Nitrates, Nitrites, Nitric Oxide and Exercise Performance

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Introduction

An increasing amount of research is focusing on lifestyle behaviors for health improvement, particularly the prevention of various chronic diseases such as coronary heart disease, cancer, and diabetes. Two key lifestyle behaviors involved in preventive health are healthy dietary practices and a proper exercise program. In a similar fashion, given the importance of sport in today’s society, considerable research effort is devoted to sport performance enhancement. Again, proper dietary and exercise practices are two of the key factors that may underlie improvement in sport performance.

Diet and exercise training may enhance both health and sport performance in a variety of ways. For example, healthy diets may contain natural substances, such as omega-3 fatty acids, antioxidants, and various phytonutrients that may help prevent some disease processes (Williams, 2010), while exercise may produce various cytokines (myokines) that may reduce many of the traditional risk factors associated with development of chronic diseases (Brandt and Pedersen, 2010).

One factor common to both diet and exercise training that may have favorable effects on both health and sport performance involves byproducts of nitrogen metabolism, particularly the role of dietary nitrates and exercise training in the formation of nitric oxide in the body.

Nitrogen, Nitrates and Nitrites

Nitrogen (N₂), as a gas, is all around us, constituting about 79 percent of atmospheric gases. Nitrogen is an inert gas, but nitrogen-fixing bacteria in the earth’s soil and in the roots of plants can convert nitrogen into nitrate (NO₃⁻) and ammonium (NH₄⁺). Lightning flashes also may convert nitrogen to nitrate and nitrite, which are stored in soil. Additionally, agricultural industries can also convert nitrogen to fertilizer, which contains nitrate and ammonium to enrich soil. Nitrates may leach from soil to lakes and rivers, which may be sources of drinking water (Provin and Hossner, 2001). As plants develop, they store nitrogen as nitrates. Plants also store nitrogen as amino acids, which are produced in plants from nitrogen-containing sources.

Nitrogen is an essential element for humans. For example, all amino acids necessary for protein formation, which underlies body structure and function, contain nitrogen, as does the DNA in our genes. Humans obtain nitrogen from various sources, including nitrates found in plants and drinking water and amino acids in plants and animal products. Considerable research
has focused on the positive health or performance-enhancing effects of various amino acids. Other nitrogen byproducts, particularly nitrates and nitrites (NO$_2$), have also been studied for similar purposes.

As noted, nitrates are natural inorganic components of plant foods. Hord and others (2009) note that approximately 80 percent of human dietary nitrate intake is derived from vegetable consumption, but also note that the total dietary nitrate intake is determined by the type of vegetables consumed, the levels of nitrate in the vegetables, and the amount of vegetables consumed. Table 1 provides a classification of vegetables based on nitrate content, given in milligrams per 100 grams (3.5 ounces) food weight. Other sources of nitrate in the human diet include sodium nitrate as a preservative in processed meats and varying amounts in drinking water.

Table 1. Classification of vegetables according to nitrate content

<table>
<thead>
<tr>
<th>Nitrate Content*</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low (&lt; 20 mg/100 g)</td>
<td>Artichoke; asparagus; garlic; onion; mushroom; pea; pepper; potato; sweet potato; tomato</td>
</tr>
<tr>
<td>Low (20-50 mg/100 g)</td>
<td>Broccoli; carrot; cauliflower; cucumber; pumpkin; chicory</td>
</tr>
<tr>
<td>Middle (50-100 mg/100g)</td>
<td>Cabbage; dill; turnip; Savoy cabbage</td>
</tr>
<tr>
<td>High (100-250 mg/100 g)</td>
<td>Celeriac (celery root); Chinese cabbage; endive; fennel; kohlrabi; leek; parsley</td>
</tr>
<tr>
<td>Very High (&gt; 250 mg/100 g)</td>
<td>Celery; cress; chervil; lettuce; red beetroot; spinach; rucola (arugula)</td>
</tr>
</tbody>
</table>

*Nitrate content in milligrams per 100 grams of fresh weight

Nitrites (NO$_2$) are also found naturally in plant foods, but to a much lesser degree than nitrates, usually much less than a milligram per 100 grams of fresh food. However, nitrite salts, such as sodium nitrite (NaNO$_2$) are added as preservatives in various foods, particularly processed meats such as bacon, ham, and hot dogs. Fresh meat contains no nitrites. For a detailed discussion regarding the nitrate and nitrite content in foods, please see the review by Hord and others (2009).

