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Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women

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Abstract

Nitric oxide (NO) has been demonstrated to enhance the maximal shortening velocity and maximal power of rodent muscle. Dietary nitrate (NO_3^-) intake has been demonstrated to increase NO bioavailability in humans. We therefore hypothesized that acute dietary NO_3^- intake (in the form of a concentrated beetroot juice (BRJ) supplement) would improve muscle speed and power in humans. To test this hypothesis, healthy men and women (n=12; age=22-50 y) were studied using a randomized, double-blind, placebo-controlled crossover design. After an overnight fast, subjects ingested 140 mL of BRJ either containing or devoid of 11.2 mmol of NO_3^-. After 2 h, knee extensor contractile function was assessed using a Biodex 4 isokinetic dynamometer. Breath NO levels were also measured periodically using a Niox Mino analyzer as a biomarker of whole-body NO production. No significant changes in breath NO were observed in the placebo trial, whereas breath NO rose by 61% ($P<0.001$; effect size=1.19) after dietary NO_3^- intake. This was accompanied by a 4% ($P<0.01$; effect size=0.74) increase in peak knee extensor power at the highest angular velocity tested (i.e., 6.28 rad/s). Calculated maximal knee extensor power was therefore greater (i.e., 7.90±0.59 vs. 7.44±0.53 W/kg; $P<0.05$; effect size=0.63) after dietary NO_3^- intake, as was the calculated maximal velocity (i.e., 14.5±0.9 vs. 13.1±0.8 rad/s; $P<0.05$; effect size=0.67). No differences in muscle function were observed during 50 consecutive knee extensions performed at 3.14 rad/s. We conclude that acute dietary NO_3^- intake increases whole-body NO production and muscle speed and power in healthy men and women.

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Keywords

dietary nitrate; nitric oxide; muscle power; isokinetic; humans

1. Introduction

Nitric oxide (NO) is a key cellular signaling molecule with pleiotropic effects on many physiological systems. These include skeletal muscle, in which NO can influence blood flow, mitochondrial respiration, substrate oxidation, and the production of reactive oxygen and/or nitrogen species (cf. Ref. 31 for review). NO therefore plays an important role in modulating the metabolic responses and hence fatigue resistance of repetitively-contracting muscle, i.e., during exercise.

In addition, though, NO can also directly or indirectly alter the contractile properties of non-fatigued muscle. Directly, NO may (cf. Refs. 15,27 for review) (or may not (6,9)) slightly suppress isometric force production, by interfering with excitation-contraction coupling at the level of Ca^{2+} release (18) and/or by directly inhibiting actomyosin via S-nitrosylation of cysteine residues (5). Indirectly, however, NO causes an overall “slow-to-fast” shift characterized, in part, by increases in the rate of force development, maximal shortening velocity, and maximal power of both single muscle fibers and isolated muscles of animals (28). These indirect effects of NO are the result of activation of soluble guanylyl cyclase (sGC) and thus enhanced production of cyclic GMP (cGMP) (15,27). Whether NO similarly influences the contractile properties of human muscle, however, has not been firmly established.

In this context, the physiological effects of dietary nitrate (NO_3^-) are of considerable interest. It is now recognized that, rather than simply being metabolically inert or, worse, a potential carcinogen, NO_3^- in the diet is a significant source of NO in the body (cf. Refs. 22-26 for review). In fact, this dietary pathway, which entails the reduction of NO_3^- to nitrite (NO_2^-) by facultative anaerobic bacteria in the mouth followed by further reduction of NO_2^- to NO by, e.g., deoxyhemoglobin, can account for a significant fraction of whole-body NO production (cf. Refs. 32,37 for review). This dietary pathway therefore serves as an important “backup” system to the more well-known NO synthase (NOS) pathway. This is likely to be especially true during exercise, since unlike the NOS pathway the dietary pathway operates well at low pO2 and is stimulated rather than inhibited by low pH (cf. 22-26), conditions that regularly exist in contracting muscle.

