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No placebo or ergogenic effect of beetroot juice during virtual-reality 20-min cycling time trials: a randomised, balanced placebo design remote study

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Abstract

A large body of evidence has shown that placebo effects of dietary supplements can improve sport performance. However, very few studies are conducted outside of the laboratory. This is important given that placebo effects may be more likely to be induced during highly controlled, artificial environments in the presence of a researcher. In the past three years, home-based, virtual-reality cycling has increased in popularity, where over 3-million athletes train or compete against athletes worldwide. The aim of this study was to examine placebo effects of an acute dose of beetroot juice during 20-minute cycling virtual reality time-trials. In line with the CONSORT statement, we used a between-participant, randomised balanced placebo design, and recruited 67 trained cyclists who performed 3x20-min cycling time-trials (familiarisation, baseline and experimental) on a virtual-reality software at home. During experimental trials, participants were randomised to one of four groups: 1) told beetroot juice/given beetroot juice, 2) told beetroot juice/given placebo, 3) told placebo/given beetroot juice, and 4) told placebo/given placebo, who received nitrate-rich beetroot juice (containing ~552 mg nitrate) or nitrate-depleted placebo (containing ~0.2 mg nitrate). Compared to baseline, performance during experimental time-trials was not different in any of the groups (effect size range: 0.00 to 0.14). Our results, indicate that placebo effects and beetroot juice do not improve virtual-reality 20-min cycling time-trial performance. These results have important considerations for future research to determine the occurrence of placebo effects and effectiveness of dietary supplements outside of the laboratory.

Introduction

Placebo effects are a positive psychobiological outcome, that can be attributed to a person's expected and/or learned response to a purported beneficial intervention (Beedie et al., 2018; Hurst, Schipof-Godart, et al., 2020). A large body of evidence has shown that placebo effects can improve sport performance when participants believe they have ingested a dietary supplement, such as caffeine (Beedie et al., 2006; Hurst et al., 2019), sodium bicarbonate (Higgins & Shabir, 2016; McClung & Collins, 2007) and carbohydrate (Clark et al., 2000). Indeed, a systematic review (Hurst, Schipof-Godart, et al., 2020) indicated that placebo effects induced by nutritional ergogenic aids can have small to medium effects on sport performance (effect size: 0.35, 95% CI = 0.20 to 0.51).

One dietary supplement popular amongst athletes is beetroot juice, which is a natural, rich source of nitrate (Jones, 2014b; Shannon et al., 2022). In the past decade, a plethora of research has investigated its influence on sport performance (Jones et al., 2020; Senefeld et al., 2020; Shannon et al., 2022), with benefits associated with vasodilation, increased blood flow and reduction in the oxygen cost of exercise (Jones, 2014a; Jones et al., 2020). However, while the purported effects are well established, meta-analytical data (Senefeld et al., 2020) report that benefits are trivial (d = 0.17). Given that placebo effects can significantly improve sport performance by similar or larger magnitude (Hurst, Schipof-Godart, et al., 2020), benefits of beetroot juice could be related, at least partially, to placebo effects (Beedie et al., 2015). To date, no research has examined placebo effects of beetroot juice on sport performance. A need therefore exists in examining whether benefits of beetroot juice are a result of the belief it has been received, the ergogenic effects or a combination of both.

To understand the interaction of placebo effects and ergogenic effects, researchers have advocated for the use of the four-treatment, balanced placebo design (Beedie et al., 2018) in which both the treatment administered (e.g., beetroot juice vs. placebo) and information about the treatment (e.g., told beetroot juice vs. told placebo) are manipulated in a 2 × 2 factorial design. Using this design, Hurst et al. (2019) reported that when participants received a placebo but were told it was caffeine, their time to complete 1000-m improved to the same magnitude as when they received caffeine and told it was caffeine. Performance improvements were associated with higher running speeds during the start of the time-trials, highlighting the importance for measuring pacing in placebo effects research. These findings are similar for other ergogenic aids using a similar design, such as sodium bicarbonate (McClung & Collins, 2007) and carbohydrate (Clark et al., 2000) and underline the need to delineate the performance contribution of placebo effects alone compared to the ergogenic effects of beetroot juice.