In nature, nitrates are readily converted to nitrites and vice versa. (Argonne, 2005). In the human body, one of the functions of nitrates and nitrites is the formation of the gas, nitric oxide.

Nitric Oxide

Nitric oxide (NO), or nitrogen monoxide, is an important functional molecule in human physiology. It functions as a signal transmitter between body cells and may be produced in
various parts of the body, including the blood vessels, heart, skeletal and other tissues. One major mechanism in the formation of nitric oxide is the metabolism of the amino acid L-arginine, and possibly other amino acids, by nitric oxide synthase (NOS) enzymes (Bescós et al., 2012). Nitric oxide may also be formed from other sources, such as via the drugs nitroglycerin and amyl nitrite.

Investigators have discovered that dietary nitrate and nitrite also can serve as a source for the production of a diverse group of nitrogen metabolites, including nitric oxide, via nitrate/nitrite reductases in tissues (Hord, 2011). Inorganic nitrate from dietary sources is converted in vivo to form nitrite, which along with dietary and other sources of nitrite, is reduced in vivo into nitric oxide and other bioactive nitrogen oxides (Hord et al, 2009; Carlström et al., 2011). In brief, after ingestion nitrate is rapidly absorbed in the upper gastrointestinal tract, circulates to the salivary glands where it is extracted, secreted in saliva into the mouth and converted to nitrite by bacteria; swallowed nitrite enters the systemic circulation which then can be further reduced in blood vessels, heart, skeletal and other tissues to form bioactive NO (Larsen et al., 2010).

Nitric oxide may affect various physiological functions important to health and exercise performance. In particular, nitric oxide is a potent vasodilator. Stamler and Meissner (2001) indicated nitric oxide also regulate several skeletal muscle functions, such as force production, blood flow, mitochondrial respiration, and glucose homeostasis. Nitric oxide is rapidly oxidized to form nitrite and nitrate and thus its direct detection in biological systems is difficult. Venous plasma nitrite concentration has been shown to be a marker of forearm NO production (Allen et al., 2005). Using such methodology, nitric oxide has been studied for its potential positive health effects over the course of the past three decades, and more recently for its potential effect on exercise performance.

**Health Effects of Dietary Nitrates and Nitrites**

There appears to be some controversy regarding the health effects of dietary nitrates and nitrites. Some evidence suggests that they may be harmful to health. Thus, some government regulations may regulate the amount found in food and water. On the other hand, some evidence suggests that they may be beneficial to health, and may underlie the rationale for proposed healthful diet plans.

**Possible adverse health effects**

In its Human Health Fact Sheet, the Argonne National Laboratory (2005) indicated that nitrates, a normal component of the human diet, by themselves are relatively nontoxic. However, after ingestion most nitrate is converted into nitrite, which may pose some health concerns. The stomachs of infants may convert more nitrate to nitrite, which may react with hemoglobin in the blood and convert it to methemoglobin. Methemoglobin cannot bind with oxygen, which may lead to a condition known as methemoglobinemia. An early sign of nitrite toxicity is a bluish tinge to the skin and lips, the so called *blue baby*, and increasing levels of methemoglobin can lead to weakness, loss of consciousness, coma, and death. All deaths from
nitrate/nitrate poisoning have been in infants, mainly associated with contaminated water used to prepare baby formula (Argonne National Laboratory, 2005).

Nitrites in the stomach may also react with food proteins to form N-nitroso compounds, or nitrosamines. In particular, nitrosamines are formed when processed meats, which may be rich sources of added nitrates and nitrites, are cooked, especially with high heat. Nitrosamines have been found to be carcinogenic in animals, particularly stomach cancer, but evidence is inconclusive relative to their potential to cause cancer in humans (Argonne National Laboratory, 2005; Gilchrist et al., 2010).

