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Influence of Sex and Acute Beetroot Juice Supplementation on 2 KM Running Performance

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Abstract: Purpose: To assess the effect of acute nitrate-rich (BJ) and nitrate-depleted (PL) beetroot juice ingestion on 2 km running performance in amateur runners, and to what extent the ergogenic effect of BJ supplementation would be influenced by the sex of the participants; Methods: Twenty-four amateur long-distance runners (14 males and 10 females) performed a 2 km time trial (TT) on an outdoor athletics track 2.5 h after ingesting either 140 mL of BJ (~12.8 mmol NO_3^-) or PL. After the tests, blood [lactate] and ratings of perceived exertion (RPE) related to the leg muscles ($RPE_{muscular}$), cardiovascular system (RPE_{cardio}) and general overall RPE ($RPE_{general}$) were assessed; Results: Compared to PL, BJ supplementation improved 2 km TT performance in both males ($p < 0.05$) with no supplement × sex interaction effect ($p > 0.05$). This improvement in 2 km running performance was a function of improved performance in the second 1 km split time in both males and females ($p < 0.05$). Supplementation with BJ did not alter post-exercise blood [lactate] ($p > 0.05$) but lowered $RPE_{general}$ ($p < 0.05$); Conclusions: acute BJ supplementation improves 2 km running performance in amateur runners by enhancing performance over the second half of the TT and lowering $RPE_{general}$ by a comparable magnitude in males and females.

Keywords: endurance performance; ergogenic aid; nitrate; nitric oxide; ratings of perceived exertion

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1. Introduction

Consumption of dietary supplements is a common pre-competition strategy for athletes competing in a variety of sports at different competitive standards in an attempt to enhance performance [1–6]. Although not unequivocal, there is evidence to suggest that dietary supplementation with inorganic nitrate (NO_3^-) can enhance exercise performance in a variety of settings [7,8]. After ingestion, NO_3^- undergoes a stepwise reduction to nitrite (NO_2^-) and then nitric oxide (NO), with the latter step augmented in conditions of acidosis and hypoxia, and believed to be responsible for the ergogenic effects of NO_3^- supplementation [7,9]. NO is a potent signalling molecule that elicits biological effects on numerous tissues and is involved in an array of physiological processes including, but not limited to, vasodilation, calcium handling, mitochondrial respiration, and neurotransmission [9]. Most of the research assessing the ergogenic potential of NO_3^- supplementation to date has been conducted in males completing cycling exercise [10–27]. This experimental approach has limited wider understanding of the settings in which NO_3^- supplementation is more and less likely to be ergogenic.

In studies assessing the effect of NO_3^- supplementation on running exercise responses, improved economy has been reported in recreationally active and moderately trained subjects, but not highly trained subjects [28–33], whereas improved time to exhaustion has been

reported in both recreationally active [29] and highly trained [28] subjects. During running time trial (TT) performance tests in well trained endurance athletes, most studies [30,32–35] have observed no change in performance over distances spanning 1.5–10 km after NO_3^- supplementation, but improved performance appears more likely over a shorter distance (1.5 km) compared to a longer distance (10 km) in this population after acute NO_3^- supplementation [36]. The effect of NO_3^- supplementation on running TT performance is less clear in recreationally active and moderately trained subjects with improvements in 3 km [32] and 5 km [37] performance having been observed in some studies, and no improvement in 5 km [36] and 10 km [38] performance having been observed in other studies. Therefore, further research is required to address the effect of NO_3^- supplementation on middle distance (<5 km) running TT performance in moderately trained subjects. Moreover, since NO_3^- supplementation has been reported to improve performance over the first half, but not the second half, of a 10 km TT [38], and to improve 5 km performance by mostly enhancing performance in the latter stages on the TT [36], further research is required to address which phases of TT performance are most likely to be enhanced by NO_3^- supplementation.

To date, the majority of studies assessing the effect of NO_3^- supplementation on running performance have either used exclusively male subjects [32–34,38], or not reported data for each sex to allow potential sex-specific effects of NO_3^- supplementation on running TT performance to be understood [30,36,37]. There is some evidence that, relative to males, females exhibit a greater increase in plasma [NO_2^-] [39] and greater improvements in muscle contractile function [40] after NO_3^- supplementation. However, females present with a more oxidative skeletal muscle phenotype compared to males [41], which could blunt NO_2^- reduction to NO and the improvement in performance after NO_3^- supplementation. Although de Castro et al. [42] reported no effect of acute NO_3^- supplementation on 3 km running TT performance in untrained women, the NO_3^- supplement was ingested 30 min prior to exercise such that plasma [NO_2^-] would not have peaked until after the TT had been completed [43]. Therefore, further research is required to assess the potential for sex-specific effects on the efficacy of NO_3^- supplementation to improve running TT performance.

