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Effects of Acute Red Spinach Extract Ingestion on Repeated Sprint Performance in Division I NCAA Female Soccer Athletes

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Abstract: Red spinach extract is high in inorganic nitrate/nitrite (NO_3/NO_2) which has been shown to enhance vascular function, cognition, and physical performance. To date, there have been no investigations as to whether red spinach extract serves as an effective strategy to improve repeated exercise performance, which is applicable to many sports and activities. The purpose of this study was to investigate the effect of acute red spinach extract ingestion on repeated sprint ability in female athletes. Eleven Division I NCAA female athletes (ages 18–24) were recruited. In a double-blinded, randomized, counterbalanced design, participants completed two separate visits each with a different treatment: placebo (placebo; tomato juice) or red spinach extract (~400 mg nitrate). For each trial, participants consumed their respective treatment two hours before exercise. Following a warm-up, participants completed 3 × 15 s Wingate Anaerobic Tests (WAnTs) separated by 2 min of recovery. A capillary blood sample was obtained pre-exercise to measure NO_2 concentrations. Performance outcomes, heart rate (heart rate), and rate of perceived exertion were measured following each WAnT. Blood lactate (La-) was obtained prior to exercise (PRE) and after the completion of the repeated sprints (POST). Each visit was separated by a minimal recovery period of 72 h. Mean power (p = 0.204), peak power (p = 0.067), heart rate (p = 0.151), and rate of perceived exertion (p = 0.379) were not significantly different between treatments. POST La- concentration was significantly higher with red spinach extract when compared to the placebo (p = 0.030). Furthermore, the fatigue index (p = 0.018) was significantly lower with red spinach extract. The results do not support the use of red spinach extract for the enhancement of power output during repeated anaerobic exercise. However, it may result in improved La-/H^+ removal from the muscle, thereby combating physical fatigue.

Keywords: nitrate; nitrite; Wingate; lactate

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1. Introduction

Amaranthus dubius, also known as red spinach, is a flowering plant native to various regions in Asia [1]. Anecdotally, the extract from red spinach has been purported to aid in digestion, improve gastrointestinal health, enhance immunity, and aid in weight loss. While red spinach extract is rich in essential minerals, it also contains high concentrations of inorganic nitrate (NO_3) which has been repeatedly shown to result in the production and accumulation of plasma nitrite (NO_2) [2]. Once consumed, NO_3 is reduced to NO_2 via nitrate reductase by anaerobic bacteria in the mouth [3]. Once NO_2 is metabolized, it is reduced further in the gastric mucosa and gives rise to the formation of nitric oxide (NO). As a biological messenger, NO induces hyperemia [4], increases metabolic efficiency [5], and contributes to the regulation of blood pressure [6]. For an in-depth view of nitrate metabolism, Lundberg et al. is recommended for greater insight, along with Figure 1 [7]. Recently, dietary enrichment with NO_3-containing or NO precursor supplements has become more common in sports and athletics due to previous reports of increased exercise performance [8–11]. However, red spinach extract has been studied little compared to other inorganic NO_3 sources, especially in regard to anaerobic exercise.

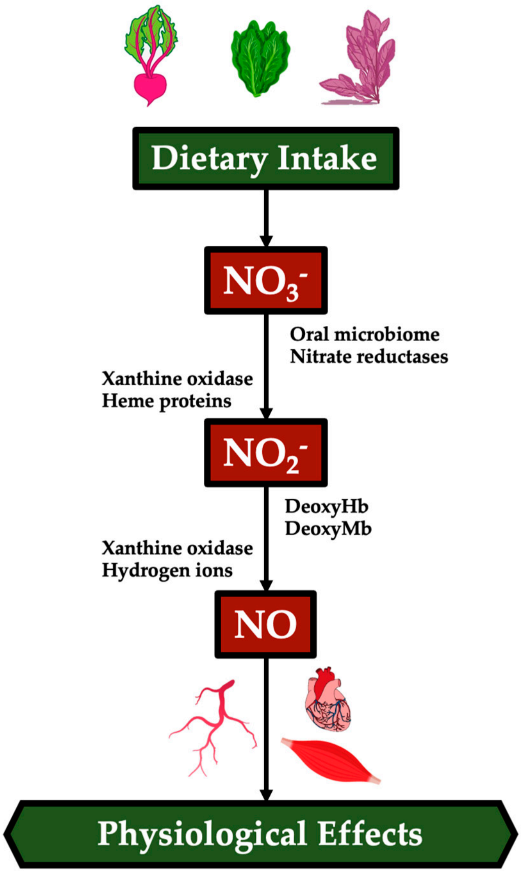


Figure 1. Summary of Nitrate-Nitrite-Nitric Oxide Pathway through diet.