Various governmental groups have developed toxicity values for dietary nitrate and nitrite intake, including water and food supplies, and particularly for food additives in processed meat and fish. Such groups include the U.S. Environmental Protection Agency (EPA), the U. S. Food and Drug Administration (FDA), the U. S. Department of Agriculture (USDA), the European Union (EU), and the World Health Organization (WHO). For example, the WHO has set the Acceptable Daily Intake (ADI) for nitrate at 3.7 mg/kg body weight and for the nitrite ion at 0.06 mg/kg body weight (Hord, et al, 2009).

The putative adverse effects of high dietary nitrate intake have been questioned by some scientists. Hord and others (2009) noted that although toxic exposures of nitrates and nitrites have occurred, the health risks appear only in specific subgroups of the population, particularly infants. In a recent review, Hord (2011) noted that current regulatory limits on nitrate intakes, based on concerns regarding potential risk of carcinogenicity and methemoglobinemia, are exceeded by normal daily intakes of single foods, such as spinach, as well as various healthful diet plans. He issued a call for regulatory bodies to consider all available data on the beneficial physiologic roles of nitrate and nitrite in order to derive rational bases for dietary recommendations.

**Possible beneficial health effects**

Rather than contributing to adverse health effects, many scientists contend that dietary nitrate and nitrite, when converted to nitric oxide, may exert some beneficial health effects, such as prevention of infection, protection of our stomach, and prevention of vascular disease (Gilchrist et al., 2010) and may serve as essential nutrients for optimal cardiovascular health (Bryan et al., 2007).

Most health-related research with nitrate supplementation, either sodium nitrate or dietary sources of nitrate, has focused on vascular aspects. Research has shown that the Dietary Approaches to Stop Hypertension (DASH) diet, which may be rich in vegetables and nitrates, is an effective means to lower blood pressure (Frisoli et al., 2011). However, the mechanism underlying this effect could be associated with other aspects of such a diet, such as a high potassium content. In their study to help identify an underlying mechanism, Larsen and others (2006) found that sodium nitrate supplementation, in amounts equivalent to 150 to 250 grams of nitrate-rich vegetables as found in the DASH diet, significantly reduced diastolic blood pressure in young, normotensive subjects. They concluded the reduced blood pressure was
associated with nitrate supplementation alone and was similar to that seen in DASH studies. Dietary nitrate content may also underlie the purported health benefits of the Mediterranean diet. Although the vasodilation effect of dietary nitrate, by increasing nitric oxide, is thought to underlie the reduction in blood pressure, Larsen and others (2006) note that the exact mechanism behind the blood pressure–lowering effect of nitrate needs to be clarified in future studies.

**Exercise Training and Nitric Oxide**

One effect of exercise training may lead to an increase in nitric oxide production, an effect that may be related to improvement in both health and exercise performance.

**Health aspects**

Proper exercise training is associated with many resultant health benefits, particularly prevention of diseases of the cardiovascular system. One such health benefit is a reduction in blood pressure; high blood pressure is one of the primary risk factors for coronary heart disease. Scientific reviews have shown reduced blood pressure following either aerobic (Kelley and Kelley, 2008) or dynamic resistance (Cornelissen et al., 2011) exercise training.

One possible mechanism underlying this blood pressure-lowering effect is an exercise-induced increased production and activity of nitric oxide. For example, several comparative epidemiological studies have shown that endurance athletes, including marathon runners, had higher nitric oxide production and basal levels compared to sedentary subjects (Rodriguez-Plaza et al., 1997; Vassalle et al., 2003). Several experimental studies have shown that both mild aerobic-endurance exercise training and short-term resistance training may increase NO production in previously healthy, sedentary older humans, which the investigators suggest may have antihypertensive effects and beneficial effects on the cardiovascular system (Maeda et al., 2006; Maeda et al., 2004). Nitric oxide production decreases with aging, which may be one factor underlying the increased risk of cardiovascular disease in the elderly. Calvert indicated that exercise can increase activity of endothelial nitric oxide synthase resulting in an increase in nitric oxide levels (Calvert, 2011) and also noted that although it is still unclear how exercise protects the heart, it is apparent that endothelial nitric oxide plays a role. (Calvert et al., 2011).