Based on the above, we hypothesized that acute dietary NO_3^- intake would increase the maximal speed and power of human muscle. To test this hypothesis, we used isokinetic dynamometry to assess the contractile function of the knee extensor muscles in healthy volunteers following ingestion of beetroot juice (BRJ), a rich natural source of NO_3^-. A number of recent studies have assessed the effects of dietary NO_3^- intake on performance during dynamic exercise lasting several minutes or longer (cf. Ref. 13 for review), and Haider and Folland (9) recently determined its influence on the contractile function of the human quadriceps muscle during electrically-stimulated and voluntary isometric contractions. The present investigation, however, is the first to determine the effects of

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dietary NO_3^- intake on the force- (or torque-) velocity and hence power-velocity relationship of human muscle.

2. Materials and Methods

2.1 Subjects

The subjects in this investigation were 7 men and 5 women with mean (±SD) age and body mass index of 36±10 y and 26.1±4.1 kg/m^2, respectively. All were healthy, as determined by history, physical examination, and standard blood chemistries, and were without significant orthopedic limitations or other contraindications to strenuous exercise. Although all were normally active, only one of the subjects exercised (by jogging 5 d/wk) on a regular basis. None of the subjects smoked. Individuals taking phosphodiesterase inhibitors (e.g., Viagra) were excluded, as these can potentiate NO effects (38). Those taking proton pump inhibitors, antacids, or xanthine oxidase inhibitors were also excluded, as these can affect reduction of NO_3^- and NO_2^- to NO (25). Women who were pregnant or lactating were also excluded. The study protocol was approved by the Institutional Review Board at Washington University School of Medicine and each subject provided written, informed consent.

2.2 Experimental design and protocol

Subjects were studied on two occasions separated by a 1-2 wk washout period using a double-blind, placebo-controlled, randomized design. To minimize variation in baseline NO levels, subjects were instructed by a dietician to avoid high NO_3^- foods for 1 wk prior to intervention and throughout the study. They were educated to record their food intake beginning with the 1st day of the washout period and ending with the completion of the 2nd study day. These food records were reviewed for adherence to the diet. Finally, they were instructed to refrain from use of an antibacterial mouthwash, brushing their teeth, or chewing gum prior to testing on study days since these can block the conversion of NO_3^- to NO_2^- by bacteria in the oral cavity (7).

On the day of each study the subject reported to the Clinical Research Unit (CRU) in the morning after avoiding food, caffeine, or alcohol intake for the previous 12 h. The time of testing was held constant for a given subject to account for diurnal or circadian variations. The level of NO in the subject's breath (a biomarker of whole-body NO production (25,30,36)) was first measured using a portable electrochemical analyzer (NIOX MINO, Aerocrine Inc., Morrisville, NC) following the American Thoracic Society/European Respiratory Society guidelines (2). This portable analyzer has been repeatedly shown to provide results closely comparable to the heretofore 'gold standard' chemiluminescent approach (1,16,28). The subject then ingested 140 mL of a commercial BRJ supplement (Beet It Sport®, James White Drinks, Ipswich, UK) either containing or essentially devoid of 11.2 mmol of NO_3^-. The placebo, which is prepared by James White Drinks by extracting NO_3^- from BRJ using an ion exchange resin, is indistinguishable in packaging, color, taste, texture, and smell from the standard product, and does not alter plasma NO_3^-/NO_2^- concentrations (19,20) or breath NO levels (see Results). Additional breath NO measurements were made 60 and 120 min after BRJ ingestion, after which the subject

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performed a 6 min walk test in a hallway of the CRU. This test (data to be reported separately) was included to assess the effects of acute dietary NO_3^- intake on aerobic exercise performance and also to serve as a warm-up for the subsequent muscle function testing (see below). Approximately 10 min after completion of the muscle function testing, breath NO was measured for a final time, after which the subject was fed lunch then released from the CRU. The timing of all measurements was based on previous studies demonstrating that plasma NO_3^- levels (15,29) and breath NO concentrations (30,36) peak approximately 2 h after dietary NO_3^- intake.