Most of the literature regarding natural supplementation with NO_3 has employed the use of beetroot juice with widespread reports of performance enhancement [8–10]. Acute beetroot juice ingestion has been shown to improve resistance exercise performance primarily in the forms of increased velocity, power, and repetition volume [8,11]. Furthermore, improvements in countermovement jump and agility performance have been noted with beetroot juice supplementation [10,11]. Increases in sprint performance have also been observed, manifesting in the enhancement of power and anaerobic capacity [12,13]. Our lab has also shown that beetroot juice effectively attenuates morning-associated declines in sprint performance and power output [9]. While still being elucidated, purported mechanisms responsible for performance enhancement may include increased skeletal muscle blood flow [14], greater metabolic recovery [9,15], and enhanced intramuscular calcium release [16], which may act independently or synergistically. While intriguing, beetroot juice has to be especially concentrated in order to provide the necessary NO_3, which may not be widely available or misleading to consumers if they are unaware of what is required to receive the optimal dose. Thus, the identification of other feasible ways of obtaining ergogenic doses of NO_3 are needed.

Among other sources rich in inorganic NO_3, red spinach extract has emerged as a distinct alternative to beetroot juice that contains no carbohydrates or oxalates, and is rich in minerals such as potassium and magnesium [17]. Indeed, red spinach extract has been shown to increase plasma NO_3 and NO_2 [18]. Red spinach extract has been shown to increase ventilatory threshold in active participants, likely indicating a greater ability to sustain aerobic metabolism as exercise intensity increases [18]. Furthermore, red spinach extract has been reported to increase time to exhaustion during high-intensity exercise and results in increased exhaled NO [19]. However, other studies investigating upper body resistance exercise performance have failed to show performance enhancement with red spinach extract [20]. Recently, Liubertas et al. showed that red spinach extract supplementation increased peak power output during a ramp cycling protocol [21]. However, no investigations to date have investigated how red spinach extract may influence repeated high-intensity exercise performance which is highly pertinent to sports and competition. Since soccer and other sports require intermittent sprinting, the use of a natural nutritional intervention to improve sprint ability could hold important implications for training and performance. Therefore, the purpose of this study was to elucidate the effects of acute red spinach extract ingestion on repeated sprint ability in Division I NCAA collegiate female soccer players.

2. Materials and Methods

2.1. Participants

An a priori power analysis was employed to determine an adequate sample size using Gpower 3.1.9.6 software (Open Access Software). Our lab recently showed that NO_3 ingestion through beetroot juice prevents losses in relative power output during repeated WAnTs [9]. The effect size of differences in performance between treatments was $\eta^2 = 0.257$. Accordingly, the following parameters were used: statistical test = repeated measures ANOVA, $\eta^2 = 0.257$, $\alpha = 0.05$, $\beta = 0.8$, correlation among repeated measures = 0.5. This yielded a minimum sample size of 8 participants. However, in order to have comparable sample sizes to previous investigations on NO_3 and exercise, a sample size of n = 11 was selected. Female soccer players (n = 11; age = 19.9 yrs $\pm$ 1.2; height = 165.1 cm $\pm$ 4.8, body mass = 69.1 kg $\pm$ 5.7) were recruited for this investigation. All participants were active on a Division I NCAA soccer roster in the past year at the time of the investigation. Participant suitability for exercise was determined using a Physical Activity Readiness Questionnaire (PAR-Q) [22]. Individuals were excluded if they had a lower-body injury within 6 months of initial testing and/or if they had previously been diagnosed with cardiovascular disease, metabolic disease, or any other health-related issue. Participants were asked to refrain from alcohol, nicotine, caffeine, and nitrate-rich foods (spinach, beets, etc.) for 12 h leading up to testing [9,10]. They were also asked to not use antibacterial mouthwash rinse on the day of testing to prevent the loss of oral bacteria needed for NO_3 reduction [3]. All experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the Samford University Institutional Review Board (EXPD-HP-22-SUM-1; June 2021).

