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Potential Effect of Beetroot Juice Supplementation on Exercise Economy in Well-Trained Females

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**Potential Effect of Beetroot Juice Supplementation on
Exercise Economy in Well-Trained Females**

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Abstract: This study examined if acute dietary nitrate supplementation (140 ml beetroot juice, BRJ) would reduce oxygen consumption (VO_2) during cycling at power outputs of 50 and 70% $\text{VO}_{2\text{max}}$ in 14 well-trained female Canadian University Ringette League athletes. BRJ had no effect on VO_2 or heart rate but significantly reduced ratings of perceived exertion (RPE) at both intensities. Individually, 4 participants responded to BRJ supplementation with a $\geq 3\%$ reduction in VO_2 at the higher power output.

- Acute BRJ supplementation did not improve exercise economy in well-trained females, but significantly reduced RPE. However, 4/14 subjects did respond with a $\geq 3\%$ VO_2 reduction.

Keywords: Nitrate, Beetroot Juice, Oxygen Consumption, Submaximal Exercise, Ratings of Perceived Exertion, Trained Female Ringette Athletes

30 Introduction

31 Since the discovery of the nitrate (NO_3^-)-nitrite (NO_2^-)-nitric oxide (NO) pathway, NO_3^- -
32 rich beetroot juice (BRJ) supplementation has become a popular performance enhancing strategy
33 based on the physiologic and metabolic roles of NO (Gao et al., 2021; Jones et al., 2021;
34 Senefeld et al., 2020). NO plays a significant role in the regulation of numerous biological
35 pathways including, but not limited to: vasodilation, sarcoplasmic reticulum calcium handling,
36 mitochondrial respiration, and neurotransmission (Bailey et al., 2012). Current findings suggest
37 that acute and chronic dietary NO_3^- supplementation can reduce oxygen consumption (VO_2)
38 during submaximal exercise and improves performance in recreationally active males with such
39 effects less likely in sedentary, well-trained, or elite males athletes (Gao et al., 2021; Jones et al.,
40 2021; Senefeld et al., 2020). Conversely, it has been reported that BRJ supplementation did not
41 decrease submaximal exercise VO_2 or performance in recreationally active females (Wickham et
42 al., 2019). These opposing responses may be supported by sex differences in the NO_3^- - NO_2^- -NO
43 pathway such as females having elevated baseline plasma $[\text{NO}_2^-]$ and greater relative increases in
44 $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ following dietary NO_3^- supplementation (Kapil et al., 2018; Wickham &
45 Spriet, 2019). However, based on the number of pathways in which NO is involved and the
46 significant underrepresentation of females in the mechanistic work (Wickham & Spriet, 2019),
47 there are several potential areas that require investigation. In fact, few studies have examined the
48 effect of dietary NO_3^- supplementation in females in general and, furthermore, only exercise
49 performance has been assessed in well-trained females with no research examining the effects of
50 BRJ supplementation on VO_2 (Wickham & Spriet, 2019).

Therefore, the purpose of this study was to examine the effects of acute BRJ supplementation on VO_2 during cycling-exercise at two submaximal power outputs (PO) in well-trained female ringette athletes.

Methods

Participants

Fourteen well-trained female athletes from the University of Guelph competitive ringette team volunteered to participate in this study ($\text{VO}_{2\text{max}}$, $45.9 \pm 5.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $20.4 \pm 1.7 \text{ y}$, body mass index (BMI), $22.3 \pm 1.6 \text{ kg} \cdot \text{m}^{-2}$). Ringette is an on-ice, high-intensity intermittent sport with high physical demands of the neuromuscular and cardiovascular systems. While the rules of play are different from ice-hockey, these sports are similar such that there are 5 skaters per team, excluding the goalie, trying to score by putting the ring in the net of the opposing team. All athletes on the University of Guelph team have 5+ years of experience playing at the AA/AAA-level. This team competes in the Canadian University Ringette (CUR) championships each year and plays against AA-level city teams throughout the season. As there is no previous data on training status in ringette athletes, the characterization of these athletes as well-trained was based on female ice-hockey athletes (Driscoll et al., 2020; Henriksson et al., 2016). During regular season, these athletes have two 1-hour on-ice practices, one 1-hour team dryland session, and a minimum of one additional individual workout/week. For 6 months prior to study, 9 participants used hormonal contraceptives (HC, $\text{VO}_{2\text{max}}$, $45.7 \pm 5.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $20.1 \pm 1.4 \text{ y}$, BMI, $22.3 \pm 1.7 \text{ kg} \cdot \text{m}^{-2}$) and 5 were eumenorrheic (EUM, $\text{VO}_{2\text{max}}$, $46.2 \pm 4.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $21.5 \pm 2.0 \text{ y}$, BMI, $22.5 \pm 1.5 \text{ kg} \cdot \text{m}^{-2}$). Participants were informed of the study requirements, potential benefits, and associated risks prior to providing oral and written consent. All

