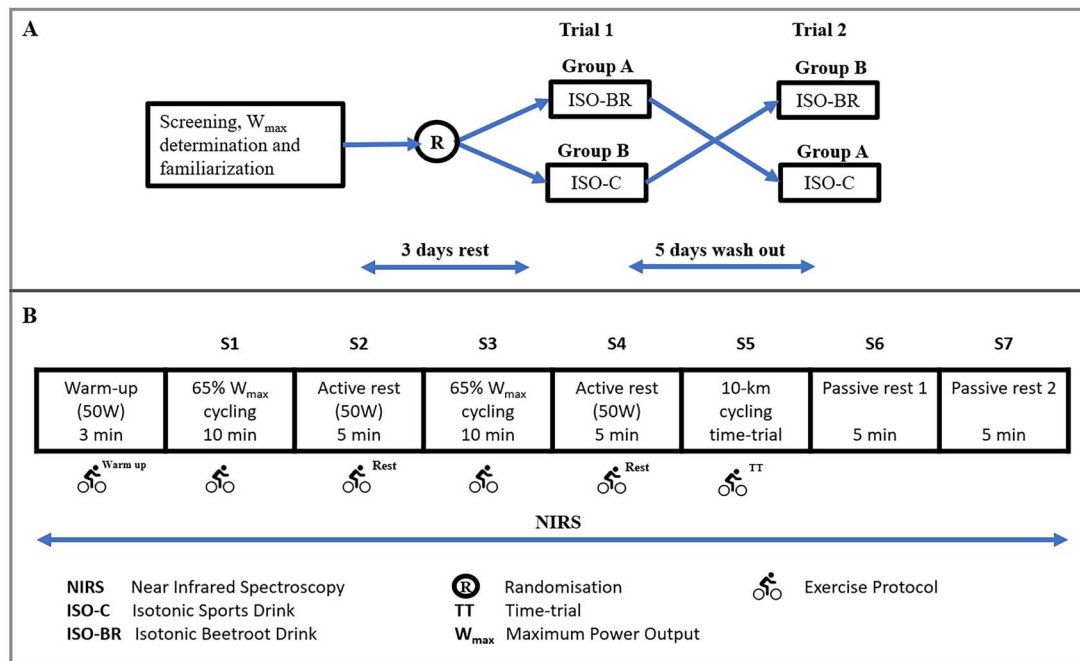


Figure 1. Experimental design (A) and exercise protocols (B). S1–S7 represented the seven stages in the exercise protocol.

personal time constraints ($n = 2$). The remaining 17 males [$M \pm SD$; age 29 ± 5 years, body mass index (BMI): 22.7 ± 2.0 kg/m²] and 7 females (age: 28 ± 5 years, BMI: 22.1 ± 1.9 kg/m²) completed all study procedures. Inclusion criteria were: (i) age: 21–40 years; (ii) Asian (for group homogeneity); (iii) healthy and injury-free (e.g., no cardiovascular disease, diabetes mellitus or orthopaedic impairment that interferes with moderate-to-vigorous exercise); (iv) blood pressure $<130/90$ mmHg; (v) engaging in a minimum of 150 min of moderate-intensity or 75 min of vigorous-intensity physical activity each week or the equivalent based on both; (vi) recreational cyclist (minimum 1-hr cycling per week); (vii) BMI: 18.5–25.0 kg/m²; and (viii) non-smoker.

Experimental design

A randomised, double-blinded, and crossover study conducted over 8 months (February 2023 to October 2023) in a controlled laboratory environment. Participants visited the laboratory three times, including a screening and familiarisation visit and two main trials. Participant randomisation for main trials was completed using a randomisation function in Microsoft Excel, and allocation was performed beforehand by an individual not involved in the data collection and analysis (a PhD student under SFB). The individual allocated participants to the study team via opaque envelopes.

The drink manufacturer coded both drinks, and the order of consumption was blinded to both researchers and participants. During each main trial, participants ingested 2×285 ml of either ISO-BR or ISO-C beverage 1 hr before performing preloaded exercise, followed by a 10-km cycling time-trial. In the subsequent trial, participants completed the same procedures with the ingested beverage reversed. Salivary total nitrate and nitrite concentrations (NO_x) were measured at the start of the morning (pre-ingestion), 1 hr after beverage consumption, and before and after the 10-km cycling time-trial. Participants filled out a sensory evaluation form concerning the drink consumed for each trial. The experimental design and exercise protocols are illustrated in Figure 1.

Screening and familiarisation

Visit one was for the screening of participants, a cycling ramp test to exhaustion and a pre-trial familiarisation. After providing written informed consent, participants had their height and body mass measured (Seca 764, Germany). Body mass index was calculated using body mass (kg) divided by the square of the height (m). Blood pressure was measured in a seated position using standard procedures (HEM 7211, Omron, Japan). Physical activity level was determined from self-report using the Global Physical Activity Questionnaire (GPAQ) (Armstrong & Bull, 2006). Participants confirmed by self-report that they were recreational cyclists (minimum 1 hr per week). Screening for potential health issues was done using a Physical Activity Readiness Questionnaire (2021 PAR-Q+) (Warburton et al., 2011).