The purpose of this study was to assess the effect of acute NO_3^- supplementation on 2 km running performance in recreational male and female runners. A 2 km running TT was selected as it is highly correlated with maximal aerobic speed [44], a key determinant of endurance performance [45]. It was hypothesized that, since plasma [NO_2^-] increases to a greater extent in females after NO_3^- supplementation [39] and the increase in [NO_2^-] post NO_3^- supplementation is correlated to improved performance [43], females would exhibit greater improvements in 2 km running performance compared to males.

2. Materials and Methods

2.1. Participants

Twenty-four long-distance runners (14 males and 10 females) participated in the present study. All participants had been training in an amateur running club for at least two years prior to participating in this study. Participants' best times for the 10 km during the two months prior to data collection were 2382 ± 338 s and 3127 ± 359 s for male and female runners, respectively. The inclusion criteria for participation in this study were: (a) > 18 years; (b) free from any cardiovascular, muscular and/or bone pathology; (c) participating regularly in amateur running competitions of 10 km (at least one competition every 3 months); (d) having not taken any medicine or nutritional supplement within 3 months prior to the beginning of the study; (e) best personal time in 10-km >30 min in males and 33 min in women. Prior to confirming their participation, all participants were informed of the study objectives and requirements. Two researchers answered any questions they had regarding their participation in the study before participants provided their written informed consent to participate in the study. Furthermore, participants were familiarised with the different perception scales which would be used subsequently

during the course of the study. This study was approved by a local ethics committee (Cod. 0045230632017) in agreement with the Declaration of Helsinki.

2.2. Anthropometric Measures

A week prior to the start of performance testing, participants reported to the laboratory where an investigator completed an anthropometric assessment. The anthropometric measurements included stature, body mass, skinfold thickness (subscapular, biceps, triceps, suprailiac, supraspinale, front thigh, medial calf and abdominal), girths (arm, flexed and tense, mid-thigh and calf) and breadths (humerus, bi-styloid and biepicondylar femur), which were conducted according to the recommendations of the International Society for the Advancement of Kinanthropometry (ISAK) with the laboratory temperature held constant at 24 °C during these measurements. The formulas used to calculate body fat mass, muscle mass and bond mass were selected according to the recommendations for this population by the Spanish Kineanthropometry Group (GREC) [46].

2.3. Design

A randomized, double blind, crossover experimental design was employed to assess the effects of ingesting nitrate-rich (BJ) and nitrate-depleted (PL) beetroot juice on performance, blood lactate concentration ([BLa]) and ratings of perceived exertion (RPE) during 2 km running TT tests. Considering the differences in physiological response [47] and performance [48] according to the diurnal variations during running test, the two experimental sessions were conducted at the same time of day (±0.5 h) and separated by 7 days, a sufficient period of time for ensuring a washout [49]. Weather conditions were similar for both testing sessions (sunny, wind speed average 1.18 ± 0.9 m/s, 27 ± 0.7 °C and 25 ± 4.2% humidity) and the tests were completed 667 m above sea level.

2.4. Warm-Up and 2 km Running Performance Tests

Participants arrived at an outdoor 400 m synthetic athletics track 2.5 h before initiating the 2 km TT. A 2 km test was selected since this distance has been reported to be completed at a similar speed to the maximal aerobic speed [44] and it was a distance that participants were familiar with completing during their group interval training sessions. After ingesting either BJ or PL, participants completed a standardized 15 min warm-up of low-intensity running. Specifically, they were instructed to run within an RPR range of 8 to 11 according to the 15-point (6–20) Borg scale [50], and subsequently completed 10 min of dynamic stretching and drills that was part of their usual warm up routine prior to training. The 2 km performance test was completed individually by participants and was hand timed to within 0.1 s by the coach and a researcher, with the mean time calculated and used for subsequent analysis. A 1 km split time was also obtained. Immediately after the 2 km TT, participants reported their RPE and a capillary blood sample was obtained within three min for [BLa] analysis.