procedures were approved by the University of Guelph Research Ethics Board and conformed to the Declaration of Helsinki.

Pre-Experimental Testing

On the first visit, participants performed an incremental cycling test to volitional exhaustion for determination of $\text{VO}_{2\text{max}}$ (Velotron™ cycle ergometer, Quarq/SRAM, South Dakota, USA). This test included the following stepwise increases in intensity: 2-minute warm-up at 60 watts (W), two minutes at 100W, an increase of 25W every two minutes until 200W, and an increase of 10W every minute until exhaustion. VO_2 was measured with a metabolic cart (MOXUS Modular VO_2 System, AEI Technologies, Pittsburgh, USA) and participants wore a heart rate (HR) monitor (POLAR H10, Polar Electro, Kempele, Finland). The day-to-day variability of VO_2 measures in female subjects in our laboratory is 2.4% at 50% $\text{VO}_{2\text{max}}$ and 2.0% at 70% $\text{VO}_{2\text{max}}$ (Wickham et al. 2019). The second visit familiarized the participants with the experimental exercise protocol and confirmed the submaximal POs for use in the study. The ergometer settings were recorded for subsequent visits.

Supplementation

In a double-blind, randomized, counter-balanced, cross-over design, participants consumed 140 ml of either NO_3^- -rich BRJ (~13 mmol NO_3^- ; Beet It, Winnipeg, CA) or NO_3^- -free blackcurrant juice placebo (PLA) (Lucozade Ribena Suntory, Uxbridge, UK) 2.5 hours prior to exercise. A third-party investigator organized the ordering, labelling, and supplying of the supplements. BRJ and PLA were provided in identical containers with coloured tape to distinguish the contents. The NO_3^- dosage and the length of the waiting period post-ingestion were determined as previously described (Wickham et al., 2019; Wylie et al., 2013).

Experimental Trials

The experimental trials were completed at the same time of day with a consistent room temperature ($20.8 \pm 0.2^{\circ}\text{C}$) and relative humidity ($33.2 \pm 0.9\%$) and were separated by 1 week. For the EUM group, experimental testing was done during the luteal phase to match the stable and low estrogen levels present with HC use (Christin-Maitre et al., 2011; Wickham et al., 2019). The participants were considered in the luteal phase on days 20-28 post menses (Janse De Jonge, 2003). Participants initially completed a 24-hour sleep, exercise, hydration, and dietary recall questionnaire and were asked to repeat these habits in the 24 hours before the experimental trials. They were also instructed to avoid intense exercise and abstain from using antibacterial mouthwash or chewing gum for 24 hour and abstain from alcohol and caffeine for 12 hours before trials. Lastly, they were instructed to brush their teeth no less than 2 hours prior to supplementation (Wickham et al., 2019). Participants were randomly assigned 140 mL of either BRJ or PLA, 2.5 hours prior to exercise. The exercise protocol consisted of a 3-min warm-up at 60 W followed by two consecutive 8-minute bouts at constant POs that were calculated to elicit 50 and 70% $\text{VO}_{2\text{max}}$. The Borg 20 Scale (Borg, 1982) was used to record the ratings of perceived exertion (RPE) at 4 and 7.5 minutes of exercise at the two POs.

