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What Is in Your Beet Juice? Nitrate and Nitrite Content of Beet Juice Products Marketed to Athletes

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Abstract

Consumption of beetroot juice (BRJ) supplements has become popular among athletes because beets tend to be rich in nitrate (NO_3^-), which can enhance exercise performance by increasing nitric oxide production. The NO_3^- content of beets can vary significantly, however, making it difficult to know how much NO_3^- any product actually contains. Samples from 45 different lots of 24 different BRJ products from 21 different companies were therefore analyzed for NO_3^- (and nitrite [NO_2^-]) concentration using high-performance liquid chromatography. The NO_3^- and NO_2^- content (i.e., amount per serving) was then calculated based on either (a) the manufacturer's recommended serving size (for prepackaged/single dose products) or (b) as used in previous studies, a volume of 500ml (for BRJ sold in bulk containers). There was moderate-to-large variability in NO_3^- content between samples of the same product, with a mean coefficient of variation of $30\% \pm 26\%$ (range 2–83%). There was even greater variability between products, with a ~50-fold range in NO_3^- content between the lowest and highest. Only five products consistently provided 5 mmol of NO_3^- /serving, which seems to be the minimal dose required to enhance exercise performance in most individuals. NO_2^- contents were generally low (i.e., 0.5% compared with NO_3^-), although two products contained 10% and 14%. The results of this study may be useful to athletes and their support staff contemplating which (if any) BRJ product to utilize. These data may also offer insight into variability in the literature with respect to the effects of BRJ on exercise performance.

Keywords

exercise; nitric oxide; supplements

Numerous studies in recent years have investigated the effects of dietary nitrate (NO_3^-) on the physiological responses to, and/or performance during, exercise. This interest stems from the fact that dietary NO_3^- is an important source of nitric oxide (NO) via the “reverse” $\text{NO}_3^- \rightarrow$ nitrite (NO_2^-) \rightarrow NO enterosalivary pathway (Kharti et al., 2017). NO_3^- induced increases in NO bioavailability have been found to enhance both endurance exercise performance (Jones et al., 2018; Van De Walle & Vukovich, 2018) as a result of

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alterations in oxygen supply, demand, and/or cellular energetics (Bailey et al., 2010), and sprint performance (Rimer et al., 2016) as a result of improvements in muscle contractile properties, that is, in maximal muscle speed and power (Coggan et al., 2015b, 2018b). For example, Lansley et al. (2011a) observed a 2.7–2.8% improvement in mean power during simulated 4- and 16.1-km cycling time trials, whereas Rimer et al. (2016) reported a 6.5% increase in maximal power during an inertial load sprint cycling test. Positive effects of BRJ seem to be most apparent in untrained or moderately trained individuals (Van De Walle & Vukovich, 2018) or patient groups, (Coggan et al., 2015a, 2018a) and less evident or even absent in highly trained athletes (e.g., Bescós et al., 2012). Notably, however, some studies (Hoon et al., 2015; Oskarsson & McGawley, 2018) have yielded negative results for reasons that do not seem to be related to differences in subject selection or other aspects of the experimental design.

Most studies of dietary NO_3^- and exercise have used beetroot juice (BRJ) as a source. This is because beets are relatively rich in NO_3^- (Santamaria, 2006) and are readily juiced. Consequently, lay publications frequently contain articles extolling the virtues of BRJ supplementation as an ergogenic aid, and a large number of BRJ supplements in various forms (i.e., powders, mixed drinks, concentrates, bulk juice) are now marketed to athletes. However, the NO_3^- content of beets or BRJ depends heavily on a number of factors, including the growing conditions, the variety of beet, and the method of preparation (i.e., fresh vs. processed; Corleto et al., 2018; dos Santos Baião et al., 2016; Wruss et al., 2015). Furthermore, most BRJ supplements are not labeled with their NO_3^- content, which in any case has only rarely been independently tested. Even some scientific studies have not verified the NO_3^- content of the BRJ supplement used and/or measured any biomarkers of NO bioavailability (e.g., plasma NO_3^- or NO_2^- , breath NO) to ensure that an adequate dose of NO_3^- has been provided (e.g., Hoon et al., 2015; Oskarsson & McGawley, 2018). Athletes and/or their support staff have therefore generally relied on blind faith that any BRJ supplement contains sufficient NO_3^- to provide physiological benefits.