2.2. Procedures

2.2.1. Study Design

Using a double-blind, counterbalanced, crossover design approach, healthy female college soccer players completed 2 visits each with a different experimental treatment: placebo or red spinach extract. Following ingestion, blood collection to quantify plasma NO_2 and lactate (La-) was performed, followed by 3 $\times$ 15-s Wingate Anaerobic Tests (WAnTs). The rate of perceived exertion and heart rate were recorded after each WAnT. Immediately upon the completion of the last WAnT, blood La- was measured again. Mean power, peak power, fatigue index, rate of perceived exertion, heart rate, La-, and NO_2 were analyzed and compared between the placebo and red spinach extract. Each trial was separated by a 72 h washout period [8,9].

2.2.2. Supplementation and Plasma NO_2

For red spinach extract supplementation, ~4.4 g of a red spinach extract supplement (Spin Boost, Nutrigardens; Portland, OR, USA) with a standardized NO_3 content of ~400 mg was dissolved in water (~100 mL). For placebo treatment, tomato juice (Campbells, Camden, NJ, USA) was given in an identical manner and volume [23,24]. Treatments were ingested 2 h prior to testing. The entirety of the supplement had to be consumed within 5 min.

Participants were not aware of any experimental hypotheses. Supplements were distributed by an independent researcher not involved in data collection, and the distribution order was only divulged to researchers at the completion of all data analyses.

Measurement of plasma [NO_2] was completed as previously reported by our lab [10]. Briefly, ~500 µL of capillary blood was collected 2 h after the corresponding treatment ingestion through a finger prick [25]. A 2.0 mm depth blade lancet (17 gauge) was used to initiate bleeding on either the third or fourth finger. Blood lactate (La-) was measured using a portable lactate meter (Lactate Plus, Nova Biomedical, Waltham, MA, USA) and a massage method was used to generate a steady blood flow. Whole blood was collected via capillary action into potassium EDTA-coated microvette® tubes (SARSTEDT, Newton, NC, USA). Whole blood was then centrifuged at 10,000 rpm for 10 min. The plasma was decanted, and subsequently frozen at −80 °C until the end of data collection was performed. Plasma concentrations of [NO_2] were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Abor, MI, USA) [26,27]. All samples were analyzed in duplicate and according to the manufacturer's instructions.

2.2.3. Protocol

For each visit, participants donned a chest strap heart rate monitor (Polar, NY, USA), and completed a 5 min warm-up on a mechanically braked cycle ergometer (Monark, Varberg, Sweden) to a metronome set to 60 bpm. Participants completed 3 × 15 s WAnTs on an electronically braked cycle ergometer (Velotron, Racermate Inc., Seattle, WA, USA). The seat height on the cycle ergometer was adjusted so that no more than 5 degrees of knee flexion was present when the leg was fully extended, and their feet were secured to the pedals with toe straps [12]. Seat height was recorded on the first visit and used subsequently for the next visit. Pedal resistance was calculated using 7.5% of the participant's body mass. Participants started each WAnT with a 10 s lead-in phase to allow for the attainment of maximal revolutions per minutes. Resistance was then immediately applied at 7.5% of the participant's body mass and they pedaled maximally for 15 s. Each test was separated by 2 min of active recovery pedaling at 50 watts at their own pace. After each WAnT, power measurements, heart rate, and rate of perceived exertion (1–10 scale) were collected. Upon the completion of the last WAnT, blood La- collection was repeated and performance variable were obtained via Velotron Software (Racermate Inc, Seattle, WA, USA) and documented for further analysis. The fatigue index was calculated as $[(W_{max} - W_{min})/\text{test duration}]$.