Data Analysis and Statistics

During the ramp incremental cycling test, once the participant reached steady state where two 10-second VO_2 readings were within 50 mL of O_2/min of each other (~ 1.5 minutes), the mean of the remaining data for each intensity was calculated. During the familiarization and experimental trials, the VO_2 , HR and RPM at each PO were determined by calculating the mean over the final 3 minutes of each intensity. For the RPE, the two values recorded at each intensity were averaged. Statistical analyses were performed using IBM SPSS 27 (IBM Corp., Armonk,

New York, USA) and significance was accepted as $P < 0.05$. Data are presented as mean $\pm$ SD. Two-way repeated measure analyses of variance (treatment and exercise intensity) were used to analyze VO_2 , HR and RPE and separately on HC and EUM groups. For all tests, partial eta squared (η^2) values were used to calculate the effect size and their interpretations were based on the following criteria: 0.1=small, 0.6=medium, 0.138=large (Cohen, 1988). While this study was not set up to specifically examine individual responses, responders were arbitrarily defined as a participant who demonstrated a $\geq 3\%$ reduction following BRJ supplementation. This is in accordance with previous work (Wylie et al., 2013) and outside the measures of day-to-day variability of 2.4% at 50% $\text{VO}_{2\text{max}}$ and 2.0% at 70% $\text{VO}_{2\text{max}}$ previously reported in females in our laboratory (Wickham et al., 2019).

Results

Oxygen Consumption

VO_2 was higher at 70 versus 50% $\text{VO}_{2\text{max}}$ ($P < 0.001$, $\eta^2 = 0.943$) but the ingestion of NO_3^- did not affect VO_2 ($P = 0.588$, $\eta^2 = 0.023$) (Figure 1). There was no interaction between treatment and intensity for VO_2 ($P = 0.668$, $\eta^2 = 0.015$). The results were similar when the HC and EUM groups were analyzed separately. Three participants (1 HC, 2 EUM) demonstrated a $\geq 3\%$ reduction in VO_2 following BRJ supplementation when compared to PLA at both 50 and 70% $\text{VO}_{2\text{max}}$ and one participant (HC) demonstrated a $\geq 3\%$ reduction in VO_2 only at 70% $\text{VO}_{2\text{max}}$.

Ratings of Perceived Exertion

RPE was higher at 70 versus 50% $\text{VO}_{2\text{max}}$ ($P < 0.001$, $\eta^2 = 0.92$) and was reduced during the BRJ trial compared to PLA ($P = 0.023$, $\eta^2 = 0.340$) (Figure 1). However, there was no interaction between treatment and intensity for RPE ($P = 0.768$, $\eta^2 = 0.007$). When analyzed

separately, the significant main effect for intensity remained for HC ($P<0.001$, $\eta^2=0.912$) and EUM ($P<0.001$, $\eta^2=0.971$). The significant main effect for treatment also remained for HC ($n=9$, $P=0.019$, $\eta^2=0.520$) but was not present for the smaller EUM group ($n=5$, $P=0.710$, $\eta^2=0.038$).

Heart Rate

HR was higher at 70 versus 50% $\text{VO}_{2\text{max}}$ (50%, 134 ± 11 ; 70%, 164 ± 11 bpm, $P<0.001$, $\eta^2=0.965$) but the ingestion of NO_3^- did not affect HR (PLA, 149 ± 19 ; BRJ, 150 ± 18 bpm, $P=0.712$, $\eta^2=0.012$). There was also no interaction between treatment and intensity for HR ($P=0.613$, $\eta^2=0.015$) and the results were similar when HC and EUM groups were analyzed.

Discussion

This is the first study to investigate the effect of acute BRJ supplementation on exercise economy in a group of exclusively well-trained females. The main findings were that BRJ supplementation did not reduce VO_2 or alter HR during submaximal exercise in well-trained females. However, we did find an overall significant reduction in RPE with acute BRJ supplementation. These results were also found in the group of athletes using HC.

Studies with well-trained females have reported no effect of acute BRJ supplementation on performance and none assess exercise economy (Buck et al., 2015; Glaister et al., 2015; Lane et al., 2014). Interestingly, only one of these studies considered menstrual status and collected data during the follicular phase for EUM participants (Buck et al., 2015). An additional study did include 2 well-trained females in a group of 9 participants and reported that acute BRJ supplementation did not reduce VO_2 during submaximal treadmill running (Oskarsson & McGawley, 2018), but the applicability of these findings to females is limited as the number of

females examined was too low. As such, the results from the present study are the first to provide evidence that BRJ supplementation does not improve exercise economy in well-trained females. We also controlled for menstrual status and report similar findings for the group of 9 women using HC.

