Ingestion of a nitric oxide enhancing supplement improves resistance exercise performance

Original Investigation

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Abstract

Studies have established that supplementation of nitrate increases nitrous oxide which in turn improves exercise performance. The current study aimed to investigate the effects of nitrate ingestion on performance of bench press resistance exercise till failure. Twelve recreationally active (age, 21 ± 2yrs, height, 177.2 ± 4.0 cm, weight, 82.49 ± 9.78 kg) resistance trained males participated in the study. The study utilised a double blind randomized cross-over design, where participants ingested either 70 ml of “BEET It Sport ®” nitrate shot containing 6.4 millimoles (mmol/L) or 400 mg of nitrate; or a blackcurrant placebo drink. Participants completed a resistance exercise session, consisting of bench press exercise at an intensity of 60% of their established 1 repetition maximum (1-RM), for three sets until failure with 2 minute rest interval between sets. The repetitions completed, total weight lifted, local and general rate of perceived exertion (RPE), and blood lactate were all measured. The results showed a significant difference in repetitions to failure (p < 0.001) and total weight lifted (p < 0.001). However there were no significant difference between blood lactate over the two trials (p = 0.238), and no difference in Local (p = 0.807) or general (p = 0.420) indicators of fatigue as measured by RPE. This study demonstrates that nitrate supplementation has the potential to improve resistance training performance and work output compared to a placebo.

Key words: Nitrate, Nitric oxide, Exercise performance, Resistance training, Supplementation.
INTRODUCTION

Nitric Oxide (NO) is an influential signalling molecule which rapidly acts on vascular smooth muscle via guanylate cyclase (18). The result of this causes increases in cyclic guanosine mono-phosphate, which facilitates relaxation effects in smooth muscle, further producing vascular dilatation (13). Dietary nitrate (NO$_3^-$) typically obtained from green leafy vegetables and in particular from beetroot, is reduced to NO$_2^-$ by nitrate reductase (31), causing a sustained increase in circulating NO$_2^-$ levels (23). It is principally a vasodilator, producing a relaxation effect upon the vascular endothelium (19). Dietary NO$_3^-$ is 100% bio-available (14, 21) and is readily absorbed in the upper gastrointestinal tract (28). Dietary nitrate and nitrite can therefore be considered storage pools for NO bioactivity (14, 23).

Dietary supplementation with either sodium nitrate or nitrate-rich beetroot juice (BR) has consistently been shown to improve exercise time to exhaustion during both endurance running (20) and cycling (6). It has also been shown to improve cycling time trial (11) and repeated intermittent rowing performance (9, 12, 20) as well as improving exercise tolerance at altitude (27). Such improvements in performance have typically ranged from between 1.2 and 2.9%, but some recent studies with highly trained participants have observed no improvement in performance following the acute single dose ingestion of BR (12, 29). However, a relatively common finding in studies examining the ergogenic effects of nitrate on endurance exercise performance is that heart rate, lactate concentration, CO$_2$ production, minute ventilation, and respiratory-exchange ratio all show no significant change between nitrate and placebo groups.

There have been only four studies (4, 17, 22, 26) that have investigated the effects of BR supplementation or betaine (an amino acid constituent in beetroot juice) on resistance training. Results regarding the use of betaine are equivocal, but suggest a modest increase in resistance exercise performance. Bailey et al., (4) recruited seven recreationally active males that consumed either 0.5 L.day$^{-1}$ of BR (5.1 mmol.day$^{-1}$ NO$_3^-$) or a placebo for six days. During the last three days of supplementation, participants completed low and high (15% and 30% maximal voluntary isometric
contractions, respectively) intensity “step” knee extension tests. Their data showed that BR increased plasma nitrite concentrations by 240% and decreased pulmonary VO₂ by 25% from rest to low-intensity exercise. Beetroot juice consumption also reduced by the amount of phosphocreatine (PCr) degraded during low-intensity exercise and high-intensity exercise by 36% and 59% respectively compared to placebo. Reductions in PCr usage were also accompanied by a reduced total ATP utilization during both high and low-density exercise, suggesting potentially ergogenic effects for this type of exercise. This is the first study that has investigated the effects of a prolonged dose of BR prior to resistance exercise. Therefore the aim of the present study was to investigate the effects of nitrate supplementation on the time to fatigue and subjective ratings of exertion during resistance exercise performance and recovery.

METHODS

Experimental Approach to the Problem

This experimental paradigm was based on the use of a beetroot juice supplement as an ergogenic aid to improve performance of recreational, weight training athletes in terms of fatigue. We decided to measure this by using the number of repetitions until fatigue without increasing physiological as measured by blood lactate or psychological fatigue as measured by RPE indicators.