2.3 Measurement of skeletal muscle contractile function

A Biodex 4 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) was used to measure each subject's maximal voluntary force (torque) production and hence power during knee extension exercise performed with their dominant leg at angular velocities of (in order) 0, 1.57, 3.14, 4.71, and 6.28 rad/s (0, 90, 180, 270, and 360 °/s). These velocities were specifically chosen to approximately span the ascending limb of the power-velocity relationship, thus aiding accurate calculation of maximal speed and power (see below) while minimizing errors due to the influence of acceleration, impact torque, etc. The chair back-chair seat angle was set to 85° and the dynamometer adjusted to place the axis of rotation of the lever arm adjacent to the lateral femoral epicondyle. To minimize extraneous movement, straps were placed across the torso, hips, and thigh, and subjects were not allowed to use the handholds. After determining the range of motion, the subject performed 3-4 knee extensions at each velocity, with the isometric testing being conducted at a knee angle of 1.22 rad (70°). Two minutes of rest was allowed after each group of contractions. Subsequently, the subject performed a 50 contraction fatigue test (at 3.14 rad/s) to determine whether dietary NO_3^- influences fatigue resistance during repetitive, maximal activation. Subjects were explicitly instructed to go "all out" (i.e., to not pace themselves) during this test, and strong verbal encouragement was provided throughout all portions of the isokinetic testing.

2.4 Data analyses

To eliminate artifacts, the isokinetic dynamometer data were windowed to isolate the isokinetic phase, filtered using a nine point weighted moving average, then smoothed as previously described (34). The highest torque generated at each velocity was multiplied by the velocity to determine the peak power at that velocity. The resulting power-velocity data were then fit with a 2nd order polynomial function (i.e., $y=ax^2+bx+c$) that was solved to determine the subject's maximal knee extensor velocity (Vmax; $=-b/a$) and power (Pmax; $=(4ac-b^2)/4a$) as described by Yamauchi et al. (40). Data from a representative subject are shown in Figure 1.

Data were analyzed using either paired t-tests or two-way ANOVA for repeated measures, as appropriate, and presented as mean±SE. Post-hoc testing was performed using the Šidák-Holms multiple comparison approach. A P value of <0.05 was considered significant. Effect sizes (ES) were calculated using Cohen's d. In addition, univariate regression was used to explore the relationships between changes in measured variables.

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3. Results

As intended, NO_3^- ingestion significantly (P<0.001; ES=0.85 to 1.53) increased NO bioavailability, as indicated by the amount of NO in breath (Fig. 2). There was no significant difference in maximal isometric torque (2.60±0.13 Nm/kg NO_3^- vs. 2.64±0.13 Nm/kg placebo; P=0.61; ES=-0.15) or in peak isokinetic torque or power at 1.57-4.17 rad/s (Table 1; ES=-0.21 to 0.15). However, a significant (P<0.05) trial-by-velocity interaction effect existed for peak torque, as dietary NO_3^- tended (P=0.12; ES=0.74) to increase peak torque at the highest angular velocity tested, i.e., at 6.28 rad/s. This difference at 6.28 rad/s was significant when data were expressed as a percentage of maximal isometric torque (P<0.01; ES=0.59), or as power instead of torque (P<0.01; ES=0.74). Vmax was therefore higher (P<0.05; ES=0.67) in the NO_3^- trial (Fig. 3, top panel), as was Pmax (P<0.05; ES=0.63) (Fig. 3, bottom panel). Nine of the subjects exhibited an increase in Vmax of, on average, 16±5%, whereas in the other three subjects Vmax was essentially unaltered (i.e., -1±1%). Similarly, Pmax increased by 9±3% in nine subjects, but was unchanged in the other three (i.e., -3±1%). However, there was no significant relationship between the absolute or relative increase in breath NO levels and the absolute or relative increase in peak torque/power at 6.28 rad/s or Vmax or Pmax. The strongest relationship was between the relative increase in breath NO and the relative increase in Pmax, where R^2=0.12 (P=0.21); all other R^2 values were lower. Finally, no significant differences were found in torque, power, work, or rate of fatigue development (i.e., work last ⅓:work first⅓) during the 50 contraction fatigue test performed at 3.14 rad/s (Table 2). ES ranged from -0.20 for work during the last ⅓ of the test to 0.32 for average peak power.