Suitable participants then undertook a ramp test to determine their maximum power output measured on a cycle ergometer (Lode Excalibur Sport Ergometer, The Netherlands). Ergometric parameters during the ramp test were controlled and monitored by software (The Lode Ergometry Manager). Participants started the exercise protocol with a standardised warm-up of 5 min of cycling at 50 W. Immediately after warm-up, the ramp test began at 50 W, with the power output increasing by 25 W (for males) or 15 W (for females) each minute until volitional exhaustion. These settings were chosen to obtain a loaded test duration of 8–12 min as recommended by the American College of Sports Medicine 2009 (Rozenberg et al., 2015, American College of Sports and Thompson, 2009). Heart rate and rate of perceived exertion were monitored during the test to confirm maximum effort. Following the ramp test, participants rested for 30 min to recover before continuing with the familiarisation procedures that mirrored the main trials.

Main trials

Participants were instructed to avoid antiseptic mouthwash for all main trials as the consumption blunts any increase in salivary or plasma nitrite (Kapil et al., 2013). Participants reported to the laboratory in the morning (8:00 a.m. to 10:00 a.m.) for each main

trial after an 8–10 hr overnight fast (water only). Before their first main trial, they were briefed and instructed to record their physical activity and food items consumed. This was verified through an exercise and food intake log to ensure participants did not consume high-nitrate foods (such as celery, beetroot, spinach, etc.), health supplements (containing nitrate), or chewing gum or sweets that could contain any bactericidal substance in the 24 hr before the test sessions. There was a minimum of a 5-day washout period between main trials. Female participants were asked to self-report their menstrual cycle and were tested only within days 7–14 of their cycle (follicular phase) after any bleeding had stopped. While this provides experimental consistency in the testing dates, there is acknowledged variability of menstrual cycle length, although the extent to which this affects exercise performance is debated (Isenmann et al., 2024; Taylor et al., 2024). One female participant declared that she was using oral contraception; the remaining six reported they were not.

Participants were provided with their drink (ISO-C or ISO-BR) and, 1 hr afterwards, started the exercise protocol on the cycle ergometer. The exercise consisted of a 3-min warm-up (50 W) followed by 30 min of a four stage (S1–S4) preload cycling protocol during which exercise intensity was varied: 65% W_{max} for 10 min (S1), 50 W for 5 min (S2), 65% W_{max} for 10 min (S3), and 50 W for 5 min (S4). Based on previous studies, this preload protocol was tested in a pilot trial, and the load was adjusted as described above for these recreational athletes to be achievable (Che Jusoh et al., 2015; Jeukendrup et al., 1996). It was employed to create some peripheral fatigue and challenge to the muscle oxygen store before the main time trial, in line with the hypothesis that NO production is facilitated in low oxygen environments. At the end of the preload cycling, participants performed a 10 km time-trial performance test on a cadence-dependent (linear mode) cycle ergometer with an “ α -factor” of $(0.85 \times W_{max})/(95 \times 95)$. This setting has previously been employed (Saunders et al., 2017), and allows participants to cycle at their own pace and cadence to complete the 10 km time-trial as fast as possible. When cadence reaches 95 revolutions per minute (rpm), the load is equivalent to 85% W_{max} (Glaister et al., 2015; Saunders et al., 2017).

A NIRS device (Portamon, Artinis Medical System, Einsteinweg, The Netherlands) was attached to the vastus lateralis of the participant's outer right thigh to measure muscle oxygenation. Based on previous recommendations, the device was placed at 2/3 of the distance from the anterior spina iliaca superior to the lateral side of the patella (Hermens et al., 1999). The device was secured with surgical tape and shrouded with a dark-coloured elastic bandage around the leg to eliminate the influence of external light. Before placement, the area was cleaned and shaved for measurement consistency where necessary. The device's location was marked with a surgical skin marker pen to ensure the exact device placement for the subsequent trial. The NIRS device transmits two wavelengths (± 760 nm and ± 850 nm) from three optodes at 30, 35, and 40 mm from the receiver. The sampling frequency was set at 5 Hz and recorded with Oxysoft software (Artinis Medical Systems, The Netherlands). The relative changes of tissue saturation index (ΔTSI) in percentage and concentrations (μM) of oxyhaemoglobin + oxymyoglobin (ΔO_2Hb), deoxyhaemoglobin + deoxymyoglobin (ΔHHb), and total haemoglobin + myoglobin (ΔtHb , sum of ΔO_2Hb and ΔHHb) were tabulated by subtracting the value at specified time points (last 10 s of each stage, S1–S7) (Figure 1) from baseline during the resting period before performing the exercise protocol (Tan et al., 2022; Volino-Souza et al., 2020). The magnitude of ΔTSI , ΔO_2Hb , ΔHHb , and ΔtHb were calculated based on the difference between the baseline of

each stage (10 s before the start of each stage) and the endpoint (10 s before the end of each stage) (the difference between the baseline and the endpoint of each stage was interpreted as the rate of change of each stage) (Papadopoulos et al., 2018). The magnitude analysis aimed to understand the specific changes (the magnitude or rate of changes) in particular stages, i.e., during the exercise stage or active recovery stages. The NIRS measurements were taken to enhance understanding of the impact of nitrate supplementation on muscle blood flow, oxygen extraction, mitochondrial respiration, and, indirectly, NO production in a low-oxygen environment. In addition, the muscle deoxygenation rate has been suggested as a non-invasive indicator for estimating fractional muscle oxygen extraction during exercise (Rowland et al., 2023).