**Exercise performance aspects**

Proper exercise training is essential to enhancement of sport performance, and numerous associated physiological, psychological, and biomechanical mechanisms may underlie such improvement. One such physiological mechanism may be an increase in nitric oxide.

Research indicates that exercise increases nitric oxide production. Studies have shown that eight weeks of mild aerobic exercise training could increase the plasma markers of nitric oxide production in both younger and older individuals, but these levels decreased to the base levels following eight weeks of detraining (Maeda et al., 2004; Maeda et al., 2001; Wang, 2005). Short-term resistance training also has been shown to increase nitric oxide production in healthy older men (Maeda et al., 2006).
Some investigators theorize that nitric oxide may be a major factor supporting improvement in exercise performance (Gilchrist et al., 2010). Increased markers of nitric oxide support an effect to promote vasodilation and increase blood supply to exercising muscle, improving exercise performance in patients with peripheral arterial disease (PAD), a condition in which a failure to adequately supply blood and oxygen to active muscles presents as claudication pain during simple exercise tasks, such as walking (Allen et al., 2010; Kenjale et al., 2011). Other studies with healthy subjects reported (1) that increases in markers of nitric oxide synthesis during exercise were positively correlated with exercise performance and that an impaired increase in plasma nitrite may limit exercise capacity (Rassaf et al., 2007), (2) that there is a favorable effect of plasma nitrite concentration on high-intensity endurance exercise (Dreissigacker et al., 2010), and (3) that subjects who could exercise hardest in a treadmill VO₂peak test also produced the most nitric oxide (Allen et al., 2005). Although a vigorous exercise training program may be a very effective means to increase nitric oxide production, some athletes may attempt similar increases via other means in attempts to go beyond training and obtain a competitive edge.

**Supplementation Protocols to Enhance Nitric Oxide Production and Exercise Performance**

As noted below, numerous studies have evaluated the effects of various means in attempts to increase nitric oxide production and concomitantly, enhance exercise or sports performance. Most studies cited have used well designed experimental methods, including appropriate dosages and double-blind, placebo, crossover protocols.

Given the potential performance-enhancement effects of nitric oxide, an increase in its production during sports competition could be beneficial to many athletes. Although the role of nitric oxide was unknown at the time, various reports indicate extensive use of ergogenic substances by athletes, including the nitric oxide producing drug nitroglycerin, in the late 19th century (Ferro, 2007; Mayes, 2010). Fast forward to the 21st century in which recent reports indicate nitric oxide dietary supplements are popular within the sport and bodybuilding communities (Bloomer et al., 2011; Bloomer et al., 2010). Maughan and others (2011) recently noted that the use of both nitrate and arginine is growing.

Various substances have been used to increase production of nitric oxide in humans. Drugs such as nitroglycerin and amyl nitrate are potent vasodilators via nitric oxide production. Although such drugs may be purchased on the Internet, their use may pose some serious health threats and will not be discussed relative to their ergogenic potential. Inhalation of nitrogen gas preparations may also increase nitric oxide production, but such use also will not be discussed. Inorganic nitrate and nitrite salts may also increase nitric oxide levels. Both salts may be used as food additives and both can be classified as either a drug or a food depending on how they are administered (Allen, 2011). Several studies have used sodium nitrate to evaluate the effect of nitric oxide on exercise performance, and the results will be presented below. However, as noted in the following section dealing with cautions in using nitric oxide-producing agents, use of such salts is not recommended. Dietary supplements, particularly L-arginine, and...
food sources of nitrates have also been studied as a means to increase nitric oxide and enhance exercise performance, and such protocols constitute the majority of current research studies.