The purpose of this study was therefore to determine the NO_3^- (and NO_2^-) content of various commercial BRJ supplements. The results may be useful to athletes, coaches, and scientists contemplating which (if any) BRJ product to use and may also offer some insight into factors contributing to variability in the literature with respect to the effects of BRJ on exercise performance.

Methods

To aid interpretation of the data, we chose to test products that (a) contained BRJ as the primary ingredient, (b) did not contain other ingredients (e.g., L-arginine) intended to alter NO bioavailability, and (c) were marketed toward athletes and/or athletic performance or were available from sources readily accessible by athletes. Products were purchased from local grocers, large online retailers, specialized “health food” websites, or directly from the producer. Our initial goal was to obtain samples from three different lots of each product, but in many instances, infrequent production runs limited us to testing just one or two different lots of a given product. We ultimately obtained samples from 45 different lots of 24 different BRJ products (i.e., $n = 6$ each of powders, mixed beverages, concentrates, and bulk

juice) produced by 21 different companies. All samples were purchased between September 2017 and June 2018, stored for 2 weeks at room temperature or under refrigeration as recommended for that product, and tested before the declared expiration date.

Powdered products were first reconstituted by thoroughly mixing one serving, that is, an entire packet or scoop, with 100 ml of NO_3^- and NO_2^- free, double-distilled water. A small aliquot of each reconstituted powder or original liquid product was then diluted 1,000-fold and the NO_3^- and NO_2^- concentration measured by injecting 10 μl into a dedicated high-performance liquid chromatography analyzer (ENO-30; Eicom USA, San Diego, CA). This analyzer has previously been described in detail (Coggan et al. 2015a; Troutman et al., 2018). The high-performance liquid chromatography analyzer was calibrated prior to each use by injecting known standards. The NO_3^- and NO_2^- content of each product (i.e., mmol/serving) was calculated from these concentration measurements based on either (a) the manufacturer's recommended serving size or (b) for BRJ products sold in bulk containers, a volume of 500 ml as used in previous studies of BRJ and athletic performance (e.g., Bailey et al., 2010).

Because of the limited number of samples available per product, no attempt was made to compare different products statistically. However, the mean NO_3^- concentration of different types of BRJ products (i.e., powders vs. mixed beverages vs. concentrates vs. bulk juices) was compared using one-way analysis of variance. A $p < .05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism (version 7.02; GraphPad Software, La Jolla, CA).

Results

Data for NO_3^- and NO_2^- concentration (i.e., per g or ml) and content (i.e., per recommended serving) for each sample are shown in Figures 1 and 2, whereas average values for content are provided in Table 1. The NO_3^- concentration of powders (i.e., $174 \pm 63 \mu\text{mol/g}$; mean \pm SD) was significantly higher (i.e., $p < .001$) than that of concentrates ($70 \pm 39 \mu\text{mol/ml}$), which in turn was higher ($p < .05$) than that of mixed drinks ($13 \pm 5 \mu\text{mol/ml}$) or bulk juices ($18 \pm 11 \mu\text{mol/ml}$). However, regardless of the type of product, there was considerable variability in NO_3^- concentration/content between products and often even between samples of the same product. Specifically, there was almost a 50-fold range in NO_3^- content between different products (albeit partially driven by the assumed 500 ml serving size for bulk juices), whereas the coefficient of variation for the NO_3^- concentration or content of samples of the same product averaged $30\% \pm 26\%$ (range 2–83%).

Although traces of NO_2^- were detectable in all products, the content was generally very low (i.e., 0.06 mmol/serving) except for two products (i.e., Superbeets and BeetElite, both from HumanN), which on average contained 0.14 and 0.22 mmol/serving, respectively. The potential significance of the higher NO_2^- content of the latter two products is considered in the Discussion section.