At the end of the cycling, participants completed the last post-exercise salivary NO_x test and a post-exercise short questionnaire related to the perception and the expected effects of the drink.

Supplementation

Drinks were supplied by a third-party beverage manufacturer (Fraser & Neave Limited, Singapore) and produced in a Good Manufacturing Practice standard food laboratory in Singapore. ISO-BR was prepared by adding beetroot extract to a typical isotonic sports drink (ISO-C) by reconstituting beetroot juice concentrate into ISO-C to standardise the nitrate content to 400 mg/285 ml. Citric acid and salts were adjusted to both ISO-C and ISO-BR to meet the osmolality level. Both drinks were pasteurised (95 °C for 30 s) to ensure safe for consumption before filling into bottles. The supplier provided a certificate of analysis for each drink to ensure the products were safe for consumption and met the target specification, including osmolality (Table 1).

Participants ingested 2×285 ml of beverage (either ISO-BR with 12.9 mmol of nitrate in total or ISO-C with 0 mmol of nitrate) within 10 min, and 1.5 hr before performing the time-trial test. Previous research suggested that beetroot juice should be ingested at least 1.5 hr before the exercise performance intervention (Jones et al., 2021). Our previous study also showed that peak salivary and plasma total nitrate/nitrite occurred within 60–90 min post-ingestion of ISO-BR (Wong et al., 2024). Both drinks had standardised colour and flavourings for blinding purposes and contained citric acid, salts, flavourings, and vitamins (B3, B6, B12). ISO-BR contained ascorbic acids (0.31 g/285 ml), which may play specific roles in nitrate reduction and metabolism (Peri et al., 2005). The drink manufacturer coded both drinks, and the order of consumption was blinded to both researchers and participants.

Sensory evaluation

Immediately after the drink ingestion, participants were given an online sensory evaluation form via QR code. An acceptance and preference test was used to evaluate the sensory properties of each drink. A 7-point hedonic scale (Meilgaard et al., 2007) was used, ranging from 1 (dislike extremely) to 7 (like extremely) for scoring the overall liking. The hedonic data were categorised into three groups: the top three boxes' overall liking rating (T3B), the bottom three boxes' overall disliking rating (B3B), and neutral overall liking (Neutral) to determine the preferences of the participant.

NO_x salivary test

A commercially available NO salivary test strip (Berkeley Fit, USA) was used to measure salivary NO_x before supplementation, one hour after supplementation, before the time trial and post time-trial exercise. The test strip measure is a composite measure of

Table 1. The specification of ISO-C and ISO-BR drinks. The additional carbohydrates, proteins, potassium, and sodium in ISO-BR (compared to ISO-C) were contributed by beetroot juice concentrate. Each bottle of ISO-BR (285 ml) contained 400 mg of nitrate.

Specification	ISO-C	ISO-BR
Nitrate content (mg/285 ml)	0	400 ± 30
Carbohydrates (g/285 ml)	14.8 ± 0.5	17.5 ± 0.5
Total sugars (g/285 ml)	14.0 ± 0.5	12.2 ± 0.5
Protein (g/285 ml)	0	1.4 ± 0.2
Sodium (mg/285 ml)	121 ± 5	219 ± 5
Initial osmolality (mOsm/kg)	290 ± 10	290 ± 10
Major ingredients	Sugars	Beetroot juice concentrate ^a
Minor ingredients	Citric acid, salts, vitamins (B3, B6, B12), permitted flavourings, colourings and preservative	Citric acid, salts, natural sweeteners, vitamins (C, B3, B6, B12), permitted flavourings and preservative

Note. Specifications were provided by the drinks manufacturer (Fraser and Neave Limited, Singapore). ^aReconstitution of 7% beetroot juice concentrate in ISO-BR equal to 35% beetroot juice content. Source of beetroot juice concentrate: *Beta vulgaris* cultivated in Karnataka, India (Sami-Sabinsa Group Limited).

saliva-derived NO analytes (nitrate and nitrite, NO_x) (Green & Green, 2017), although they are a better reflection of nitrite in the saliva as the strips are based on a modified Griess reagent reaction (Babateen et al., 2019; Modi et al., 2017). One researcher compared and ranked the colour on the test pad to the NO_x scale. The test strip was also scanned with a Nitric Oxide App (Berkeley Fit, USA) for a second reading. The average number of the two readings was used for subsequent statistical analysis.