Participants

Twelve recreationally active males (age, 21 ±SD 2 yr, height, 177.2 ± 4.0 cm, weight, 82.5 ± 9.8 kg) were recruited for this study. All participants were familiar with performing resistance exercises, and had a minimum of three years training experience (4.2 ± 0.9 yr), and a minimum weekly training frequency of three days a week (4 ± 1 days), and three hours per week (6 ± 2 hr). Written informed consent was obtained from all participants prior to completing health screening for contraindications to exercise. Participants were required to abstain from ingesting ergogenic supplements during the data collection period. Furthermore, they were also required to replicate dietary intakes for the 24 hr prior to each laboratory visit, avoid exhaustive exercise (9) and limit
caffeine consumption (15). Prior to each laboratory visit the participants were instructed to eat no less than three hours prior to the trials and consume only water in the final hour before testing commenced (9). Participants were requested to continue their normal dietary intake during the study and this was verbally checked with each individual prior to testing. The study was approved by the Departmental Research Ethics Committee.

Procedures

The initial laboratory visit required each participant’s bench press one repetition maximum (1-RM) to be determined using a previously validated protocol (3). Participants completed a warm up set of 12 repetitions at 10% of estimated 1-RM which was performed using a Smith machine (Hammer Strength, Life Fitness, Ely, UK) followed by a one minute rest period. The load was then increased by 20% and a set of six repetitions was then completed followed by a two minute rest period. A near maximal load was then estimated, this was loaded onto the equipment for the participant to complete three repetitions, followed by a 2-3 min rest period. After these initial warm up sets, the participants estimated 1-RM was established and one attempt was made followed by a 2-4 minute rest. If completed, 1.25 kg, 2.5 kg or 5 kg was progressively loaded to attempt to find the actual 1-RM. The 1-RM was obtained within three to six sets for all participants.

Following the establishment of 1-RM, participants attended the laboratory on two further occasions. The study utilized a double blind, randomized cross-over design requiring participants to complete a six day ingestion period of either a 70 ml BEET It Sport ® nitrate shot, or a blackcurrant placebo drink (6). The nitrate shot contained 6.4 mmol.l⁻¹ or 400 mg of nitrate (2). The order in which participants consumed the nitrate or placebo was administered by a laboratory technician, and this order was only divulged to investigators on completion of all of the data collection. The supplementation strategy required participants to ingest either a 400 mg nitrate shot or placebo on each of the six consecutive days. No participants reported any adverse gastrointestinal discomfort during either of the ingestion periods. Two experimental trials were then performed, one for each
of the ingestion strategies, which were separated by a minimum wash out period of 72 hours, a strategy which has previously been used in a study with a similar design (30). These trials took place on the last day of ingestion at the same time of day (10). The experimental trials consisted of a warm up of two sets of 12 repetitions of bench press. The first set was performed at 10% and the second at 20% of established 1-RM using the Smith machine. This was followed by a one minute rest period that took place after each warm up set. Testing procedures then began performing sets of repetitions to failure using a load of 60% of 1-RM, with each set separated by a two minute rest period (8, 15). Number of repetitions, total weight lifted, local ratings of perceived exertion (RPE) were recorded on completion of each set using 1-10 scales where 1 represented no exertion at all and 10 being maximal exertion. Pre and post exercise blood lactate was measured from capillary blood samples (Micro-stat P-GM7, Analox, USA).

Statistical Analyses
All data were assessed for normality using standard graphical methods. Between condition analysis for all variables measured after each set were analysed using repeated measures ANOVA. Violations to sphericity were determined using a Mauchley’s test. If a significant violation was observed, significance was adjusted using either the Huynh-Feldt or Greenhouse Geisser correction technique, for where for sphericity > 0.75 and < 0.75 respectively. Post hoc analysis was conducted using the Bonferroni pairwise comparison. Total weight lifted and total number of repetitions were analysed using paired t-tests for both condition effects and order effects. Statistical significance was set to $p < 0.05$ for all tests. Calculations of effect sizes were done using $\eta^2$ for ANOVA and Cohen’s d for for t-tests. All data were analysed using SPSS v22 (IBM, Portsmouth, UK).

RESULTS
There was a significant improvement in the total number of repetitions in all three sets (Figure 1) in the nitrate trial compared to the placebo ($F = 29.62, p < 0.001, \eta^2 = 0.70$). This resulted in
significantly greater total repetitions completed (mean difference = 6.92, t = 5.44, p < 0.001, d = 0.96) and total weight lifted (mean difference = 411.3 kg, t = 5.00, p < 0.001, d = 0.52) in the nitrate trials (Figure 1 and Table 1 respectively). **No order effect on resistance exercise performance was observed**, since neither total weight lifted nor total repetitions differed between trial 1 and 2 (mean difference = 56.7 kg, t = -0.41, p = 0.69, d = 0.16; and mean difference = 1.6, t = -0.73, p = 0.48, d = 0.29 respectively). Despite the observed increases in resistance exercise performance in the nitrate condition, there were no significant differences in the lactate responses (Table 1) between the trials (F = 1.79, p = 0.20, $\eta^2_p = 0.12$), but in both experimental conditions the performance of the bench press protocols did cause significant increases in lactate concentrations (f = 306.68, p < 0.001, $\eta^2_p = 0.96$), without an observed condition*time interaction (F = 4.31, p = 0.058, $\eta^2_p = 0.25$). Interestingly, despite increases in resistance exercise performance in the nitrate condition, there were no observed differences in either RPE or RPE-L between conditions (F = 0.69, p = 0.42, $\eta^2_p = 0.05$ and (F = 0.06, p = 0.81, $\eta^2_p = 0.01$ respectively) although there were significant increases in RPE-L between each of the sets (F = 24.09, p < 0.001, $\eta^2_p = 0.65$).