In most placebo effect and beetroot juice research, outcomes are often assessed within artificial, highly controlled laboratory environments (Shannon et al., 2022), whereby a researcher administers the intervention to participants in person. However, such a procedure rarely occurs in the real world and it is likely that the interaction between the researcher administering the intervention and the participant receiving it, plays a large role for inducing placebo effects (Beedie et al., 2018; Roelands & Hurst, 2020). Recently, remote-based, virtual-reality software has become increasingly popular, whereby cyclists ride on a stationary bike at home and train and compete against other athletes worldwide. Such virtual-reality platforms has begun to receive scientific attention (McIlroy et al., 2021), with Matta et al. (2022) recently reporting that performance during 20-min cycling time-trials on Zwift is highly reproducible (coefficient of variation = 3.7%). A novel opportunity exists for researchers to harness this technology and examine placebo effects whereby athletes administer an intervention themselves and complete the time-trial without interaction from the researcher. Given this, we used a balanced placebo design to examine both placebo and ergogenic effects of beetroot juice during 20-min cycling, virtual reality cycling time-trials.

Methods

The methods section of the present study is reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Schulz et al., 2010). The lead author's institutional human research ethics committee approved the study in compliance with the Declaration of Helsinki (ref: ETH2021-0238) and all participants provided digital informed consent before participation.

Trial design and randomisation. We used a randomised, repeated-measures, between-participant, balanced-placebo design. Participants performed 3x20-min time-trials (1 familiarisation, 1 baseline and 1 experimental) on a virtual-reality software (i.e., Zwift) interspersed by 5-7 days at the same time of day (± 2 h). After completing familiarisation, participants were randomly assigned to one of four experimental groups:

1. Informed beetroot juice/given beetroot juice (BRJ/BRJ)
2. Informed beetroot juice/given placebo (BRJ/PLA)
3. Informed placebo/given beetroot juice (PLA/BRJ)
4. Informed placebo/given placebo (PLA/PLA)

The lead researcher was responsible for enrolling and randomising participants to each group but was blinded to participants' allocation of beetroot juice or placebo until all statistical analyses had been completed. Participants were randomly assigned sequentially numbered codes using computer-generated software

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(www.randomization.com) in a 1:1:1:1 ratio. No other members of the research team were aware of randomised allocations, and one researcher not involved with data collection, labelled beetroot juice and placebos for random assignment (i.e., W, X, Y, Z). Participants were unaware of the existence of other groups until completion of the final trial.

Sample size and participants. Sample size was calculated *a priori* (G*Power, 3.9.1.7). With a statistical power of 0.80, a error 0.05 and effect size 0.35 (for 4 groups and 3 measurements), 64 participants were required. Effect sizes were based on results of a previous systematic review examining placebo effects on sports performance (Hurst, Schipof-Godart, et al., 2020). Eligibility criteria stipulated participants were 1) UK-based cyclists; 2) between 18 and 60 years old; 3) non-smokers; 4) free of injury; 5) had used Zwift for more than 4 months; 5) had been cycling more than 3 times per week; and 6) had not experienced any COVID-19 symptoms (i.e., defined as high temperature, new, continuous cough and loss or change to a sense of smell/taste) in the 2 months preceding participation. A flow diagram displaying the progress of the participants and the reasons for exclusion can be found in Figure 1.

Outcome measures. Our main outcome was mean power output (relative [W] and absolute [W/kg]) collected during each 20-minute time-trial. Secondary outcomes included power output speed (km·h^{-1}), distance covered (km), heart rate (beats·min^{-1}) and cadence (rev·min^{-1}) in 2-min intervals. We also assessed beliefs about the effectiveness of sport supplements prior to the study, whereby participants were asked to rate on a 6-point Likert-type scale, anchored by 1 (strongly disagree) to 6 (strongly agree), how much they agreed with the following statement: "Sport Supplements will improve my performance".