Statistical analysis

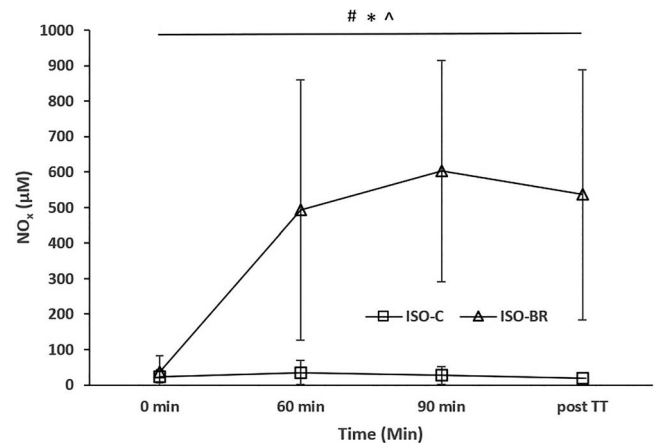
Data were analysed using SPSS version 26.0 (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp., USA) and Microsoft Excel version 2008 (Microsoft Corporation, USA). All data were assessed for normal distribution using visual inspection (histogram and quantile-quantile plot) and the Shapiro-Wilk test. Two-way repeated measures ANOVA were used to compare the effect of the drinks on salivary NO_x and relative changes in ΔTSI, ΔO₂Hb, ΔHHb, and ΔtHb (treatment × time). Paired t-tests were performed to compare differences in completion time, the magnitude of changes for each stage (ΔTSI, ΔO₂Hb, ΔHHb, and ΔtHb), average load, maximum load, average rpm, and maximum rpm during the time-trial performance test. Statistical tests for sensory evaluation were performed using XLSTAT (Version 2021.4; Addinsoft, Paris, France). The participants' preferences were analysed using the Wilcoxon signed-rank test. The overall liking among the samples was analysed and compared using paired t-tests. Tukey's test was used to determine the significant differences between the drinks. Unless otherwise stated, all data are reported as M ± SD. Significance was set at $p < .05$.

Results

All participants tolerated both drinks based on a questionnaire provided during their consumption confirming if they could accept the drink and whether they could finish it within 10 min. There were no self-reported adverse effects. Participants were unaware of the expected effects of each treatment, and 67% of participants could not identify both drinks correctly.

Salivary NO_x concentration

Salivary NO_x remained the same as pre-ingestion baseline concentrations after consuming the ISO-C drink throughout the trial but increased more than 13-fold after consuming ISO-BRial (main effect of drink, $p < .001$, partial $\eta^2 = .47$; main effect of time $p < .001$, partial $\eta^2 = .23$; interaction effect, $p < .001$, partial $\eta^2 = .22$). Post-hoc Bonferroni comparisons indicated that salivary NO_x was

**Figure 2.** Salivary ($n = 24$) total nitrate and nitrite (NO_x) responses after ISO-C and ISO-BR supplementation. Data presented as M ± SD.

*Significant main effect of time ($p < .001$). # Significant main effect of drink ($p < .001$). ^Significant interaction effect ($p < .001$).

significantly higher with ISO-BR than ISO-C at 60 min, 90 min, and post TT (all $p < .001$) (Figure 2).

Time-trial performance

Compared to ISO-C, cycling time-trial performance significantly improved by 6.4% following ISO-BR supplementation (Table 2). The average load (W) and cadence (rpm) increased significantly by 5.3% and 3.8% on ISO-BR compared with ISO-C supplementation. There was no significant difference in maximum load and cadence between drinks, which differed by −1.8% and −0.8%, respectively.

Sensory evaluation

Table 3 shows the sensory evaluation outcomes from both trials. There was no significant difference in overall liking and preference between drinks.

Muscle oxygenation

The relative changes of ΔTSI, ΔO₂Hb, ΔHHb, and ΔtHb from baseline are illustrated in Figure 3. There were substantial changes in all four parameters over time (all main effects of time, $p < .05$). However, there was no significant interaction between drinks and stages (S1–S7, please refer to Figure 1) for all four parameters (all $p > .05$). The ΔO₂Hb (Figure 3B) was significantly higher (main effect of drink, $p = .03$, partial $\eta^2 = .015$), and ΔHHb (Figure 3C) was substantially lower (main effect of drink, $p = .01$, partial $\eta^2 = .021$) on ISO-BR than ISO-C supplementation. There were no significant

Table 2. Effects of ISO-C or ISO-BR supplementation on 10 km cycling time-trial performance ($n = 24$).

Variables ($n = 24$)	ISO-C	ISO-BR	<i>p</i> value	Cohen's <i>d</i>	% (ISO-C versus ISO-BR)
Time (s)	1141.0 $\pm$ 381.8	1068.4 $\pm$ 333.3 ^a	<.001	0.20	-6.4
Average load (W)	146.2 $\pm$ 48.4	153.9 $\pm$ 47.7 ^a	<.001	0.16	5.3
Maximum load (W)	306.9 $\pm$ 94.5	301.3 $\pm$ 86.2	.636	0.06	-1.8
Average cadence (rpm)	80 $\pm$ 7	83 $\pm$ 6 ^a	<.001	0.36	3.8
Maximum cadence (rpm)	117 $\pm$ 14	116 $\pm$ 11	.658	0.07	-0.8

Note. Values are $M \pm SD$. ISO-C = Isotonic drink; ISO-BR = Isotonic beetroot drink. ^aSignificant difference compared with ISO-C, $p < .05$. Statistical significance was determined using two-tailed paired *t*-tests.