2.5. Betroot Juice Supplementation and Diet Control

Forty-eight hours prior to both 2 km TTs, participants were instructed to follow a standardized diet to ensure similar macronutrient (10% protein, 60% carbohydrate and 30% lipid) and micronutrient intake, in order to avoid dietary variation confounding our results. Furthermore, during the 24 h preceding both tests, participants were instructed to avoid the intake of NO_3^--rich foods (beetroot, ruccula, celery, turnip, spinach, lettuce, leak, cabbage, parsley, endives) and caffeine. Participants were also instructed to avoid brushing their teeth and use of antibacterial mouthwashes for 24 h and 7 days, respectively, prior to both performance tests. On the experimental testing days, participants consumed 140 mL of a beetroot juice supplement that was either enriched in NO_3^- (~12.8 mmol NO_3^-; BJ) (Beet IT; James White Drinks Ltd., Ipswich, UK) or a depleted of NO_3^- (PL), with the latter prepared as described previously [51]. The timing of BJ ingestion relative to the performance test was selected based on the pharmacokinetic data presented by

Wylie et al. [43] where plasma [nitrite] peaks 2–4 h post the ingestion of 140 mL of BJ. Participants started the standardized warm-up 135 min after supplement ingestion, and the 2 km time trial (TT) 150 min post supplementation ingestion, ensuring that the TT coincided with the plasma [nitrite] peak after supplementation.

2.6. Ratings of Perceived Exertion (RPE)

The 15-point (6–20) Borg scale was used to record RPE [50]. The scale was "anchored" by explaining that a score of 20 should equate to a previous memory of absolute exhaustion. As described previously [52], participants were asked to indicate the RPE related to their leg muscles ($RPE_{muscular}$), cardiovascular system (RPE_{cardio}) and general overall RPE ($RPE_{general}$).

2.7. Measurement of Blood Lactate Concentrations

Post-test [BLa] was determined from capillarised blood samples (5 µL) taken from the left index finger using the Lactate ProTM 2 LT-1710 blood analyzer (Arkray Factory Inc., KDK Corporation, Shiga, Japan).

2.8. Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences 24.0 (IBM, Armonk, NY, USA). Data were initially checked for normality of distribution by Shapiro–Wilk test, equality of variances and sphericity as appropriate. When sphericity was violated, a Greenhouse–Geisser correction was employed. The 2 km completion times, 1 km split times, RPE and [BLa] were compared across supplementation conditions and sex using mixed model ANOVAs, with repeated measures for supplement. Effect sizes were calculated using partial eta-squared (η_p^2) considering values <0.25, 0.26–0.63 and >0.63 as small, medium and large effect sizes, respectively [53]. When a significant effect was detected, pairwise comparisons were assessed using the Holm–Bonferroni test. Pearson's correlation coefficient was used to determine whether 2 km completion time in the PL condition and the change in 2 km time between the PL and BJ conditions were related to determine whether the ergogenic potential of BJ supplementation was influenced by baseline fitness. Values are provided as mean (M) ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Anthropometrics Measures

The anthropometric data of the male and female participants are presented in the Table 1.

Table 1. Anthropometric data of the subjects.

Variable	Males	Females
Age (years)	38.7 ± 9.2	36.6 ± 8.2
Height (m)	1.74 ± 0.05	1.64 ± 0.08
Body mass (kg)	69.1 ± 7.1	57.1 ± 7.8
Body mass index ($kg \cdot m^{-2}$)	22.8 ± 2.2	21.2 ± 2.2
Fat mass (kg)	8.9 ± 2.9	11.0 ± 3.6
Fat mass (%)	12.8 ± 3.1	18.9 ± 4.2
Muscle mass (kg)	36.1 ± 2.6	25.8 ± 2.5
Muscle mass (%)	52.6 ± 4.1	45.5 ± 3.3
Bone mass (kg)	12.4 ± 2.3	9.4 ± 0.9
Bone mass (%)	16.3 ± 2.2	16.7 ± 1.6

Data are shown as mean ± standard deviation.

3.2. Performance and Blood Lactate Concentrations

There was a main effect for supplement on 2 km TT completion time and the second 1 km split time ($p = 0.002$, $\eta_p^2 = 0.372$ and 0.282, respectively) with these values being

faster in the BJ condition compared to the PL condition (Table 2, Figure 1). There was a main effect of sex on 2 km TT performance and the first and the second 1 km split times with these being faster in males than females ($p < 0.001$, $\eta_p^2 = 0.564$–0.628) (Table 2). No differences across supplementation conditions and sex were found for [BLa] (Table 2).