2.3. Data Analysis

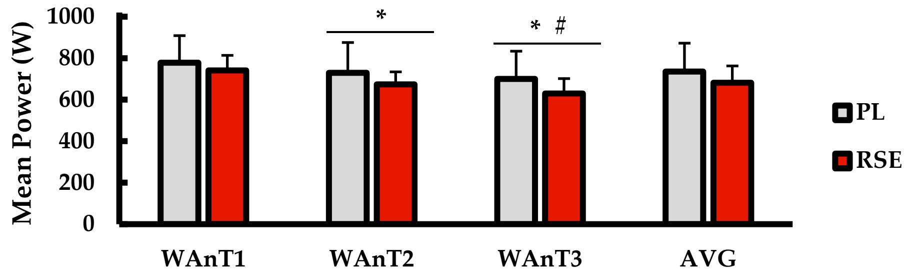
Data analysis was completed using Jamovi software (Version 0.9; Sydney, Australia). Confirmation of data normality was conducted using a Shapiro–Wilk test. Plasma NO_2 was analyzed using a pairwise *t*-test. Blood lactate was analyzed using a 2 × 2 repeated measures ANOVA [condition × time]. All other variables were analyzed using a 2 × 3 repeated measures ANOVA [condition × test] to detect main effects. In this regard, data are shown for individual WAnTs and all WAnTs are averaged which effectively represents main effects for treatment. A Bonnferroni–Holm post hoc test was used to determine differences in means for significant main effects.

3. Results

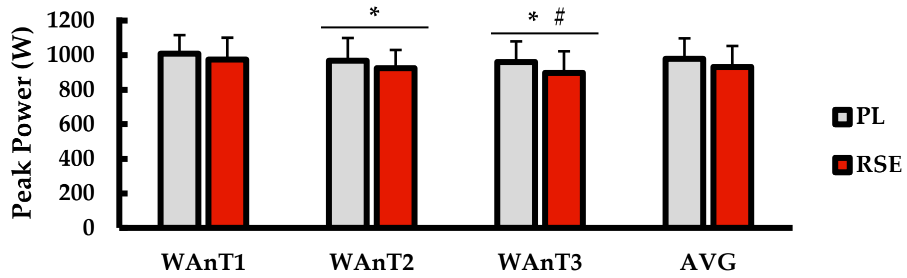
3.1. Anaerobic Performance

Mean power, peak power, and fatigue index are shown in Figure 2. For mean power (watts; Figure 2a), there was a main effect for test ($p < 0.001$ $\eta^2 = 0.116$) but not for treatment ($p = 0.204$; $\eta^2 = 0.056$). There was no interaction between test and treatment ($p = 0.166$; $\eta^2 = 0.003$). Mean power during WAnT1 was higher than WAnT2 ($p < 0.001$) and WAnT3 ($p< 0.001$). Peak power during WAnT2 was also higher than WAnT3 ($p < 0.001$). For peak power (watts; Figure 2b), there was a main effect for test ($p < 0.001$ $\eta^2 = 0.048$) but not for treatment ($p = 0.076$; $\eta^2 = 0.037$). No interaction between test and treatment existed ($p = 0.370$; $\eta^2 = 0.003$). Specifically, peak power during WAnT1 was higher than WAnT2

($p = 0.003$) and WAnT3 ($p = 0.001$). Peak power during WAnT2 was also higher than WAnT3 ($p = 0.050$). Peak power during WAnT2 was also higher than WAnT3 ($p < 0.001$). For the fatigue index (watts/s; Figure 2c), there was a main effect for test ($p < 0.001$ $\eta^2 = 0.088$) and treatment ($p = 0.034$; $\eta^2 = 0.075$). No interaction between test and treatment existed ($p = 0.771$; $\eta^2 = 0.006$). For treatment, the fatigue index was significantly lower for red spinach extract versus placebo ($p = 0.034$). The fatigue index during WAnT1 was lower than WAnT2 ($p = 0.006$) and WAnT3 ($p = 0.019$).