4. Discussion

This study was designed to test the hypothesis that by increasing NO availability, acute dietary NO_3^- intake would increase the maximal speed and power of human muscle. Consistent with this hypothesis, we found that NO_3^- ingestion significantly increased force/torque, and hence power, output during isokinetic knee extensions performed at a moderate-to-high angular velocity. This was associated with significant increases in the estimated maximal speed and power of knee extension. The present study is therefore the first to demonstrate that dietary NO_3^- enhances the speed and power of human muscle during voluntary exercise. Moreover, as NO_3^- ingestion increased NO availability in breath and hence presumably throughout the body, the current results support the hypothesis that NO influences the contractile properties of human muscle, as previously shown in animals (15,18,27,31). The present study therefore adds to the body of recent literature demonstrating that dietary NO_3^- intake can improve performance under other exercise conditions, in particular, during high-intensity dynamic exercise (e.g., cycling, running) lasting several minutes or longer (13).

As indicated above, ingestion of NO_3^- increased estimated maximal knee extensor velocity and power by, on average, 11 and 6%, respectively. Although relatively small, these changes are in line with the effects of NO on the speed and power of animal muscle (27,31). More importantly, however, improvements of this magnitude could be of considerable practical significance in both power-based sports and in clinical populations. For an elite athlete, for

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example, even a 1% increase in performance ability could double their chances of winning a particular event (11). Future studies should therefore determine whether NO_3^- ingestion enhances maximal speed and/or power during multi-muscle/multi-joint exercise (e.g., cycling), more typical of athletic competitions. Similarly, it is important to determine whether dietary NO_3^- can improve muscle speed and/or power in, e.g., the elderly, patients with heart failure, etc., in whom muscle function is reduced (17,34,35).

The improved muscular performance in the NO_3^- trial was accompanied by increased production of NO, as indicated by the increase in breath NO levels. The latter was presumably responsible for the former, as the only difference between treatments was provision of NO_3^-, which must be reduced to NO_2^- and then to NO to exert its biological effects. Beyond this, however, the specific mechanisms involved cannot be determined from the present data. Based on animal studies, the most likely explanation is that the NO_3^--induced increase in NO led to activation of sGC and hence an increase in cGMP and subsequent phosphorylation of myosin (27). Other, more direct effects of NO (e.g., nitrosation or S-nitrosylation of various proteins) seem unlikely, as these generally inhibit rather than enhance muscle function (28). Somewhat along the same lines, previous studies of aerobic exercise have implicated dietary NO_3^--induced changes in ATP demand (3) and/or mitochondrial coupling (21) as being responsible for improvements in performance under such conditions. Logically, however, there is no reason to expect such alterations in energy supply/demand to improve speed or force of muscle during a single contraction. Furthermore, we found no improvement in performance during more sustained exercise, i.e., the 50 contraction (~1 min) fatigue test, implying that ingestion of NO_3^- did not alter muscle energetics in the present subjects. This may have been the result of the acute nature of the current investigation, as most prior studies showing a positive effect of dietary NO_3^- supplementation on performance during high-intensity exercise have utilized 3-6 d of treatment (13).

Notably, not all of the subjects in the present study exhibited an increase in muscle function following dietary NO_3^- intake. This is consistent with previous reports that there are responders and non-responders to NO_3^- ingestion in terms of changes in blood pressure (10) or endurance exercise performance (39). Nonetheless, why some of the present subjects did not benefit is not clear. It does not appear to be due to a lack of conversion of NO_3^- to NO_2^- and then NO, as breath NO rose similarly in these three individuals vs. the other nine (i.e., +19±8 vs. +13±5 ppm; P=0.98; ES=0.46). There was also no correlation between the magnitude of the change in breath NO and the magnitude of the change in muscle speed and/or power. It is possible, however, that the magnitude of the changes in breath NO were not directly reflective of the magnitude of changes/lack-of-changes in NO (or more importantly/directly, cGMP) within muscle itself. Since the biological effects of NO are most prominent in fast-twitch, or type II, muscle fibers (27), another possibility is that these three subjects had a low percentage of this fiber type, and thus responded less/not at all to dietary NO_3^- supplementation. Additional studies will be required to determine why NO_3^- intake does not alter muscle contractile properties in all individuals, perhaps by examining genetic differences in endogenous NO production (10).