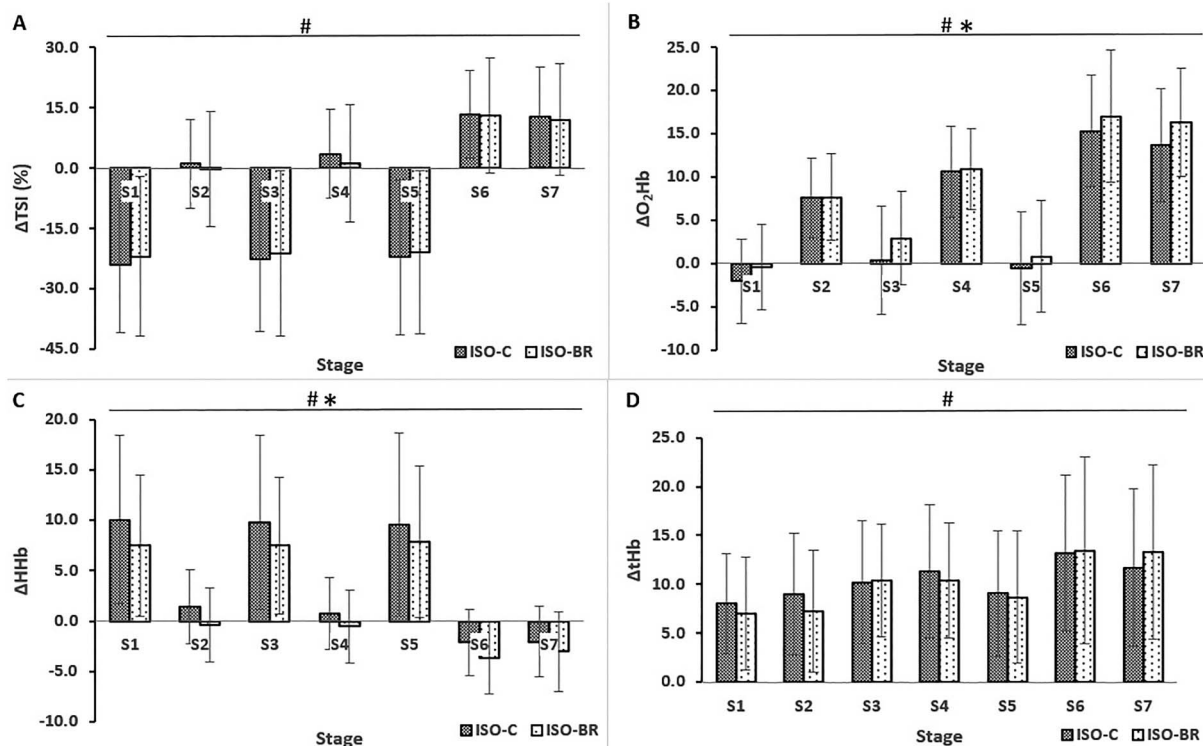


Figure 3. The Δ TSI (A), Δ O₂Hb (B), Δ HHb (C), and Δ tHb (D) from baseline ($n = 24$) after ISO-C and ISO-BR supplementation. (B) ISO-BR had significantly higher Δ O₂Hb than ISO-C supplementation (main effect of drink, $p = .03$, partial $\eta^2 = 0.015$). (C) ISO-BR had significantly lower Δ HHb than ISO-C supplementation (main effect of drink, $p = .01$, partial $\eta^2 = 0.021$). All data are presented as $M \pm SD$. #Significant main effect of time ($p < .05$). *Significant main effect of drink ($p < .05$). No interaction effects observed.

Table 3. Summary of overall liking and preference test of ISO-C and ISO-BR drinks ($n = 24$).

Overall liking and preference (7-point hedonic scale)	ISO-C	ISO-BR
Top 3 box overall liking (T3B)	79%	63%
Neutral overall liking (neutral)	13%	13%
Bottom 3 box overall liking (B3B)	8%	25%
Average mean score	5.25 ^a	4.50 ^a
Preference	53% ^a	47% ^a

Note. Different superscript alphabet letter (a) refers to significant differences between samples, $p < .05$. There was no significant difference in overall liking and preference between drinks.

differences between ISO-C and ISO-BR for Δ TSI (Figure 3A) and Δ tHb (Figure 3D).

The magnitude of Δ TSI, Δ O₂Hb, Δ HHb, and Δ tHb are shown in Table 4. The magnitudes were calculated based on the differences between the baseline of each stage (before the start of each stage) and the end of each stage (please refer to Figure 1 for exercise intensity of each stage). In contrast to the relative changes of

muscle oxygenation, which referred to the resting baseline, the magnitude calculation compared the baseline value at each individual stage which can vary; thus, paired *t*-tests were performed to compare the differences between drinks. Compared to ISO-C, the magnitude of Δ TSI was significantly lower during active recovery stages S2 and S4 following ISO-BR supplementation (both $p < .05$). In contrast, although some evidence of higher Δ TSI was present in exercise stages S3 and S5, the difference between drinks did not meet conventional levels of statistical significance (all $p > .05$). The magnitudes of Δ O₂Hb, after ISO-BR ingestion, was significantly lower compared with ISO-C at the active recovery stage S2 and S4, and exercise stage S3. The magnitudes of Δ HHb were significantly lower compared with ISO-C at S1 and S4. Lastly, the magnitudes of Δ tHb were significantly higher compared with ISO-C at exercise stage S3 and significantly lower at active recovery stage S4.