(**a**)



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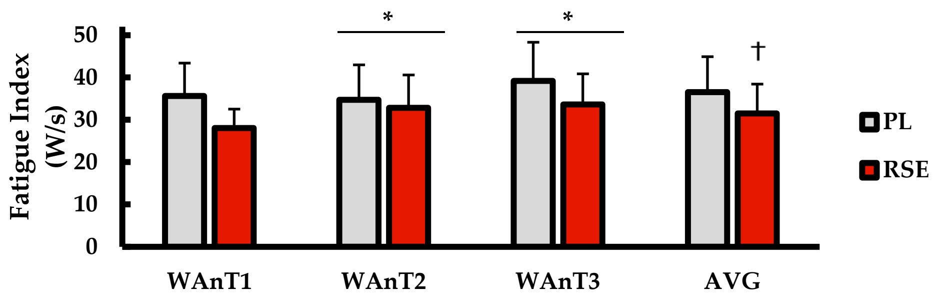
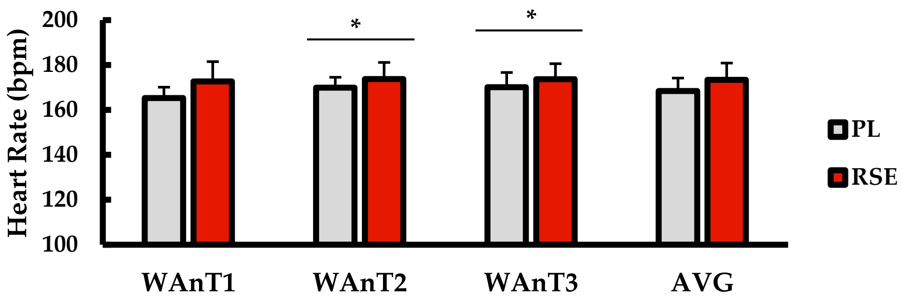


(**c**)

Figure 2. Changes in (**a**) mean power (W), (**b**) peak power (W), and (**c**) fatigue index (W/s) for WAnT1, WAnT2, WAnT3, and all WAnTs averaged together (AVG) between placebo (placebo; grey bars) and red spinach extract (dark red bars). Data are presented as mean ± SD. * indicates significantly different from WAnT1 ($p < 0.05$). # indicates significantly different from WAnT2 ($p < 0.05$).

3.2. Heart Rate and Rate of Perceived Exertion

Heart rate and rate of perceived exertion are shown in Figure 3. For heart rate (bpm; Figure 3a), there was a main effect for test ($p = 0.009$ $\eta^2 = 0.064$) but not for treatment ($p = 0.151$; $\eta^2 = 0.051$). There was no interaction between test and treatment ($p = 0.520$; $\eta^2 = 0.003$). Heart rate during WAnT1 was lower than WAnT2 ($p = 0.043$) and WAnT3 ($p = 0.016$). The rate of perceived exertion (1–10 scale; Figure 3b) showed a main effect for test ($p < 0.001$ $\eta^2 = 0.432$) but not for treatment ($p = 0.379$; $\eta^2 = 0.009$). No interaction between test and treatment existed ($p = 0.132$; $\eta^2 = 0.004$). The rate of perceived exertion during WAnT1 was lower than WAnT2 ($p < 0.001$) and WAnT3 ($p < 0.001$). The rate of perceived exertion during WAnT2 was lower than WAnT3 ($p = 0.002$).



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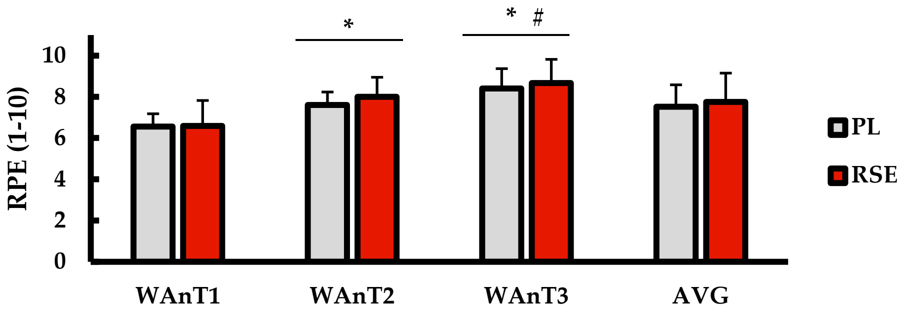

(**b**)

Figure 3. Changes in (**a**) heart rate (bpm) and (**b**) rate of perceived exertion (1–10 scale) for WAnT1, WAnT2, WAnT3, and all WAnTs averaged together (AVG) between placebo (placebo; grey bars) and red spinach extract (red spinach extract; dark red bars). Data are presented as mean ± SD. * indicates significantly different from WAnT1 ($p < 0.05$). # indicates significantly different from WAnT2 ($p < 0.05$).