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As in previous studies (e.g., 14,36), the subjects in the present investigation were studied after a short (i.e., 7 d) period of controlled dietary NO_3^- intake. This intervention was apparently successful in minimizing the potentially confounding effect of day-to-day variations in NO bioavailability, as indicated by a high correlation (i.e., R^2=0.87; P<0.001) in individual baseline breath NO levels between the placebo and NO_3^- trials. While in theory this diet may have led to a reduction in NO_3^- to below-normal levels, Jungersten et al. (14) and Miller et al. (29) previously found that variations in dietary NO_3^- intake within the usual range of 0-2.5 mmol/d had limited influence on plasma NO_3^- levels; marked changes were observed only after acute ingestion of a much larger dose (i.e., 5-11 mmol). Nonetheless, it is possible that the effects of dietary NO_3^- intake observed in the present were magnified as a result of this aspect of the experimental design. Even if this was true, however, it would not undermine our primary conclusion, which is that dietary NO_3^- intake influences the speed and power of human muscle.

While this study was in progress, Haider and Folland (9) reported that 7 d of dietary NO_3^- intake enhanced the rate of force development and peak force output of the human quadriceps muscle during electrically-stimulated isometric contractions. These effects were absent, however, during voluntary isometric contractions. Along with Fulford et al. (6), we also found no influence of dietary NO_3^- during voluntary isometric exercise. The reason for this apparent difference between electrically-evoked and voluntary isometric contractions is not immediately clear. It may, however, be related to differences in the pattern of motor unit recruitment. Specifically, percutaneous electrical stimulation of a motor nerve inverts the normal orderly recruitment of motor units, preferentially depolarizing larger alpha motor neurons innervating type II muscle fibers (cf. Ref. 8 for review). As indicated above, at least in animals the effects of NO on muscle are greatest in such fibers; thus, electrical stimulation as used by Haider and Folland (9) might favor finding a positive effect. In contrast, during a voluntary isometric contraction both slow-twitch, or type I, as well as type II fibers would be recruited, thus potentially "diluting" the effects of NO produced via the $NO_3^- \rightarrow NO_2^- \rightarrow$ NO pathway. A greater impact of NO_3^--derived NO in type II vs. type I fibers would also explain why in the present study dietary NO_3^- intake only improved muscle function at the highest angular velocity tested, since under these conditions the relative contribution of type II fibers to force/torque development would be greatest (4,12,33).

There are several limitations to the present study. We did not measure changes in plasma NO_3^- or NO_2^-, and therefore cannot determine whether interindividual differences in NO_3^- absorption and/or reduction to NO_2^- accounted for the lack of improvement in muscle function in some subjects. We did, however, measure the ultimate effector molecule, i.e., NO, albeit only in breath. We also did not obtain muscle biopsies, and hence cannot determine whether the improvements in muscle contractile performance that were observed were the result of an increase in cGMP production or the result of some other mechanism. Such data are not required, however, to conclude that dietary NO_3^- intake increases the speed and power of human muscle. We only studied the effects of a single (acute) dose of NO_3^- in subjects who were consuming a low NO_3^- diet. We therefore cannot draw any conclusions about the effects of a more prolonged period of supplementation, and as stated previously it possible that the differences observed were magnified as a result of the dietary

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intervention. Finally, the effects of NO_3^- ingestion on muscle function were assessed only during unilateral knee extensor exercise, and were evident only at a moderate-to-high angular velocity (i.e., ~½½ Vmax). As stated above, it therefore remains to be determined whether NO_3^- intake results in comparable improvements in muscle speed and/or power during other forms of exercise.

In summary, the present study is the first to demonstrate that dietary NO_3^- increases the maximal speed and power of human muscle during voluntary exercise, apparently by increasing NO availability. The magnitudes of these improvements, although relatively small, are such that they may be relevant to both athletic and clinical populations. Additional, larger-scale studies are required to test this hypothesis, as well to determine the precise mechanisms involved.