Interventions. Before the start of the experimental time-trial, participants self-administered either 70 mL of nitrate-rich beetroot juice (˜552 mg nitrate; Beet It Sport; James White Drinks Ltd.®, Ipswich, United Kingdom, UK) or a placebo composed of nitrate-depleted beetroot juice (˜0.2 mg mmol nitrate; Beet It Sport; James White Drinks Ltd., Ipswich, United Kingdom, UK). Both drinks were indistinguishable in taste and smell, and bottles were identical. Participants were asked to self-administer the substance 2 h before the start of the time-trial and to fast during the time between the supplementation and the start of the warm-up. They were also requested to complete a food diary 24 h before the start of each time-trial, to ensure the diet was consistent between time-trials and that they administered the drink 2 h before the start.

To enhance belief in the benefits of beetroot juice, and in line with expert consensus of placebo effect research (Beedie et al., 2018), participants were emailed leaflets containing detailed information about the benefits of beetroot juice on cycling performance, which included key studies reporting the ergogenic benefits of beetroot juice supplementation (Lansley et al., 2011; Senefeld et al., 2020) and an infographic developed by the Australian Institute of Sport. Information provided was standardised among participants for each group, minimising potential differences in presentation. Further, and as encouraged by an expert consensus Delphi study for studies examining beetroot juice on sport performance (Shannon et al., 2022), we independently verified the nitrate content for both the beetroot juice and placebo drinks using ion-pair high-performing liquid chromatography (HPLC). The mean nitrate concentration in the beetroot juice and placebo drinks were 552 $\pm$ 13 mg and 0.2 $\pm$ 0.1 mg per 70 mL, respectively (for further information on how we assessed the nitrate content, please see supplementary material).

Procedure. Two days before each time-trial, participants were instructed to maintain their normal diet, be consistent in their intake of nitrate-rich foods (Boorsma et al., 2014; Lansley et al., 2011) avoid high-intensity, long-duration exercise and the use of anti-bacterial mouthwash, and produce the highest possible power output during each 20-min time-trial. The day before the start, participants were e-mailed standardised instructions and requested to calibrate their equipment according to the manufacturer's guidelines. All time-trials were performed on participants' setup and Zwift account, of which they navigated their on-screen avatar through the virtual road that simulated outdoor conditions. The time-trial protocol was developed by the research team through the lead researcher's Zwift account, which was exported as a workout file (.zwo) and sent to participants' e-mail, who then imported the file to their accounts. Participants were provided with detailed instructions, containing a step-by-step guide about how to import the workout file to their accounts.

Participants performed all time-trials individually and used their virtual time-trial bike—available to everyone on Zwift—which removes the drafting effect feature, caused by overtaking other riders. They first performed a 10-min warm-up at their habitual self-selected intensity (i.e., defined during familiarisation and replicated throughout), followed by 5-min rest, and completed the 20-min trial at the virtual "Tempus Fugit" course, which is designed as an out and back flat course, available to all users, containing 17.3 km and 16 m of elevation gain. The 20-min time-trial was chosen as it is a standard performance measure among cyclists (MacInnis et al., 2018), familiar to Zwift users and has high reproducibility (Matta et al., 2022).

After each time-trial, participants exported their data file in a Flexible and Interoperable Data Transfer (FIT) format and emailed it to the lead researcher. Given that there might be differences in the performance data generated by Zwift and external power meters devices (e.g., Garmin or SRM), participants were requested to export the file from their Zwift account folder instead of the file generated by other sources. Between each time-trial, participants were instructed to keep their diet, fluid intake, equipment (i.e., bike and/or trainer) and environment (i.e., the position of a fan, place and start time) the same as the previous trial.

Twenty-four hours before experimental trials, participants were informed whether they received the beetroot juice drink (i.e., BRJ/BRJ and BRJ/PLA groups) -described as an active substance that could induce ergogenic effects based on extensive previous research or placebo drink (i.e., PLA/BRJ and PLA/PLA groups)-described as an inert substance that would not induce any ergogenic effects. They then completed the time-trial in the exact same manner as baseline. Following completion of all trials, participants were debriefed about the true nature of the study in line with the American Psychological Association guidelines for deceptive research (American Psychological Association, 2017).