Discussion

This study examined how adding dietary nitrate (beetroot juice) to an isotonic drink can affect high-intensity cycling time-trial

Table 4. Magnitude of change ($n = 24$) of Δ TSI, Δ O₂Hb, Δ HHb, and Δ tHb after ISO-C and ISO-BR supplementation ($n = 24$).

	Variables ($n = 24$)	Δ TSI	p value	Δ O ₂ Hb (μ M)	p value	Δ HHb (μ M)	p value	Δ tHb (μ M)	p value
S1	ISO-C	-21.1 ± 16.6	.440	-2.0 ± 4.8	.067	10.1 ± 8.8	.002*	8.0 ± 5.1	.322
	ISO-BR	-21.9 ± 19.8		-0.5 ± 5.8		7.5 ± 7.5		7.0 ± 5.6	
S2	ISO-C	25.2 ± 12.1	.048*	9.9 ± 6.5	.006*	-8.6 ± 5.9	.073	1.0 ± 2.4	.141
	ISO-BR	21.7 ± 11.2		8.1 ± 6.6		-7.9 ± 6.0		0.3 ± 1.8	
S3	ISO-C	-23.8 ± 12.0	.053	-7.2 ± 7.2	<.001*	8.4 ± 6.2	.175	1.2 ± 2.9	<.001*
	ISO-BR	-21.0 ± 10.8		-4.4 ± 7.3		7.9 ± 5.9		3.1 ± 3.0	
S4	ISO-C	26.2 ± 12.1	.003*	10.2 ± 7.0		-9.1 ± 6.3	.005*	1.2 ± 1.7	
	ISO-BR	22.4 ± 11.3		8.0 ± 6.5	<.001*	-8.0 ± 5.8		0.0 ± 1.9	<.001*
S5	ISO-C	-25.7 ± 13.7	.071	-11.1 ± 8.1		8.8 ± 6.7	.266	-2.3 ± 3.6	
	ISO-BR	-22.1 ± 11.0		-10.1 ± 7.4	.091	8.4 ± 6.7		-1.7 ± 3.6	.354
S6	ISO-C	35.5 ± 15.6	.476	15.8 ± 8.9		-11.7 ± 7.9	.760	4.1 ± 5.6	
	ISO-BR	34.0 ± 13.6		16.6 ± 9.7	.709	-11.5 ± 8.2		4.7 ± 7.9	.700
S7	ISO-C	-0.5 ± 4.9	.573	-1.6 ± 4.3		0.1 ± 2.3	.382	-1.5 ± 5.3	
	ISO-BR	-1.0 ± 4.2		-0.7 ± 4.4	.504	0.6 ± 3.1		-0.1 ± 6.9	.412

Note. Values are M $\pm$ SD. ISO-C = Isotonic drink; ISO-BR = Isotonic beetroot drink. *Significant difference compared with ISO-C, $p < .05$. Statistical significance was determined using two-tailed paired t-tests.

performance. Compared with an ISO-C drink, acute supplementation with ISO-BR significantly increased salivary NO_x levels, and saw a faster 10 km cycling time trial time (~6.4%) and greater average cadence during the trial by 3.8%. The ISO-BR supplementation yielded small but substantial enhancements in performance compared with the comparison ISO-C drink and demonstrates the advantages of a nitrate-containing drink over a relatively short time trial. Importantly, the ISO-BR showed good acceptance among the participants, with the two drinks similar in overall liking and preference.

The ISO-BR was created by adding beetroot extract to a typical isotonic sports drink, ISO-C. Both drinks were standardised in colour to help with blinding. In addition, the ISO-BR drink contained 4.5 mg/100 ml ascorbic acid, which may play specific roles in nitrate reduction and metabolism (Duncan et al., 1995; Flueck et al., 2016; Lbban et al., 2023). We previously hypothesised that providing dietary nitrate in an isotonic drink might facilitate the absorption and nitrate reduction process (Wong et al., 2024) potentially by promoting the reduction of nitrate to nitrite via the enterosalivary circulation pathway. This is partly because isotonic drinks have been reported to increase salivary flow rate by ~15% compared with baseline measures (Siow et al., 2017). Based on the observed data in the present study, the concentration of salivary NO_x was substantially elevated with ISO-BR compared with ISO-C. However, we did not analyse plasma nitrate/nitrite directly. Nevertheless, a previous study from our group has confirmed that salivary NO_x data are associated with plasma NO_x data after consuming an ISO-BR drink (Wong et al., 2024). Moreover, previous research shows that measures using the salivary test strips employed here reflect a strong association with salivary nitrite measured using either chemiluminescence or high-performance liquid chromatography coupled with the Griess reaction (Babateen et al., 2019; Modi et al., 2017). The salivary test strips have also been shown to exhibit good inter-observer reproducibility and intra-observer repeatability, and there is a strong association with established methods of measuring salivary nitrite, although actual limits of agreement are wide (Babateen et al., 2019; Modi et al., 2017).