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Highlights

- Acute dietary NO_3^- intake increased NO availability (breath NO) by 61% ($P<0.001$; effect size=1.19)
- Maximal velocity of knee extension increased by 11% ($P<0.05$; effect size=0.67)
- Maximal knee extensor power also increased by 6% ($P<0.05$; effect size=0.63)
- Performance during a 50 contraction fatigue test did not differ between placebo and nitrate trials
- Dietary NO_3^- increases muscle speed and power in healthy men and women

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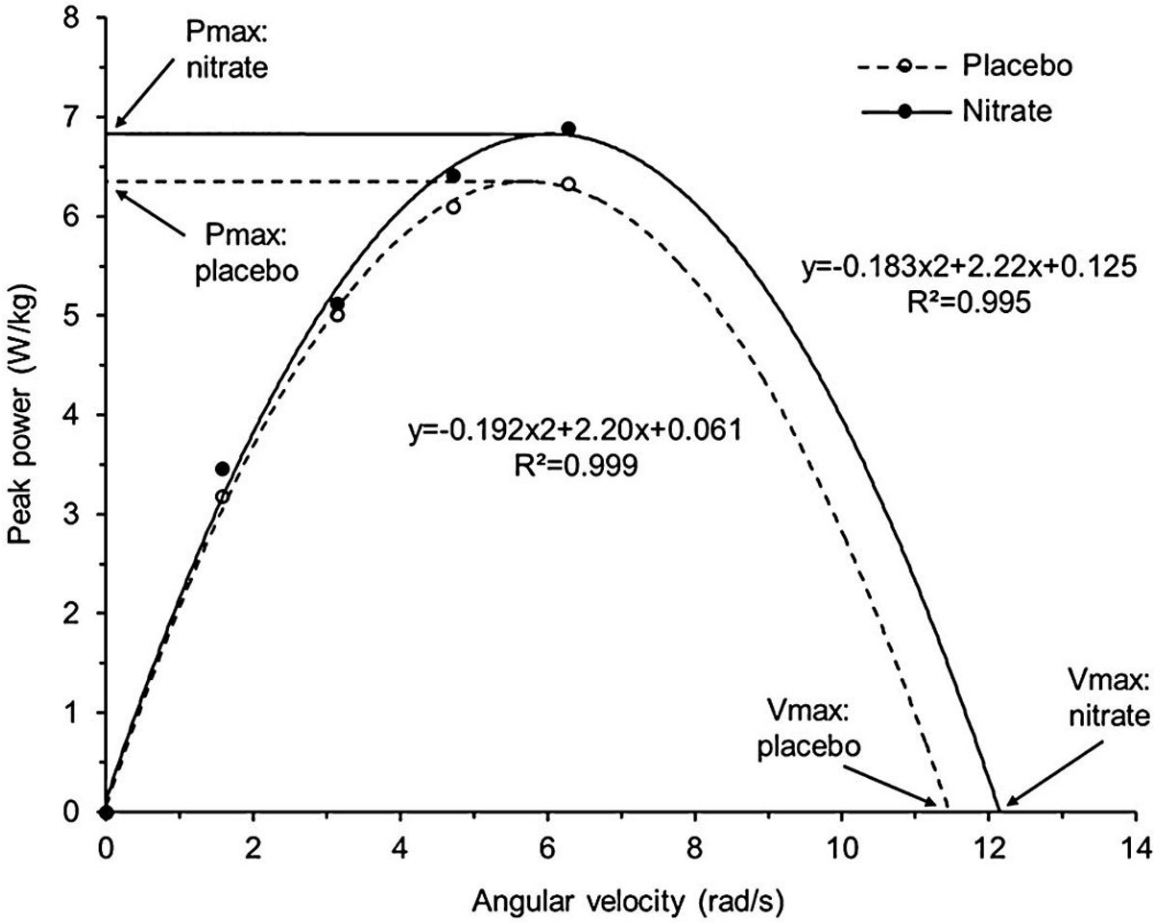

Figure 1.

Calculation of maximal knee extensor velocity (Vmax) and power (Pmax) for a representative subject. *Open circles and dashed line*, placebo trial. *Closed circles and solid line*, NO_3^- trial.