Table 2. Effect of nitrate-rich and nitrate-depleted beetroot juice supplementation on 2 km running performance and post-exercise blood lactate concentration.

Parameter	Condition	Males	Females	Analysis Type	F	η_p^2
0–1 km (s)	PL	213.6 ± 26.6	276.1 ± 32.4	Suppl. × sex	0	0.014
	BJ	214.4 ± 26.1	276.9 ± 30.5	Suppl.	0.322	0.014
				Sex	28.427	0.564 §
1–2 km (s)	PL	223.0 ± 27.8	304.5 ± 37.1	Suppl. × sex	0.199	0.009
	BJ	218.4 ± 27.3	298.2 ± 39.2	Suppl.	8.656	0.282 †
				Sex	37.1	0.628 §
2 km (s)	PL	436.6 ± 52.7	580.7 ± 67.0	Suppl. × sex	0.4	0.018
	BJ	432.7 ± 52.9	575.1 ± 68.6	Suppl.	13.014	0.372 †
				Sex	34.023	0.607 §
[BLa] (mmol/L)	PL	15.2 ± 5.0	15.6 ± 2.7	Suppl. × sex	0.011	0
	BJ	15.8 ± 6.1	16.4 ± 3.7	Suppl.	0.489	0.022
				Sex	0.077	0.003

Data are shown as mean ± standard deviation. Abbreviations: F: adjusted values for test statistic; η_p^2: partial eta squared; PL: nitrate-depleted juice; BJ: nitrate-rich beetroot juice; [BLa]: blood lactate concentration. Degrees of freedom were always 1 and 22. Significant differences were set at $p < 0.05$ († $p < 0.01$, § $p < 0.001$).

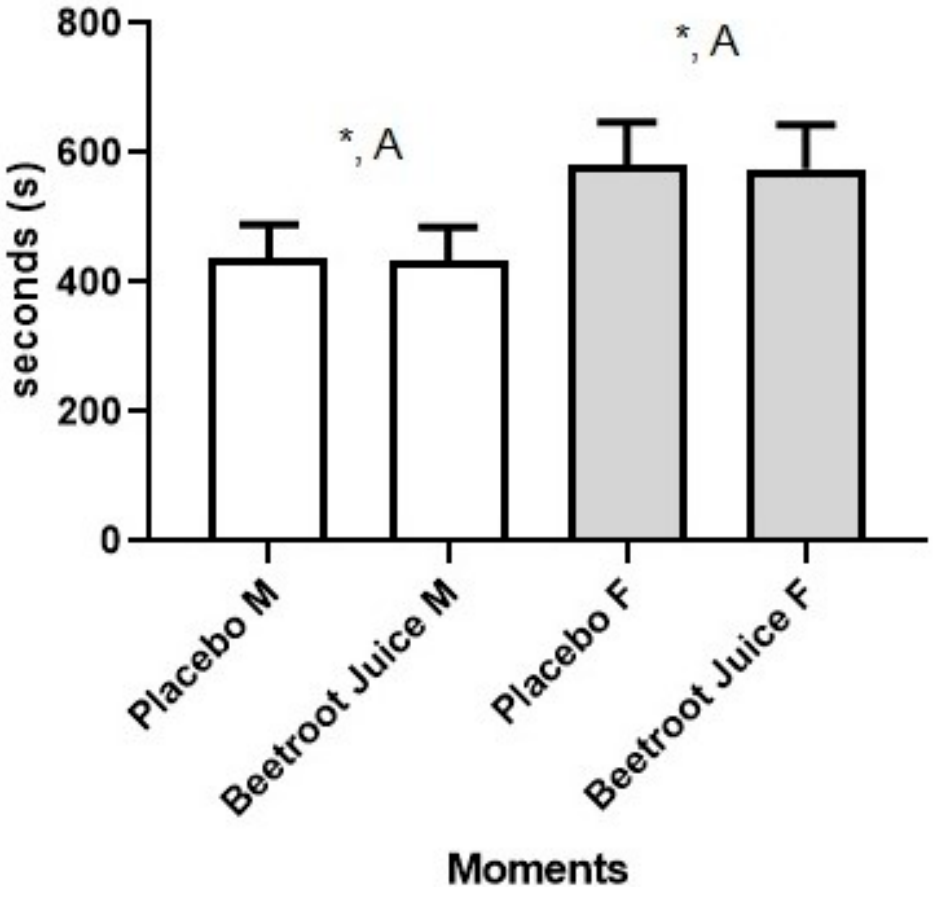


Figure 1. Two km completion times in nitrate-rich beetroot juice and nitrate-depleted beetroot juice (placebo) conditions. Data presented as mean ± standard deviation. * means statistical differences between beetroot juice and placebo ($p < 0.05$); A means significant gender ($p < 0.05$).