3.3. Plasma Nitrite (NO_2) and Blood Lactate (La-)

Plasma NO_2 concentrations taken 2 h after the ingestion of the corresponding supplement along with pre- and post-exercise blood La- are shown in Figure 4. For plasma NO_2 (μM; Figure 4a), the ingestion of red spinach extract resulted in significantly higher NO_2 levels compared to placebo ($p = 0.018$). For blood La- (mmol/L; Figure 4b), there was a main effect for time ($p < 0.001$ $\eta^2 = 0.841$) and treatment ($p = 0.012$; $\eta^2 = 0.005$). There was also an interaction between test and treatment ($p = 0.026$; $\eta^2 = 0.003$). Blood La- was significantly higher post- compared to pre-exercise ($p < 0.001$) and for red spinach extract compared to placebo ($p = 0.012$). At the post time point, La- was significantly higher with red spinach extract compared to placebo ($p = 0.030$).

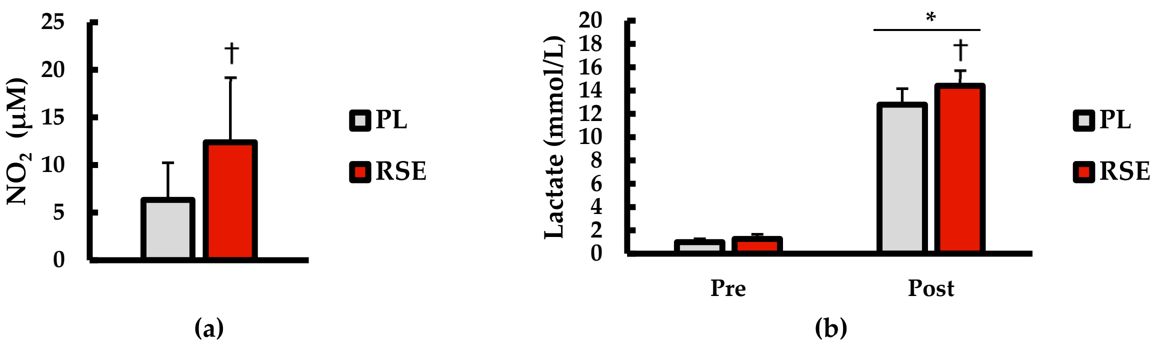

Figure 4. Changes in (**a**) plasma nitrite (NO_2; μM) and (**b**) pre- and post-exercise blood lactate (mmol/L; La-) between placebo (placebo; grey bars) and red spinach extract (red spinach extract; dark red bars). Data are presented as mean ± SD. * indicated significantly different from pre-exercise ($p < 0.05$). † indicates significantly different from placebo ($p < 0.05$).

4. Discussion

Acute red spinach extract ingestion has been previously reported to instill ergogenic benefits during high-intensity exercise [18,28]. However, no studies to date have investigated the effects of acute red spinach extract ingestion on repeated exercise performance in athletic populations. Currently, the results from this study suggest that despite marked increases in NO_2, red spinach extract does not increase power-generating capabilities during repeated sprints. However, red spinach extract ingestion resulted in lower fatigue indices and higher post-exercise lactate values compared to placebo. While underpinning mechanisms were not comprehensively determined, present results from this study indicate red spinach extract effectively increases plasma NO_2 levels and may aid in attenuating fatigue during repeated sprint exercises.