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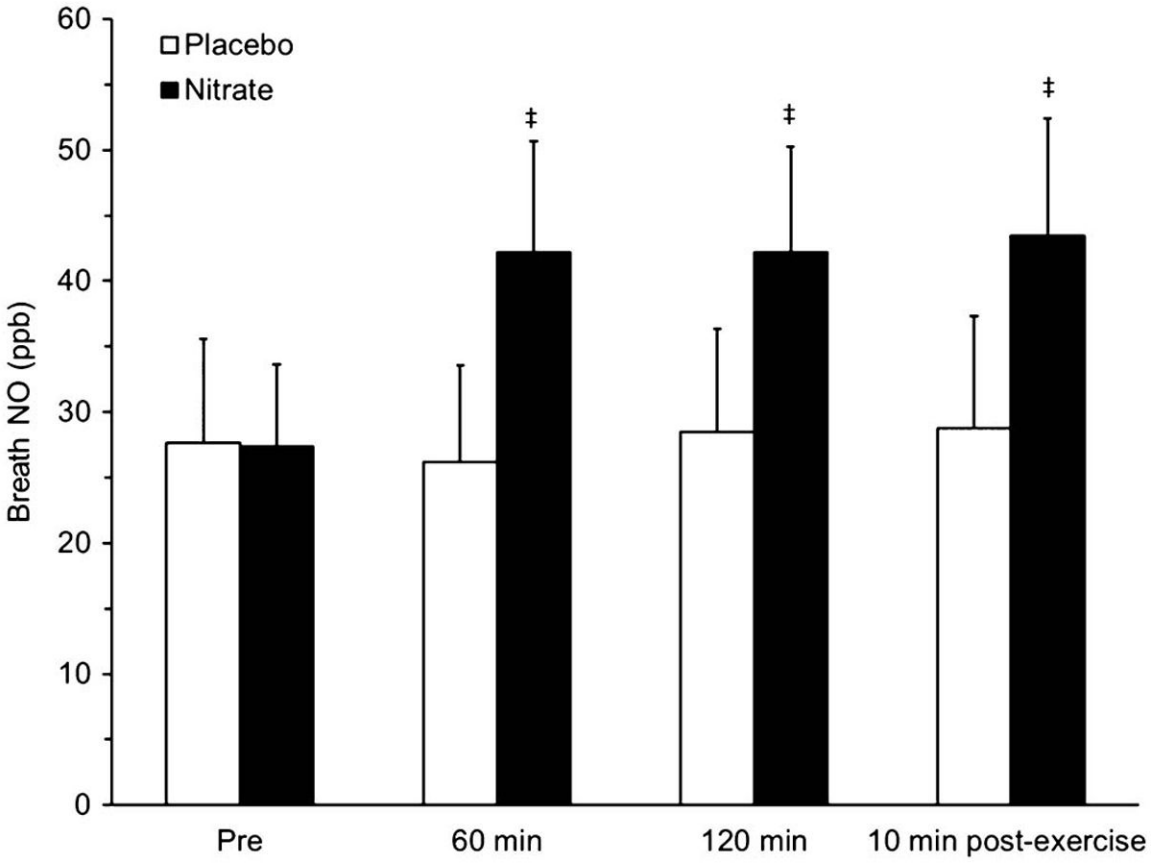

Figure 2.

Effect of acute dietary NO_3^- intake on breath NO levels. *Open bars*, placebo trial. *Closed bars*, NO_3^- trial. Values are mean ± S.E. for n=12. NO_3^- trial significantly higher than placebo trial at same time point: ‡P<0.001.