Animal models and human studies indicate low pH and oxygen environments stimulate NO production (Jones et al., 2021; Lundberg et al., 2008). Thus, nitrate supplementation might be particularly effective in short-duration, high-intensity endurance events,

where intense effort quickly depletes oxygen (Wong et al., 2022). It appears that both acute (>5 mmol) and chronic supplementation regimens may be beneficial (Jones et al., 2021; Senefeld et al., 2020). A relatively high nitrate dosage (12.9 mmol/570 ml) was provided in the present study. It is possible that this dose could elevate blood and muscle nitrate levels. In addition, previous research shows that excess nitrate and nitrite can be stored in the blood and tissues as a “reservoir” of NO precursors, ready to be converted to bioactive NO under conditions of low oxygen or acidity, potentially enhancing exercise performance (Jones et al., 2021; Lundberg et al., 2008). Several studies have demonstrated that nitrate supplementation can improve endurance performance by lowering the oxygen cost of exercise (Bailey et al., 2009; Lansley et al., 2011; Larsen et al., 2007; Wylie et al., 2013). Furthermore, nitrate supplementation may alter the delicate equilibrium between oxygen delivery and consumption within skeletal muscle, particularly in the vastus lateralis, during high-intensity cycling exercise. The present study provides some support for this suggestion as illustrated by the lower Δ HHb with ISO-BR during cycling.

Contrary to earlier studies suggesting improved blood flow to type II muscle fibres with nitrate supplementation (Bourdillon et al., 2015; Ferguson et al., 2015; Rokkedal-Lausch et al., 2019), NIRS measurements in this study revealed no significant difference in total blood flow (Δ tHb) to the vastus lateralis during exercise, compared to ISO-C. However, oxygenated blood (Δ O₂Hb) was significantly higher, and deoxygenated blood (Δ HHb) was significantly lower with ISO-BR than ISO-C during exercise. The observation suggests that nitrate supplementation prioritised efficient oxygen extraction. This may have occurred via augmented NO production, which has been implicated in reducing the adenosine triphosphate cost of muscle contraction, metabolic stress, and improving mitochondrial function (Larsen et al., 2011; Shannon et al., 2017). Furthermore, nitrate’s potential performance benefits may not be solely mediated by vasodilation or blood flow improvement but also involve boosting type II muscle calcium handling and contractile function (Hernández et al., 2012), ultimately enhancing high-intensity exercise performance, although we do not have evidence of this in the present study. This contrasts with low-intensity exercise, where the performance benefits may be mediated by blood flow or vasodilation, which primarily rely on aerobic metabolism via type I muscle fibre recruitment.

In the magnitude of change analysis (Table 4), the ΔHHb was significantly higher with ISO-BR than ISO-C in stage 1 (first preload high-intensity cycling with 65% W_{max}). During the active recovery stages (S2 and S4) and exercise stage S3, the magnitude of $\Delta\text{O}_2\text{Hb}$ was significantly higher with ISO-C than with ISO-BR. This observation may again suggest that nitrate supplementation promoted efficient oxygen extraction, particularly during first preload cycling, and less oxygenated blood flow was required for muscle recovery during S2 and S4. There were no significant differences in the magnitude of change for $\Delta\text{O}_2\text{Hb}$ and ΔHHb during the time-trial (S5). Likely, the magnitude of change for ISO-BR at stage 5 was blunted by the lower ΔHHb and higher $\Delta\text{O}_2\text{Hb}$ baseline before the start of stage 5 (Table 4). Therefore, it is important to be cautious about the interpretation of the results of the magnitude of changes between conditions, as the baseline of a certain stage can be influenced by the previous stage. The relative changes of ΔTSI , $\Delta\text{O}_2\text{Hb}$, ΔHHb , and ΔtHb from the resting baseline are more appropriate to represent muscle oxygenation dynamics throughout the trials.

Our observations related to muscle oxygenation are aligned with a study examining trained cyclists ($n = 10$) undertaking high-intensity interval training (cycling at 50% above functional threshold power for 75 s, followed by a 2-min recovery at 50% of functional threshold power until exhaustion) where the $\Delta\text{O}_2\text{Hb}$ concentrations were significantly higher, while ΔHHb concentrations were substantially lower during the exercise trials after 125 mg nitrate and 20 mg nitrite supplementation compared with placebo (Broeder et al., 2021). The study also suggested that the high intensity interval training work segment tolerance was enhanced with a complimentary recovery effect after supplementation (Broeder et al., 2021). However, not all studies showed similar muscle oxygenation effects (Shannon et al., 2017; Songdhasn et al., 2023; Volino-Souza et al., 2020), potentially from variations in the exercise modalities employed.