There was no correlation between 2 km completion time in the PL condition and the change in 2 km time between the PL and BJ conditions in either males or females ($r < 0.056$, $p > 0.796$).

3.3. Ratings of Perceived Exertion (RPE)

There was a main effect for supplement on $RPE_{general}$ ($p = 0.01$, $\eta_p^2 = 0.26$) (Table 3) with the BJ condition lower than in the PL condition. There was a main effect for sex on $RPE_{muscular}$ ($p = 0.003$, $\eta_p^2 = 0.291$) (Table 3), being higher in males than females.

Table 3. Effect of nitrate-rich and nitrate-depleted beetroot juice supplementation on ratings of perceived exertion (RPE) responses after a 2 km running time trial.

Parameter	Condition	Males	Females	Analysis type	F	η_p^2
$RPE_{muscular}$	PL	17.1 ± 1.2	15.1 ± 2.6	Suppl. × sex	0.162	0.007
	BJ	16.5 ± 2.3	14.1 ± 2.6	Suppl.	2.179	0.09
				Sex	9.048	0.291 †
RPE_{cardio}	PL	18 ± 1.5	17.5 ± 1.8	Suppl. × sex	0.607	0.027
	BJ	17.29 ± 1.4	17.4 ± 1.8	Suppl.	1.067	0.046
				Sex	0.129	0.006
$RPE_{general}$	PL	17.9 ± 0.9	17.9 ± 1.2	Suppl. × sex	0.774	0.034
	BJ	17.4 ± 1.1	16.8 ± 2.6	Suppl.	7.739	0.26 *
				Sex	0.292	0.013

Data are shown as mean ± standard deviation. Abbreviations: F: adjusted values for test statistic; η_p^2: partial eta squared; PL: nitrate-depleted juice; BJ: nitrate-rich beetroot juice; Suppl.: supplementation. Degrees of freedom were always 1 and 22. Significant differences were set at $p < 0.05$ (* $p < 0.05$, † $p < 0.01$).

4. Discussion

The principal novel findings of this study were that acute BJ supplementation improved 2 km running performance with a comparable improvement in male and female recreational runners. There was no change in [BLa], but $RPE_{general}$ was lower after the 2 km TT following BJ compared to PL supplementation, in both males and females. Therefore, our original findings indicate that BJ supplementation is equally effective at improving 2 km running performance in males and females and this ergogenic effect appears to be linked to lower $RPE_{general}$.

Although plasma [NO_2^-] was not measured in the current study, it has been unequivocally demonstrated that adopting the BJ supplementation strategy administered in the current study is effective at increasing plasma [NO_2^-] (e.g., Wylie et al. [43]). This elevated plasma [NO_2^-] then serves as a circulating substrate pool to be reduced to NO and subsequently elicit NO-mediated physiological responses such as improving skeletal muscle perfusion, metabolic control and contractility [7]. Importantly, the reduction of NO_2^- to NO is enhanced in acidosis and hypoxia [7], which is consistent with a lowering in plasma [NO_2^-] during high-intensity exhaustive exercise which evokes acidosis, but not during lower intensity exercise that markedly perturbs acid–base balance [35]. Since [BLa] was substantially elevated post the 2 km TTs in the present study, this would have generated the physiological conditions to aid NO_2^- reduction to NO.

Improved 2 km running TT performance in the present study is consistent with previous observations of improved 3 km [32] and 5 km [36] running TT performance in some studies, but conflicts with other previous studies reporting no change in 5 km [37] and 10 km [38] running TT performance after NO_3^- supplementation. However, in the study by de Castro et al. [38] assessing 10 km running TT performance, the first 5 km of the TT was completed more rapidly after BJ supplementation. Collectively, the existing evidence suggests that BJ supplementation is more likely to be ergogenic in shorter duration running TT performance tests in recreational runners, as observed previously in trained runners/triathletes [35]. There was no change in the 0–1 km split time in the current study, whereas performance was enhanced over the 1–2 km split. This observation is consistent

with increased running velocity over the final third, but not the first two thirds, of a 5 km TT after BJ supplementation [36], and with BJ supplementation being more likely to improve skeletal muscle contractile function as the muscle becomes more fatigued [54]. Therefore, BJ supplementation might boost middle distance running performance by attenuating fatigue development over the latter stages of the race.