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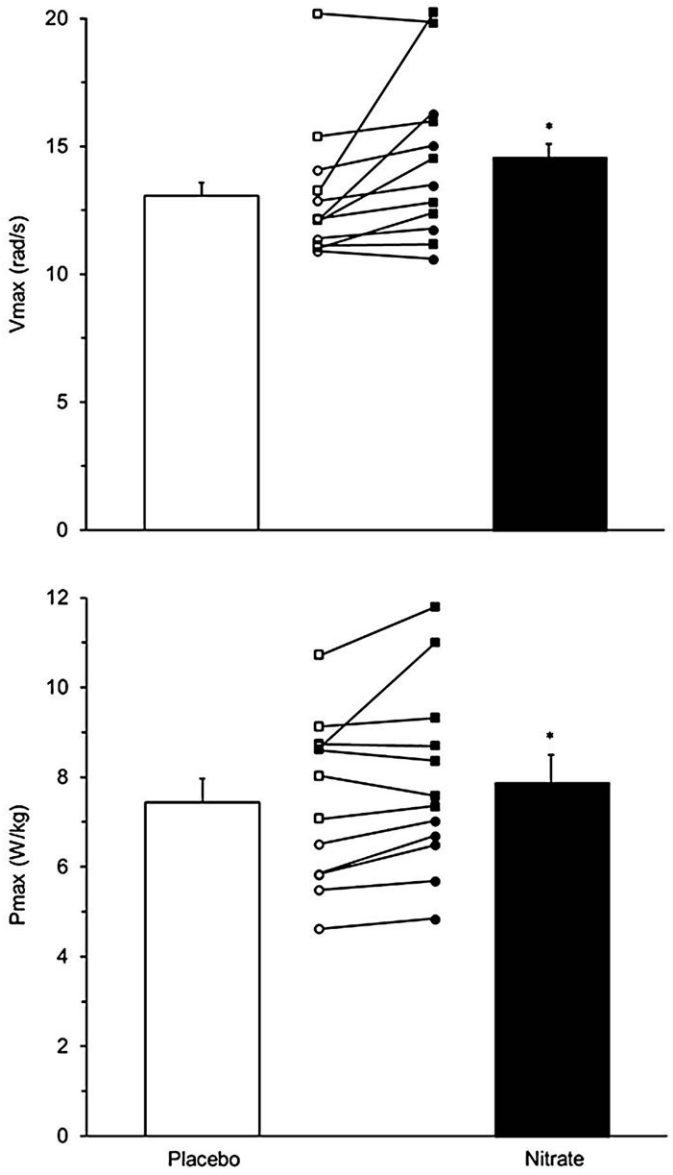

Figure 3.

Effect of acute dietary NO_3^- intake on estimated maximal knee extensor velocity (Vmax; top panel) and power velocity (Pmax; bottom panel). The *open bars* and *closed bars* indicate the mean (± S.E.) values for the placebo and NO_3^- trials, respectively, whereas the *squares* and *circles* illustrate the responses of individual male and female subjects, respectively. NO_3^- trial significantly higher than placebo trial: *P<0.05.

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Table 1

Effect of acute dietary NO_3^- intake on knee extensor torque and power

		Angular velocity (rad/s)			
		1.57	**3.14**	**4.17**	**6.28**
Peak torque (Nm/kg)	Placebo	2.15±0.11	1.74±0.12	1.42±0.11	1.17±0.08
	NO_3^-	2.11±0.10	1.71±0.10	1.42±0.10	1.22±0.08
Peak torque (% of isometric)	Placebo	81.9±2.7	66.6±3.8	54.1±3.5	44.3±1.9
	NO_3^-	81.6±2.4	66.6±3.4	55.0±3.2	46.8†±2.1
Peak power (W/kg)	Placebo	3.38±0.21	5.48±0.38	6.67±0.50	7.34±0.54
	NO_3^-	3.31±0.16	5.38±0.32	6.67±0.46	7.64†±0.52

Values are mean ± S.E. for n=12. NO_3^- trial significantly higher than placebo trial:

†P<0.01

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Table 2

Results of 50 contraction fatigue test at 3.14 rad/s

	Peak torque (Nm/kg)		Peak power (W/kg)		Total work (J/kg)			
	Highest	Average	Highest	Average	First third	Last third	Ratio	Total
Placebo	1.66±0.10	0.96±0.07	2.61± 0.16	1.36±0.11	26.4±1.3	9.2±1.1	0.35±0.03	51.4±3.4
NO_3^-	1.67±0.10	0.97±0.05	2.63±0.15	1.42±0.09	26.3±1.5	8.9±1.0	0.34±0.03	51.0±3.3

Values are mean ± S.E. for n=12.