**Nitrate Salts**

Nitrate salt supplementation has been studied for its ergogenic potential, as have some new salt preparations marketed as dietary supplements. In one study, cyclists consumed sodium nitrate (10 mg/kg body weight) prior to an ergometer test consisting of four 6-minute submaximal workloads with increasing intensity and then, after a short rest period, an incremental exercise test until exhaustion. The supplement increased plasma nitrate and nitrite, but significantly reduced peak oxygen uptake and the ratio between oxygen consumption and power at maximal intensity. This reduction of oxygen consumption occurred without changes in the time to exhaustion (Bescós et al., 2011). In another study, subjects received dietary supplementation with sodium nitrate for two days before undertaking maximal exercise tests, consisting of an incremental exercise to exhaustion with combined arm and leg cranking on two separate ergometers. Similar to the preceding study, supplementation was associated with a decrease in maximal oxygen uptake with a trend towards an increase in exercise time to exhaustion (Larsen et al., 2010). As noted in a following discussion, the findings of these two studies may be associated with enhanced performance.

**L-arginine supplements**

As noted previously, L-arginine and other amino acids may serve as substrate for the production of nitric oxide in the body. Most dietary supplements promoting nitric acid production contain L-arginine (Bloomer et al., 2010). Citrulline, another amino acid, is taken up by the kidney and metabolized to generate arginine. Hickner and others (2006) noted that citrulline supplementation increases plasma arginine levels to a higher level than arginine supplementation.

Positive effect on performance In an earlier review, Cheng and Baldwin (2001) reported that oral arginine supplementation in several small studies has been shown to improve exercise ability in coronary heart disease patients, but noted that larger, well-designed studies are required to confirm its effect before therapy can be routinely recommended. More recent studies have shown that L-arginine supplementation could enhance exercise performance in patients with chronic stable heart failure (Doutreleau et al., 2006) and in patients following a heart transplant (Doutreleau et al., 2010).

Research findings are scant regarding improved exercise performance in healthy subjects. Bailey and others (2010A) reported that L-arginine supplementation (6 grams) one hour prior to a series of moderate- and severe-intensity cycling exercise bouts reduced oxygen uptake and increased the time to exhaustion in the severe-intensity exercise tests. They concluded that L-arginine supplementation elicited positive effects on exercise performance similar to those associated with dietary nitrate supplementation, as discussed below.
**No effect on performance** Most studies report no ergogenic effect of L-arginine supplementation on various tests of aerobic endurance, anaerobic performance, or resistance exercise in patients and healthy subjects.

Relative to aerobic exercise, Wilson and others (2007) found that long term L-arginine supplementation (3 grams daily of 6 months) did not improve treadmill NO production or walking performance in patients with peripheral arterial disease. McConell and others (2006) actually infused arginine into endurance-trained cyclists during a cycling exercise protocol, and reported no effect in a 15-minute maximal cycling time trial following two hours of cycling at 72 percent of maximal oxygen uptake. In another endurance cycling study, Abel and others (2005) reported that arginine aspartate supplementation had no effect on cycling endurance to exhaustion.

Several studies have found no effect on tests of anaerobic exercise performance. Olek and others (2010) studied the effect of a single 2-gram dose of arginine prior to three 30-second all-out supramaximal Wingate Anaerobic Tests, but found no improvement in exercise performance compared to the placebo trial. Liu and others (2009) evaluated the effect of L-arginine, 6 grams daily for 3 days, on performance by well-trained male judo athletes in an intermittent cycle ergometer anaerobic exercise test. Although plasma L-arginine levels increased, there was no effect on plasma nitrate or nitrite or on peak and average power in the exercise test.

Studies also have reported no ergogenic effect of L-arginine supplementation on resistance exercise tests. Altars and others (2012) evaluated the acute effects of L-arginine supplementation (6 grams) consumed 80 minutes before tests of biceps strength performance. Although muscle blood flow increased to the exercising muscles, there was no effect of the supplement on nitric oxide or various measures of strength performance, such as peak torque and total work.

The majority of studies indicate that L-arginine supplementation does not enhance exercise performance, and the major reason may be that while L-arginine supplementation may increase plasma levels of L-arginine, supplementation has rather consistently been shown to not increase nitric oxide or blood flow to the exercising muscle (Bescós et al., 2009; Tang et al., 2011; Willoughby et al., 2011).