**DISCUSSION**

This study investigated the effects of six days of nitrate supplementation in the form of beetroot juice, on resistance exercise performance. It was hypothesised that nitrate would increase the number of repetitions until fatigue without increasing physiological or psychological fatigue indicators. The principle findings of this study, the first of its kind, established that total work increased in terms of total weight lifted, and repetitions until failure at 60% of 1RM. Both improvements occurred without significantly affecting physiological responses in terms of blood lactate concentration and psychological fatigue indicators of local and general rate of perceived exertion.
Nitrate in the form of beetroot juice has been shown to increase plasma nitrite (6, 21) and these increases have been identified as a significant factor in influencing exercise tolerance (24). This has been investigated by Bailey et al. (4) providing substantial evidence that increased plasma levels of nitrate are associated with a 25% increase in intermittent exercise performance during a two legged knee extensor exercise protocol. The current study found similar percentage increases in resistance exercise performance to failure with a mean average of 19.4% in total repetitions and mean average of 18.9% in total weight lifted. Although blood plasma nitrite levels were not taken within the current study it is presumed that plasma levels increased due to the reports from previous studies and increased performance within this study. The increased tolerance is a result of increased energy yield through PC stores (7), which is supported by findings from the work of Bailey and colleagues (4). They established that with high intensity exercise, specifically leg extension exercise, there was a reduction in PC degradation and a decrease in the production of Adenosine di-phosphates and inorganic phosphates, which are metabolites that have been linked to fatigue within muscles (8). This work concludes that nitrate supplementation could provide a more sufficient rate of change between these substrates and metabolites, consequently delaying the time until they reach critical values. This in turn, enables a greater time until depletion which results in a more efficient usage of PC stores at high intensity exercise, prolonging the tolerance at the given work rate. In relation to the current study’s findings, this suggests that PC stores were used more efficiently when performing the repetitions until fatigue within a given set, allowing individuals to sustain muscle contractile performance for longer. This could explain the increase in total repetitions, more specifically increases in repetitions within each set due to the nitrate ingestion.

Dietary nitrate supplementation has also been reported to excitation-contraction coupling and efficiency (5, 16). These responses occur through an increase in sarcoplasmic reticulum calcium release and increase in force production per power stroke within type 2 fibres (16). The excitation-contraction coupling occurs when signals at the surface of the cell couple to cause releases in calcium within the sarcoplasmic reticulum, consequently this induces actin-myosin interactions.
resulting in muscle fibre contraction (1). The calcium is then withdrawn by the ATP-dependent pump (25). When multiple burst action potentials occur, calcium is sustained and greater contraction force can occur (25). These components modulate the effectiveness and efficiency of the force that is produced by this process. Greater NO production has been proposed to enhance the efficiency of coupling between the above components to produce this overall effect of greater muscle contractile function (5, 25). Hypothesizing that these given effects are apparent in humans (4) these enhancements in muscle efficiency within type 2 fibres, could result in lower overall energy turnover (5). The above evidence suggests, for our work, that when participants were completing upper body (bench press) exercise until fatigue, more effective muscle contraction was apparent with lower overall energy cost, meaning the nitrate aided in conserving energy over the given sets, thus allowing participants to complete more sets.

**PRACTICAL APPLICATIONS**

Coaches and their athletes alike, could make use of nitrate supplementation since our evidence suggests it, can be used within healthy male resistance trained population to increase exercise tolerance and improve performance. Further studies are necessary to investigate long term use and possible adaptations to resistance training with longer periods of dosing with nitrate.
References


**Figure Legend**

**Figure 1.** Mean (±SD) number of bench press repetitions for each set and condition totals. (*) Denotes a significant main effect for condition (p < 0.05).
Table 1. Mean (±SD) physiological and perceived exertional responses.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nitrate</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Weight Lifted (kg)</td>
<td>2582.8 ± 863.9‡</td>
<td>2171.5 ± 720.5</td>
</tr>
<tr>
<td>Lactate (mmol.l⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.1 ± 0.5</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Post</td>
<td>4.2 ± 0.8*</td>
<td>3.8 ± 0.6*</td>
</tr>
<tr>
<td>RPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set 1</td>
<td>4 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Set 2</td>
<td>5 ± 2</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Set 3</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>RPE-L</td>
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<td></td>
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<tr>
<td>Set 1</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Set 2</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Set 3</td>
<td>7 ± 2</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

(‡) Denotes a significant main effect for condition (p < 0.001).
(*) Denotes a significant main effect for time (p < 0.05).