Discussion

Based on studies demonstrating that BRJ ingestion can enhance exercise performance (Jones et al., 2018), a large number of BRJ-based supplements are now marketed to athletes. The NO_3^- concentration of beets, however, varies markedly (dos Santos Baião et al., 2016; Wruss et al., 2015), and few BRJ products have been independently tested to determine their NO_3^- content. Athletes and/or their support staff must therefore generally rely on claims made by producers to determine whether such supplements contain sufficient NO_3^- to impact physiological function.

To help fill this knowledge gap, we measured the NO_3^- (and NO_2^-) content of a wide variety of BRJ-based products, including powders, mixed drinks, concentrates, and bulk juices. Our data demonstrated that there can be significant variation between products and even between samples of the same product. Furthermore, only five of the products consistently contained more than 5 mmol of NO_3^- /serving, which based on previous research seems to be the minimal dose required to enhance exercise performance in most individuals (cf. Jones et al., 2018). These findings are in line with the results of a previous study that included a smaller number of BRJ products sold primarily in Europe (Wruss et al., 2015). Although individuals can always choose to consume larger-than-recommended amounts, potential disadvantages to doing so include increased cost, greater volume to ingest, and higher intake of oxalate. The present data are therefore likely to be highly useful to athletes and their support staff in guiding selection of BRJ supplements containing 5 mmol/serving). They may also adequate amounts of NO_3^- (i.e., help explain some of the negative and null findings in the scientific literature with respect to the effects of BRJ supplementation on exercise performance (Hoon et al., 2015; Oskarsson & McGawley, 2018). Indeed, given the possible variation even between samples of the same product, scientists should measure the NO_3^- content of any BRJ supplement used or at least provide the lot or batch number.

An important assumption of the present study is that any differing effects in vivo would be solely, or at least primarily, due to differences in the NO_3^- content of the supplements tested. In fact, beets and therefore BRJ-based products contain numerous other potentially biologically active compounds, including carotenoids, betalains, bioflavonoids, and ascorbic acid (Georgiev et al., 2010; Wruss et al., 2015). Indeed, there is some evidence that BRJ may be more effective than simple NO_3^- salts at reducing muscle pain after eccentric exercise (Clifford et al., 2017) or in enhancing training-induced improvements in peak oxygen consumption (Thompson et al., 2018). However, Lansley et al. (2011b) demonstrated that supplementation with NO_3^- depleted BRJ does not alter metabolism or performance during exercise, indicating that the effects of BRJ are mediated primarily if not exclusively by NO_3^- . Furthermore, by studying only liquid BRJ products, or BRJ powders likely to be consumed as liquids, we avoided any interpretative difficulties that may have arisen had we compared liquid versus solid supplements (e.g., McDonagh et al., 2018) or included products in which BRJ was not the primary ingredient. Still, it is possible that the in vivo efficacy of the various products that we tested may differ due to differences in factors other than their NO_3^- content.

Along with NO_3^- , we also measured the NO_2^- concentration and hence content of the various BRJ supplements. In general, NO_2^- levels were quite low except for two products (from the same company) containing ~ 0.2 mmol NO_2^- /serving. It is difficult to predict the impact that this higher NO_2^- content might have on in vivo responses to ingestion of these products. Previous studies of ~ 2 to ~ 4 mmol, provided in the form higher doses of NO_2^- , that is, of sodium salt, have demonstrated improvements in various measures of physical function in older individuals (Justice et al., 2015). It is not known, however, whether the smaller amount found in the present products would be sufficient to elicit comparable effects. It might be expected that these two products would have a more rapid onset of action as a result of bypassing the NO_3^- reduction step of the enterosalivary pathway of NO production. In line with this, McDonagh et al. (2018) recently reported that plasma NO_2^- levels peaked within 30 min of ingestion of one of these products (i.e., Superbeets) versus 2–3 hr after ingestion of other BRJ supplements. Unlike the other BRJ products studied, however, Superbeets did not result in a significant reduction in either systolic or mean arterial blood pressure. This is presumably due to the lower dose of NO_3^- provided (i.e., ~ 1 vs. ~ 6 mmol), which failed to sustain the initial elevation in NO_2^- .