Statistical analysis. Descriptive data are reported as mean +- standard deviation along with 95% confidence intervals (95%CI), unless otherwise stated. One-way Analysis of Co-Variance (ANCOVA) were used to assess the differences in power output (in absolute and relative values), speed, distance, heart rate and cadence during the experimental trials between the 4 experimental groups, controlling for their respective values achieved during baseline. To investigate the effect of condition on pacing, the average power output from each 2-min segment was initially percentage normalised to the average power output of the entire 20-min for each participant, hereafter referred to as normalised power output. This procedure enables the characterisation of pacing per se (Davis et al., 2020; Thomas et al., 2012), in contrast with the distribution of absolute power output that is performance dependent. Two-way repeated-measures ANOVAs were used to assess differences in pacing between trials, and changes were assessed by interaction effects only. A Spearman's rho correlation assessed relationships between changes in mean power output between baseline and experimental trials (in relative values), and participants' beliefs about the effectiveness of sport supplements. Data analyses were performed using SPSS (27.0, IBM, Armonk, USA) with statistical significance set at P [?] .05.

Results

One-hundred and two cyclists initially volunteered to participate (22 women; 44 +- 11 years old, 177 +- 9 cm, 75.8 +- 11.8 kg) and were assessed for eligibility (Figure 1). A total of 29 were excluded, leaving 73 who were randomised to one of the four groups. Five participants dropped out, leaving a final sample size of 67 (12 women; 44 +- 9 years old, 177 +- 8 cm, 73.9 +- 9.3 kg) who completed all trials and included in the analysis. All participants were performing at least 4 hours of training and 3 sessions per week.

Descriptive data for baseline and experimental trials for all variables between each group are presented in Table 1. Between baseline and experimental time-trials for all groups, no differences in mean absolute (F = .72, P = .544, $\eta_p{}^2$ = .03), relative power output (F = .55, P = .652, $\eta_p{}^2$ = .03), speed (F = .95, P = .420, $\eta_p{}^2$ = .04), distance (F = 1.59,P = .202, $\eta_p{}^2$ = .07), heart rate (F = .08, P = .970, $\eta_p{}^2$ = 0.04) and cadence (F = .16, P = .921, $\eta_p{}^2$ = 0.01) were found (Table 2).

Two-way repeated measures ANOVA revealed that pacing was not different between baseline and experimental trials for BRJ/BRJ (F = .81; P= .605; $\eta_p{}^2$ = .05; Figure 2A), BRJ/PLA (F = 1.67; P = .103; $\eta_p{}^2$ = .09; Figure 2B), PLA/BRJ (F = 1.37; P = .206; $\eta_p{}^2$ = .08; Figure 2C) and PLA/PLA (F = .22; P = .991;

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$\eta_p{}^2$ = .01; Figure 2D). Finally, there was no association between athletes' belief in changes in performance and the effectiveness of sport supplements for BRJ/BRJ (r = -.07, P = .766), BRJ/PLA (r = -.30,P = .242), PLA/BRJ (r = -.33, P = .201) and PLA/PLA (r = .12, P = .655).

Discussion

We found that both placebo effects and beetroot juice do not improve performance during virtual-reality, 20-min cycling time-trials. While we aimed to induce positive beliefs about the ergogenic effects of beetroot juice, we did not find any differences in both relative performance or pacing. Given that laboratory-based studies of placebo effects (Hurst, Schipof-Godart, et al., 2020) and beetroot juice (Senefeld et al., 2020) have shown, on average, to improve performance, our results highlight that placebo and ergogenic effects of beetroot juice were not shown when athletes performed on a virtual reality software absent from the researcher.