Individual responders to BRJ supplementation have previously been arbitrarily defined as an individual that demonstrates a reduction in $\text{VO}_2 \geq 3\%$ during submaximal exercise (Wickham et al., 2019). In this study, 3 participants responded with a reduced VO_2 at both 50 and 70% of $\text{VO}_{2\text{max}}$ and 1 additional participant responded at 70% $\text{VO}_{2\text{max}}$, suggesting that some well-trained females do respond to acute BRJ supplementation. The responders were from both the HC and EUM groups. Future studies should investigate individual variability in response to BRJ supplementation in well-trained female athletes from other sports.

Interestingly, our results revealed a significant reduction in RPE with acute BRJ supplementation. Three previous studies with well-trained females cyclists and runners reported no effect of supplementation on RPE (Glaister et al., 2015; Lane et al., 2014; Oskarsson & McGawley, 2018). One reason for these inconsistent results may be the difference in fitness levels between participants. In the present study, although the participants were characterized as well-trained, they were stop-and-go sport athletes and have comparatively lower $\text{VO}_{2\text{max}}$ values than well-trained endurance athletes. It could be that in the present study, the participants did respond to the BRJ supplementation, as demonstrated by RPE and individual responders, but the effects were not strong enough to establish a statistically significant mean reduction in VO_2 . For example, a recent study found a reduction in RPE and improved time trial performance in amateur female runners (Casado et al., 2021). Although $\text{VO}_{2\text{max}}$ was not reported, amateur endurance athletes may be expected to have $\text{VO}_{2\text{max}}$ values lower than well-trained adult

endurance athletes, but similar to well-trained stop-and-go sport athletes. Additional studies are clearly needed to better characterize the effects of NO_3^- supplementation in females, with emphasis on a variety of fitness levels and exercise types, and the potential connection to RPE. We must also note that because some of the athletes may have been able to distinguish between the BRJ and the blackcurrant juice PLA, we cannot fully discount the possibility of a placebo effect. In general, the athletes were not knowledgeable about the potential physiological and performance effects of BRJ ingestion, so this seems unlikely.

When controlling for menstrual status, the significant effect of treatment on RPE remained in the HC group but was lost in the EUM group. While these results could suggest an influence from the use of HC, it could also be attributed to the lower sample size for the EUM and therefore reduced statistical power. Female specific mechanistic investigation of the effects of dietary NO_3^- supplementation on exercise economy and performance, with emphasis on the influence of HC, is needed.

In this study, we did not measure plasma $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$, however it has been previously demonstrated that the same dose of BRJ successfully elevates plasma $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ (Wickham et al., 2019; Wylie et al., 2013). Additionally, we did not have a large enough group of EUM athletes to make definitive conclusions regarding BRJ supplementation.

Conclusions

This study demonstrated that acute BRJ supplementation did not reduce VO_2 or HR during exercise at 50 and 70% $\text{VO}_{2\text{max}}$ in 14 well-trained and competitive female ringette athletes. Similar results were also found in the group of 9 athletes using HC. However, 3 participants were identified as responders to BRJ supplementation at both POs, 1 at only the higher PO, and RPE was also reduced following BRJ supplementation. In high performance

208 athletes, slight improvements in exercise economy and reduced RPE can make a significant
209 difference for performance. Future work should examine the effects of dietary NO_3^-
210 supplementation on exercise economy in females from various sports, including assessment of
211 the individual variability.

212 **Conflict of interest statement:** The authors declare there are no competing interests.

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302 **Figure Captions**

303 Figure 1. (A) Individual data for oxygen consumption (VO_2) when cycling at 50% and 70%
304 maximum oxygen consumption ($\text{VO}_{2\text{max}}$) after supplementation with beetroot juice (BRJ) or
305 placebo (PLA). $n = 14$. There was a significant main effect for intensity. (B) Individual data for
306 ratings of perceived exertion (RPE) when cycling at 50% and 70% $\text{VO}_{2\text{max}}$ after supplementation
307 with BRJ or PLA. $n = 14$. There was a significant main effect for intensity. *Significant main
308 effect for treatment.

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(A) Individual data for oxygen consumption (VO_2) when cycling at 50% and 70% maximum oxygen consumption (VO_{2max}) after supplementation with beetroot juice (BRJ) or placebo (PLA). $n = 14$. There was a significant main effect for intensity. (B) Individual data for ratings of perceived exertion (RPE) when cycling at 50% and 70% VO_{2max} after supplementation with BRJ or PLA. $n = 14$. There was a significant main effect for intensity. *Significant main effect for treatment.

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