In summary, we have measured the NO_3^- and NO_2^- concentration and hence content of numerous BRJ supplements. Our data reveal marked variation between different products and often even between different samples of the same product. These data should be beneficial to athletes and/or their support staff interested in implementing BRJ supplementation. They also illustrate the importance of measuring the NO_3^- content of any BRJ supplements used in scientific research or the putative active ingredient(s) of any nutritional supplement.

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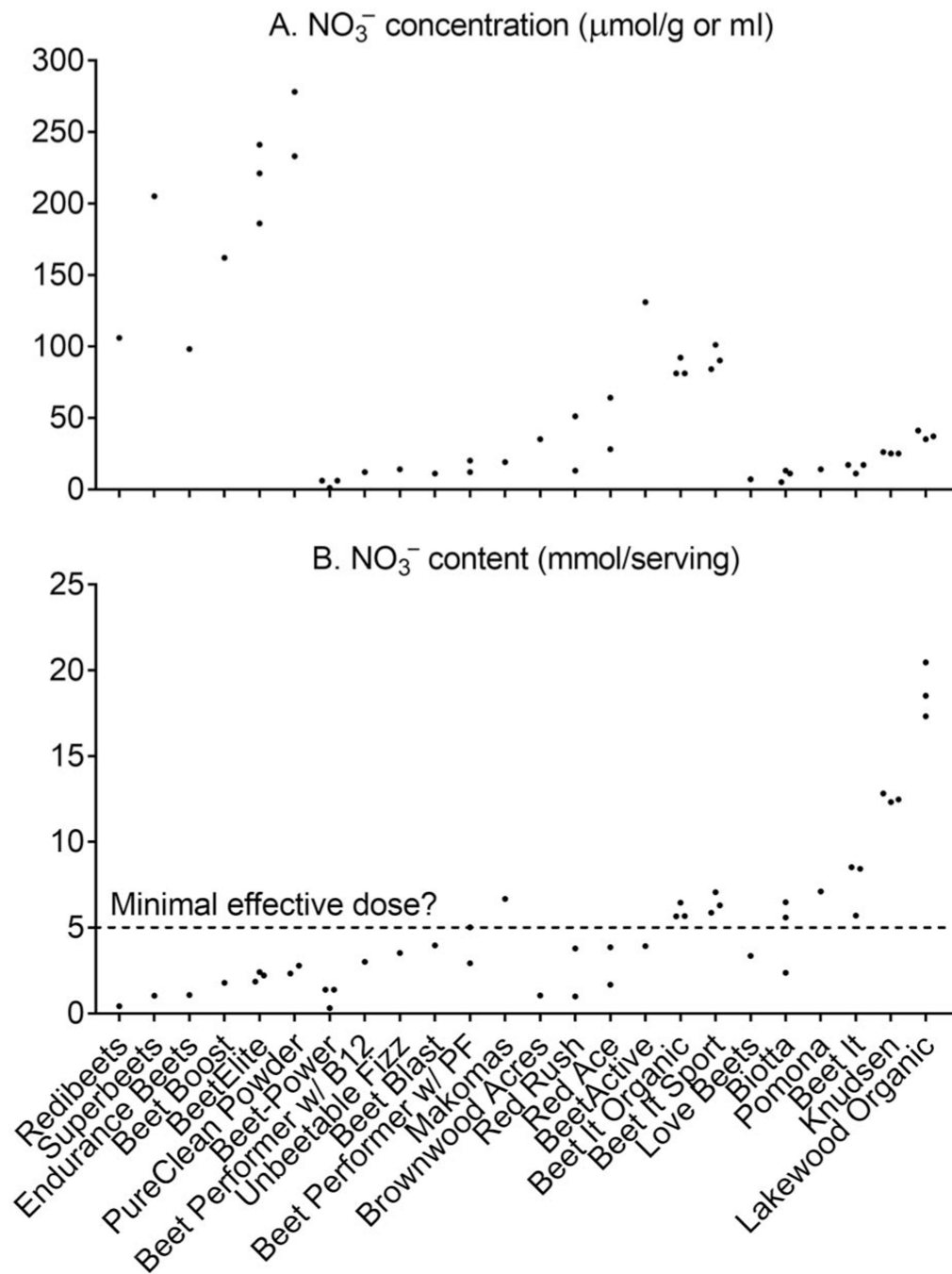