Supplementation with BJ resulted in a comparable improvement in 2 km running TT performance in the present study in males (0.9%) and females (1%). Although the effects of NO_3^- supplementation on running TT performance in males has been addressed in several studies [32–35,38], and while some previous studies have assessed running performance in female subjects [30,36,37], independent effects in males and females were not reported in the latter group of studies, which has thwarted understanding of potential sex-specific effects of NO_3^- supplementation on performance. Moreover, the only study to assess the effects of BJ supplementation on performance in recreational female runners did not observe an improvement in 3 km TT performance [42]. However, the interpretation of this finding is confounded by the fact that the authors administered an acute BJ dose 30 min prior to the TT, which was insufficient time to allow plasma [NO_2^-] to peak after NO_3^- ingestion [43]. In spite of previous reports that plasma [NO_2^-] is increased more after BJ supplementation in females than males [39] and that males might present with a preferential phenotype to aid the reduction of NO_2^- to NO [41], comparable improvements in 2 km running performance after BJ supplementation were observed across sexes in the present study. Therefore, an important novel contribution of the current study is that BJ supplementation appears similarly ergogenic in male and female recreational runners completing a 2 km TT.

Previous studies have linked the ergogenic effects of NO_3^- supplementation to improvements in skeletal muscle perfusion, metabolic control and contractility [7]. In the present study, $RPE_{general}$ was lower after the 2 km TT in the BJ compared to the PL condition in both the male and female subjects. A lower RPE has been observed in some studies that report improved running performance after BJ supplementation [28,36]. However, there are also studies reporting improved running performance in the absence of changes of RPE [32,35,38]. The lowering in $RPE_{general}$ after BJ supplementation might be a function of an increase in brain perfusion [55] or a lowering in muscle metabolite accumulation [56], which would subsequently attenuate activation of type III/IV muscle afferent feedback to the central nervous system and the potential for central fatigue development [57]. Further research is required to resolve the underlying mechanisms for improved performance following BJ supplementation. It is acknowledged that a limitation of the current study is that we did not assess plasma [NO_2^-] to ascertain whether this was increased to a greater extent in females compared to males, as reported previously [39]. In addition, the menstrual cycle of female subjects was not controlled in the current study.

Dietary supplementation with BJ has emerged as a nutritional strategy to enhance exercise performance under certain experimental conditions [7]. However, much of the existing understanding of the ergogenic potential of BJ supplementation is derived from studies conducted exclusively on male subjects. This is a limitation not just of the NO_3^- supplementation literature, but is emblematic of sport science research more generally [58]. Therefore, further research is required to elucidate whether males and females exhibit similar or divergent responses to various interventions with the potential to enhance exercise performance. Such information is vital to administer bespoke, evidence-based ergogenic interventions for males and females prior to competition. With regard to potential sex-specific effects of NO_3^- supplementation, it has been reported that females present with an oral microbiome that is more conducive to oral NO_3^- reduction and results in a plasma [NO_2^-] after NO_3^- supplementation [39]. On the other hand, males might present with a phenotype that favors NO_2^- reduction to NO during exercise [41]. Despite these divergent sex-specific characteristics with the potential to modulate different components of the NO_3^-- NO_2^--NO pathway, 2 km running TT performance was enhanced by a comparable magnitude in male and female subjects in the current study after BJ supplementation.

Therefore, the key practical application of the current study is that acute BJ supplementation appears to be equally ergogenic for middle distance running performance in male and female recreational runners.

5. Conclusions

In conclusion, acute BJ supplementation improved 2 km running TT performance in male and female subjects by a comparable magnitude. This improvement in 2 km TT performance was linked to improved performance over the second half of the TT and an improvement in post-exercise $RPE_{general}$. These findings support the ergogenic potential of acute BJ supplementation to improve middle distance running performance in male and female subjects and suggest that this improvement might be linked to a reduction in RPE.

Author Contributions: Conceptualization, R.D. and S.F.d.S.; methodology, S.F.d.S.; recruitment of the sample, A.C.; elaborating placebos and ensuring nutritional guidelines, R.D.; data collection, A.C.; data curation, R.D.; formal analysis, A.C., R.D., and S.F.d.S.; writing—original draft, A.C., R.D., and S.J.B.; A.C., R.D., S.F.d.S., and S.J.B. revised the final draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the Alfonso X El Sabio University (protocol Cod. 0045230632017, 23 February 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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