Previous studies have reported placebo effects when participants are induced to believe its effectiveness might have similar effects to the actual ergogenic aid. Hurst et al. (2019) showed that the belief of ingesting caffeine improved 1,000-m running performance when participants ingested a placebo, and Clark et al. (2000) reported improvements of 4.3% in cycling performance when athletes ingested a placebo described as carbohydrate. However, our results contrast with this and the body of placebo effect research in sport (Hurst, Schipof-Godart, et al., 2020), which is likely due to the environment in which we performed our study. That is, in previous placebo effect research, participants are often administered the placebo in person by the researcher, which has been proposed to significantly influence the likelihood of placebo effects being induced (Davis et al., 2020; Roelands & Hurst, 2020). It has also been suggested that social support can trigger neurobiological pathways to enhance sports performance as a form of placebo effect (Davis et al., 2020), which may explain the lack of differences in our study. As a result, our findings support previous suggestion that placebo effects can be highly social in nature and induced by social interactions between researchers and participants.

Data from our study indicated that pacing over 20-min time-trials did not differentiate between baseline and experimental conditions in any of the groups, partially contrasting previous findings. Hurst et al. (2019) investigated the placebo effects induced by caffeine ingestion on 1,000 m running performance and reported faster speeds at the start of the trial. Ross et al. (2015) reported a faster start during 3 km time-trials when participants self-administered a placebo injection purported to have similar effects as recombinant human erythropoietin. The lack of differences in pacing during the overt administration of beetroot juice (BRJ/BRJ group), partially agrees with previous findings showing no effects of nitrate on pacing during 40-min (Bescos et al., 2012), 60-min (Cermak et al., 2012) and 50-mile (Wilkerson et al., 2012) cycling time-trials. It is important to note that those studies reported no changes in performance after nitrate supplementation in comparison to a placebo, which might explain why pacing was not different.

We did not find any changes in performance from the open (BRJ/BRJ group) or hidden (PLA/BRJ) administration of beetroot juice, which is consistent with previous randomised controlled trials showing no ergogenic effects (Callahan et al., 2017; Cermak et al., 2012; Hoon et al., 2014; Hurst, Saunders, et al., 2020). Cermak et al. (2012) investigated whether beetroot juice affected ~60-min time-trial performance (acute dose of 8.7 mmol nitrate) and although plasma nitrite concentration was higher in comparison to placebo, performance was not different. Exploring a slightly different supplementation strategy, Callahan et al. (2017) investigated whether beetroot crystals ingested in capsules (5 mmol nitrate over 3 days) affected 4-km cycling performance in trained athletes and did not find any differences in comparison to placebo. Using a competitive setting, Hurst, Saunders, et al. (2020) investigated the effects of an acute dose of beetroot juice (~6.2 mmol nitrate) on 5-km running performance, and while times decreased when participants ingested beetroot juice, it was not different from placebo. While a meta-analysis (Senefeld et al., 2020) reported mean performance improvements of ~3% after nitrate supplementation, effect sizes are small (d = 0.17) indicating that performance changes in response to nitrate supplementation are highly susceptible to variability. By removing the researcher in our study during the administration of the intervention and the time-trials, it could be speculated that such variability may in part, be introduced by the presence of the

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researcher and the interaction they have during the administration of the intervention.

Our research must be interpreted considering the following limitations. First, while we analysed the effects of an acute dose of beetroot juice on cycling performance, there is evidence suggesting chronic doses over a longer period may be more efficacious (Shannon et al., 2022). However, the amount of nitrate chosen in our study was selected as it is more convenient to athletes, and in accordance with a previous study showing improved cycling performance with a similar amount (Lansley et al., 2011). Second, as we aimed to adopt a study replicating participants' day-to-day reality and increase ecological validity of our findings, we did not control for the amount of nitrate in their daily diet. It has been suggested that athletes with a nitrate-rich diet, may display lower responsiveness to nitrate supplementation (Jones, 2014a). Finally, although we used a software with reported high reproducibility and comparable to laboratory-based studies (Matta et al., 2022), we did not directly compare outcomes within the laboratory. Future studies should adopt similar designs in a hybrid format, involving a mix of field- and laboratory-based time-trials to better elucidate the direct impact of nitrate supplementation, the mechanisms behind potential changes and whether the physical presence of an experimenter and their expectations influence participants' performance.