A prior systematic review and meta-analysis, we conducted on high-intensity endurance time-trial performance (24 included studies), evaluated the evidence for dietary nitrate supplementation in time trial performances between 5 and 30 min on the premises that nitrate may alleviate peripheral fatigue over a shorter duration. We proposed that nitrate supplementation's ergogenic effect might arise from distinct physiological benefits over shorter time trial distances which encompassed high-intensity efforts (Wong et al., 2022). Specifically, we hypothesise that enhanced type II muscle fibre recruitment could be a pivotal contributor to performance improvement in shorter time trials, where peripheral fatigue plays a more prominent role (Shannon et al., 2017; Thomas et al., 2015). This is supported by evidence of reduced potentiated twitch in knee extensors, a marker of peripheral fatigue, during shorter bouts of intense exercise (Thomas et al., 2015). Conversely, longer-time trials are characterised by greater central fatigue, marked by reductions in voluntary activation as measured by motor nerve and cortical stimulation (Thomas et al., 2015).

Research suggests low pH and hypoxic conditions are potent stimuli for NO production (Jones et al., 2021). This aligns with the potential for nitrate supplementation to be particularly effective in high-intensity endurance efforts, where rapid and intense exertion rapidly depletes oxygen stores (Wong et al., 2022). Thus, it is suggested that a potential mechanism by which nitrate supplementation may enhance performance in such scenarios is via enhanced NO bioavailability under the metabolic stress experienced during such exercise. Certainly there was a notable reduction in tissue oxygen extraction during high-intensity

exercise following ISO-BR supplementation in comparison with ISO-C.

It is important to note that this randomised crossover study relied heavily on participant compliance with the protocol, particularly overnight fasting before the experiment. Participants were reminded to adhere strictly to the protocol and complete survey forms, but verifying compliance could not go beyond verbal confirmation. Nonetheless, salivary NO_x concentrations did rise substantially after the consumption of ISO-BR, and this was not seen with ISO-C. Furthermore, this study was limited to manipulating the nitrate content of the drinks by comparing ISO-BR with ISO-C as a placebo. Whether isotonicity of the ISO-BR drink was useful in nitrate reduction was not demonstrated here. However, our previous study did show the efficacy of ISO-BR compared to beetroot juice alone in elevating salivary nitrate/nitrite levels and improving peak power output during sprinting (Wong et al., 2024). Nonetheless, including a non-isotonic beetroot juice as a comparator in this study could have provided more comprehensive insights into the relative effectiveness of ISO-BR, but the study was powered to compare only the two conditions.

While nitrate was the primary focus of this study, it is important to acknowledge that beetroot juice also contains other bioactive compounds, such as polyphenols and ascorbic acid, (Peri et al., 2005; Wootton-Beard & Ryan, 2011). The ISO-BR drink used in this study contained 12.8 mg of ascorbic acid per 285 ml, which may have contributed synergistically to the observed improvements in muscle oxygenation and performance. Future studies should consider isolating or comparing these components to better understand their individual and combined effects.

Practical implications

Motivating factors for ergogenic aid consumption extend beyond performance enhancement alone. Athletes often seek multi-functional products addressing a range of needs, including hydration, fluid provision, and gastrointestinal comfort, adding complexity to dietary supplement selection. Identifying efficacious supplements based on individual sports' nutritional goals and competitive demands requires significant expertise and effort. This study addressed this issue by comparing the effects of a novel isotonic nitrate beverage (ISO-BR) with an isotonic drink (ISO-C) on cycling time-trial performance. While our findings demonstrate the encouraging benefits of ISO-BR, particularly in specific contexts, further research is crucial to evaluate its efficacy in meeting other athletic demands across diverse sports disciplines.

Conclusion

The present study demonstrated that providing dietary nitrate (beetroot) in an isotonic drink before a high-intensity cycling time-trial performance leads to a 6.4% increase in performance compared with an isotonic drink alone. Muscle oxygenation dynamics offer a rationale for the ergogenic effect of nitrate supplementation via improved oxygen extraction. Future research should investigate the efficacy of ISO-BR in high-intensity interval exercise paradigms and on cognitive function. Notably, our data establish that a dose of 12.9 mmol led to a better performance than an ISO-C drink, potentially guiding future studies exploring the application of ISO-BR in various athletic contexts.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

Data related to this publication will be placed in the Data Repository of the Nanyang Technological University National Institute of Education (Singapore) and made publicly available.

Author contributions

Tak Hiong Wong (Conceptualisation, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualisation, Writing—original draft, Writing—review & editing), Ian Gern Liang Lau (Data curation, Formal analysis, Investigation, Project administration, Software, Writing—review & editing), and Stephen F. Burns (Conceptualisation, Formal analysis, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing—review & editing)

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Conflicts of interest

S.F.B. and I.G.L.L. declare no conflict of interest. T.H.W. is a registered PhD candidate at Nanyang Technological University and a Fraser and Neave Limited employee. He was involved in the study design, data collection, analyses, interpretation, and manuscript writing but had no role in participant allocation, randomisation, or preparation and coding of the drinks used and was blinded to drink allocation. The funding source listed had no role in the study design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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