Figure 1 —. NO₃⁻ concentration (a) and content (b) of BRJ supplements. Data for each sample tested are shown (circles). In addition, the minimal dose apparently required to increase exercise performance (i.e., 5 mmol) (dashed line), based on a recent review by Jones et al. (2018), is also shown. NO₃⁻ = nitrate; BRJ = beetroot juice.

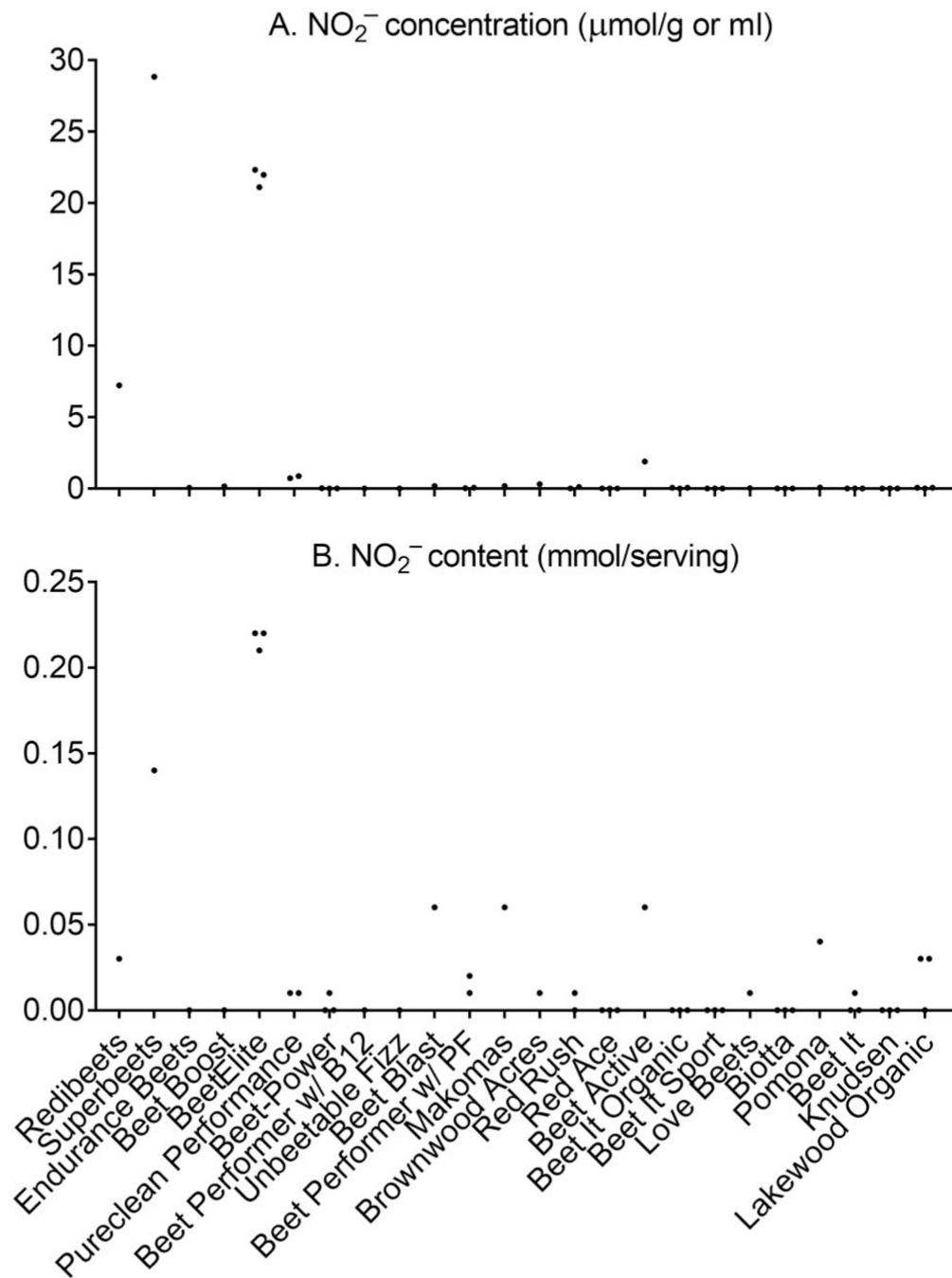


Figure 2 —. NO_2^- concentration (a) and content (b) of BRJ supplements. Data for each sample tested are shown (circles). NO_2^- = nitrite; BRJ = beetroot juice.

Table 1

BRJ Products Tested

Company	Product	Serving size (g)	Claimed NO ₃ content (mmol/serving)	Measured NO ₃ content (mmol/serving)	Measured NO ₂ content (mmol/serving)
Powders					
AIM International	RediBeets	4	–	0.43	0.03
HumanN	Superbeets	5	–	1.03	0.14
HealthySkoop	Endurance Beets	11	–	1.08	0.00
Nutrigardens	BeetBoost	11	–	1.78	0.00
HumanN	BeetElite	10	–	2.16 ± 0.28	0.22 ± 0.01
PureClean	PureClean Powder	10	–	2.56 ± 0.32	0.00 ± 0.00
Mixed drinks					
H2Bev	Beet-Power	250	–	1.02 ± 0.62	0.00 ± 0.00
CAJ Foods	Beet Performer w/ B12	250	–	3.01	0.00
Unbeetable	Unbeetable Fizz	250	4.08	3.52	0.00