**Negative effect on performance** Some studies even suggest that L-arginine or citrulline supplementation may impair endurance exercise performance. Buchman and others (1999) provided arginine or a placebo to marathon runners and speculated arginine may be ergolytic as the predicted times of the runners receiving arginine were slower than those receiving the placebo. Hickner and others (2006) reported that citrulline supplementation had no effect on treadmill run time to exhaustion, and results of their study suggested that the supplement actually impaired run time to exhaustion.

**Dietary sources of nitrate**
As noted previously, various vegetables may be excellent sources of dietary nitrate. In particular, beetroot juice has been studied for its performance-enhancing potential. Beetroot is the term used in England for the vegetable we know in the United States as the red beet. Doses used in studies approximate 300-500 milligrams of nitrate, an amount found in about 500 milliliters of beetroot juice, and there is no evidence that higher amounts are more effective (Lundberg et al., 2011). Doses used in studies may be expressed as milligrams or millimoles. One millimole of nitrate is the equivalent of 62 milligrams, so 5-8 millimoles would be the approximate equivalent of 300-500 milligrams of nitrate. In several studies, nitrate-depleted beetroot juice was used as the placebo.

Various study protocols have been used to evaluate the ergogenic effect of nitrate supplementation, including acute (several hours) and chronic (several days) supplementation time frames before testing, varying dosages, multiple dependent variables, varying levels of exercise intensity, and specific exercise tasks.

**Increase of nitric oxide** Numerous studies have shown that dietary nitrate supplementation, usually in the form of beetroot juice, increases plasma nitrite concentration, a marker for nitric oxide (Bailey et al., 2009; Lansley et al., 2011A; Lansley et al., 2011B; Vanhatalo et al., 2010). Such increases were noted after both acute and chronic supplementation.

**Reduced oxygen cost of exercise** One of the most consistent findings from studies is a reduced oxygen cost of exercise or increased oxygen efficiency following either acute or chronic dietary nitrate supplementation. Relative to acute supplementation, Kenjale and others (2011) reported that beetroot supplementation three hours prior to testing reduced the fractional oxygen extraction of the gastrocnemius muscle during submaximal walking in patients with peripheral arterial disease. Vanhatalo and others (2010) reported significant reductions, about 4 percent, in the oxygen cost of moderate-intensity cycling exercise following both acute (2.5 hours prior to testing) and chronic (daily for 5 and 15 days) supplementation. These investigators concluded that dietary nitrate supplementation acutely reduces the oxygen cost of submaximal exercise and that these effects are maintained for at least 15 days if supplementation is continued. Other studies support similar effects with chronic supplementation of beetroot juice. Lansley and others (2011B) reported a reduced oxygen cost of treadmill walking, moderate-intensity running, and severe-intensity running following 4-5 days of supplementation. Cermak and others (2012) reported a significant reduction in oxygen consumption in cyclists during 60 minutes of submaximal cycling following 6 days of supplementation, and in two studies Bailey and others (2010B; 2009) reported a reduction in the oxygen cost of low-, moderate-, and high-intensity exercise, involving either cycling or knee extension exercise, following 4-6 days of supplementation. In a competitive cycling time-trial study, Lansley and others (2011A) reported that the oxygen uptake values were not significantly different between the dietary nitrate supplement and placebo during any stage of the trial, but the power outputs were increased, suggestive of improved oxygen efficiency. In yet another study, Lansley and others (2011B) concluded that the positive effects of six days of beetroot supplementation on the physiological responses to exercise, primarily a decrease in the oxygen cost of walking and running, can be ascribed to the high nitrate content per se.
**Increased performance** As noted above, ingestion of sodium nitrate salts in the equivalence of 100-300 grams of nitrate-rich vegetables trended towards an increase in exercise time to exhaustion (Larsen et al., 2010). Research with nitrate-rich beetroot juice supports this linkage.