Notwithstanding these limitations, our data have important applications for both athletes and coaches, and other researchers examining placebo effects and dietary supplements on sport performance. Given that no placebo or ergogenic effect of beetroot juice was shown, athletes may not benefit from the acute supplementation of beetroot juice when training or competing on virtual reality software at home. Nutritionists and coaches working with athletes using Zwift should therefore consider the benefits of such an intervention. Similarly, there is a need for researchers to consider the influence their presence has in the effectiveness of dietary supplements. In a consensus statement of placebo effect research in sport and exercise (Beedie et al., 2018), authors encouraged researchers to report and detail the interaction they have with their participants. The interaction between a participant and researcher is important for inducing placebo effects (Roelands & Hurst, 2020) and as a result, is likely to influence the effectiveness of an intervention. Researchers should therefore consider the impact they have in the effectiveness of their interventions and aim to examine the influence of other dietary supplements in similar environments.

Conclusions

The results of our study indicate that ingesting an acute dose of beetroot juice does not improve virtual-reality 20-min cycling time-trial performance irrespective of whether the athlete was informed they received beetroot juice or a placebo. This suggests that the purported benefits of an acute dose of beetroot juice may be limited in enhancing cycling performance within remote exercise, virtual reality environments, such as Zwift. These results have important implications for future research delivering nutritional and placebo effect interventions and the need to further elucidate the influence of the presence of researchers in the magnitude of effects reported.

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Conflict of interest and funding statement

The authors declare there are no competing interests and received no funding for this work.

Data availability

The data used in this study is available under reasonable request. Readers may contact the lead author directly via e-mail.

Table 1. Descriptive statistics for baseline and experimental trials for each measure.

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Power output (W)
Power output (W/kg)
Distance (km)
Heart rate (beats·min^{-1})
Cadence (rev·min^{-1})

Note: Data are means ± standard deviation, with 95% confidence intervals in parentheses. BRJ/BRJ = Informed beetroot

Table 2. Differences in measures between baseline and experimental trials for each group

Measure
Power output (W)
Power output (W/kg)
Distance (km)
Heart rate (bpm)
Cadence (rpm)

Note: BRJ/BRJ = informed beetroot/given beetroot; BRJ/PLA = informed beetroot/given placebo; PLA/BRJ = informe

Figures captions

Figure 1. CONSORT flowchart displaying the progress of participant recruitment, randomisation, and reasons for exclusions.

Figure 2. Pacing adopted between baseline (BSL) and experimental (EXP) trials for each group: A) Informed beetroot juice/given beetroot juice; B) informed beetroot juice/given placebo; C) informed placebo/given beetroot juice; and D) informed placebo/given placebo.

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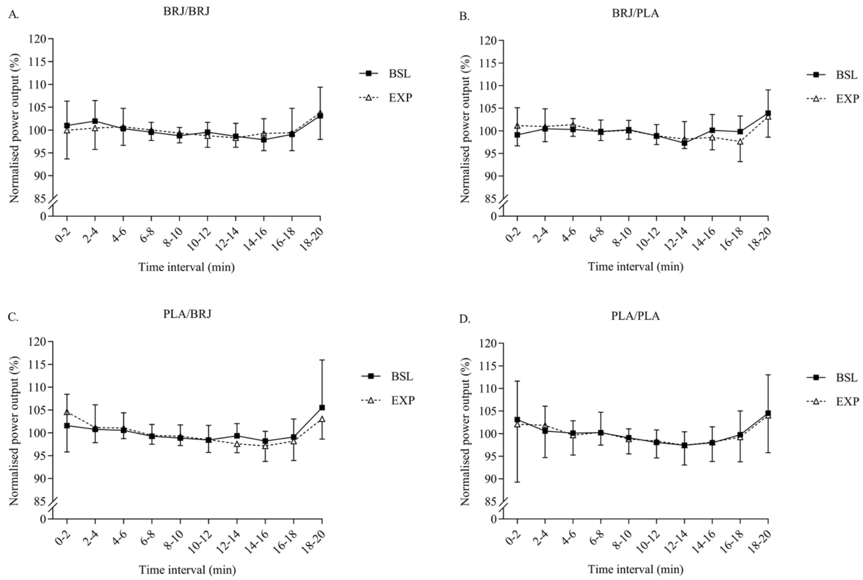

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