BluePrint Organic	Beet Blast	355	–	3.96	0.06
CAJ Foods	Beet Performer w/Passion Fruit	250	–	3.97 ± 1.47	0.02 ± 0.01
Makomas	Ginger Beet Juice	355	–	6.67	0.06
Concentrates					
Brownwood Acres	(beet juice concentrate)	30	–	1.05	0.01
AIM International	Red Rush	74	8.06	2.39 ± 1.97	0.01 ± 0.01
Red Ace Organics	Beet Performance Supplement	60	–	2.76 ± 1.54	0.00
CherryActive	BeetActive	30	3.69	3.93	0.06
James White Drinks	Beet It Organic Beetroot Shot	70	4.84	5.93 ± 0.45	0.00 ± 0.00
James White Drinks	Beet It Sport Pro-Elite Shot	70	6.45	6.41 ± 0.60	0.00 ± 0.00
Bulk beet juice					
Love Beets	(beet juice)	500 ^a	–	3.35	0.01
CAJ Foods	Biotta beet juice	500 ^a	–	4.81 ± 2.16	0.00 ± 0.00
Pomona Organic	(beet juice)	500 ^a	–	7.11	0.04
James White Drinks	Beet It beet juice	500 ^a	–	7.55 ± 1.61	0.00 ± 0.00

Company	Product	Serving size	Claimed NO ₃ ⁻ content (mmol/serving)	Measured NO ₃ ⁻ content (mmol/serving)	Measured NO ₂ ⁻ content (mmol/serving)
Knudsen and Sons	(beet juice)	500 ^a	–	12.54 ± 0.25	0.00 ± 0.00
Lakewood Organic	(beet juice)	500 ^a	–	18.77 ± 1.59	0.02 ± 0.02

Note: BRJ = beetroot juice; NO₃⁻ = nitrate; NO₂⁻ = nitrite.

^a Assumed serving size.