Red spinach extract has been repeatedly shown to result in increases in plasma NO_3 and NO_2, which the current results support. For example, Subramanian et al. showed that increases in NO_3 and NO_2 levels may begin as soon as 30 min post-ingestion of red spinach extract and last for 8 h thereafter [29]. Of particular importance is that red spinach extract treatment currently resulted in increases in NO_2 to similar concentrations to those previously reported with beetroot juice [10]. NO_2 is the direct precursor to NO and likely the mediator of physiological benefits. However, power output remained unaltered despite these changes. This is in contrast to prior studies which may in part be due to the current mode of exercise. Previous studies utilizing red spinach extract during exercise have investigated supplementation in the context of high-intensity aerobic exercise [18,28]. The high reliance on oxidative metabolism during these activities may have allowed possible alterations in blood flow from red spinach extract to more optimally aid in energy regulation during exercise. While increases in oxygen/blood flow to skeletal muscle may aid in phosphocreatine recovery [30], this may not have been fully realized from red spinach extract supplementation and, thus, did not enhance performance. However, it should be acknowledged that other forms of nitrate supplementation (i.e., beetroot juice) have been shown to improve repeated sprint ability [9]. Future studies directly comparing various NO_3 sources will be needed to elucidate possible differences in effectiveness.

Despite the lack of changes in mean and peak power, red spinach extract resulted in a lower fatigue index compared to the placebo. This suggests that while red spinach extract may not have enhanced power output, it may have allowed for a greater maintenance of power over the course of the WAnTs. Furthermore, post-exercise blood La- was significantly higher compared to the placebo. Taken together, this may suggest that red spinach extract imposed attenuation in fatigue, possibly through alterations in muscle La- removal. Indeed, La- removal from muscle is tightly linked with monocarboxylate transporter (MCT) capacity and fatigue indices [31]. Sources of dietary NO_3 have also been implicated in promoting skeletal muscle blood flow even in the absence of increased oxygen uptake [32]. Although purely speculative, red spinach extract may have resulted in an increased skeletal muscle blood flow which could have allowed for a greater removal of La- from the muscle into the circulation. In turn, the increases in post-exercise La- may reflect greater removal from muscle to the blood during exercise, thereby resulting in a lower fatigue index. However, the reader is cautioned that this cannot be deduced from current data alone, and future studies quantifying glycolytic flux and MCT activity with red spinach extract will be necessary to confirm the conclusions from the study.

While this study provides novel information regarding red spinach extract supplementation and repeated exercise performance in athletes, there were limitations. As a preliminary investigation, the sample size of athletes was small, albeit desirable from a training status perspective. While adequately powered statistically, more comprehensive studies with larger and more diverse samples (i.e., different sports, expertise, age, etc.) will be needed in order to generalize findings through collegiate athletics. Additionally, the menstrual cycle of athletes was not strictly controlled, and we cannot rule out the possibility of it influencing the results. Baseline NO^- levels in women may differ at varying cycles, even without supplementation [33]. This could in turn partially indicate that the effects of RSE may be higher when NO^- is already elevated at baseline. Thus, we cannot be certain that plasma NO^- was the same among all participants. However, it is worth noting that our rationale for omitting this control was due to previous evidence showing that anaerobic performance is unchanged regardless of the menstrual cycle phase in addition to the fact that females are grossly understudied in exercise research [34,35]. Future studies will need to consider this for their population and investigate if red spinach extract remains efficacious throughout the entire cycle.

5. Conclusions

In conclusion, red spinach extract ingestion induced large increases in plasma NO_2 but failed to improve mean and peak power during repeated sprints. Heart rate and rate of perceived exertion were largely unaffected by treatment. However, red spinach extract lowered the fatigue index and resulted in elevated post-exercise La- levels compared to placebo. This may suggest an improved La- clearance and ability to sustain repeated exercise. From a practical standpoint and related to the current population, soccer involves long bouts of intermittent sprinting which may result in an increased risk of fatigue compared to other steady-state exercises. Current data suggest that acute red spinach extract ingestion may help to prevent fatigue during intermittent high-intensity sprinting, which may translate to better competition and/or game-play. However, testing on sports-specific skills is warranted to understand how current findings translate to the field of gameplay.

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Institutional Review Board Statement: All experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the Samford University Institutional Review Board (EXPD-HP-21-SUM-5; June 2021).

Informed Consent Statement: Prior to any data collection, verbal and written informed consent was obtained from each participant.

Data Availability Statement: All data are freely available within this manuscript.

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

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