**Time to exhaustion** As a measure of exercise performance, many studies use tests that involve exercise to exhaustion, such as the subject can no longer continue to exercise at a given rate or stops because of complete fatigue. Using such protocols, investigators have reported significant improvement in exercise tests to exhaustion following beetroot supplementation. Kenjale and others (2011) reported that patients with peripheral arterial disease improved peak walking time by 17 percent in a cardiopulmonary exercise test three hours after supplementation. Lansley and others (2011B) reported improved treadmill run time to exhaustion in a severe-intensity treadmill test after 4 and 5 days of supplementation. Bailey and others (2010B; 2009) utilized various protocols involving high-intensity exhaustive knee-extension and cycling tests and found that 4-6 days of supplementation improved exercise time to exhaustion. Vanhatalo and others (2011), studied the effect of dietary nitrate supplementation under conditions of hypoxia and found that one day after supplementation, performance in a knee-extension test to the limits of tolerance under hypoxic conditions was restored to values observed in normoxia. In their study of both acute and chronic supplementation protocols, Vanhatalo and others (2010) reported that supplementation increased both work rate and peak power in a ramp incremental cycle ergometer exercise protocol.

**Sport performance-specific research** When conducting research specific to exercise or sport performance, scientists generally recommend consideration of two factors. One, the exercise task should be as applicable as possible to actual sport performance, and two, subjects should be trained in the sport or exercise task. Although exercise tests to exhaustion may be useful to study the effects of performance-enhancing substances, they do not replicate actual sports performance. A more relevant approach involves sport-like competitions, such as time trials under laboratory conditions, which are attempts to mimic actual types of sport performance. Relative to the training status of subjects in studies of dietary nitrate supplementation, Bescós and others (2012) noted that most studies showing enhancement of exercise performance have used untrained males as subjects.

However, two studies using a simulated sport competition protocol and trained cyclists have reported performance-enhancing effects of both acute and chronic beetroot juice supplementation. In one study, nine club-level competitive male cyclists consumed beetroot juice 2.5 hours before testing. Compared to the placebo trial, the cyclists significantly improved both power output and performance in both a 4-kilometer and 16.1-kilometer cycling time trial. Oxygen consumption was similar during the stages of the time trial, suggesting beetroot juice improves cycling economy (Lansley et al., 2011A). In the second study, trained male cyclists consumed beetroot juice for 6 days, and on test day performed 60 minutes of submaximal cycling followed by a 10-kilometer time trial. Similar to the acute supplementation study, both power output and time-trial performance were significantly improved with beetroot.
supplementation, although the performance difference between the two trials was relatively small (Cermak et al., 2012).

Collectively, both sets of these data provide rather strong support for a performance-enhancing effect of dietary nitrate supplementation.

**Possible Mechanisms of Nitrate Supplementation on Performance Enhancement**

Dietary nitrate supplementation, as noted, may be related to favorable effects on both cardiovascular health and exercise performance. Machha and Schechter (2011) note that multiple mechanisms may underlie such beneficial effects, and such mechanisms may be applicable to both health and exercise performance. Specific to exercise performance, Bescós and others (2012) suggested that improvements following supplementation with dietary nitrates may be related to the increase in nitric oxide production and subsequent enhancement of oxygen and nutrient delivery to active muscles. As noted below, increased oxygen delivery may be one of the key mechanisms, but research relative to a performance-enhancing effect of nutrient delivery is very limited, and that which is available is not supportive. For example, Cermak and others (2012) reported no effect of dietary nitrate supplementation on whole-body fuel selection and plasma glucose or lactate concentrations during a 10-kilometer cycling time trial. However, Bailey and others (2010B) reported a slight shift in substrate utilization toward a relatively greater use of carbohydrate, possibly attributed to an nitric oxide-mediated increase in muscle glucose uptake, which could reduce oxygen uptake. They recommend additional research to evaluate this possibility.

Larsen and others (2010), noting the effect of dietary nitrate supplementation to reduce the oxygen cost of exercise during maximal exercise suggested that two separate mechanisms are involved: one that reduces oxygen uptake and another that improves the energetic function of the working muscles.

The vasodilative effect of dietary nitrate appears to be a major factor involved in the reduction of oxygen uptake during exercise. Several factors may be involved. Jones and others (2011) note that the VO₂ slow component, a slowly developing increase in VO₂ during constant-work-rate exercise performed above the lactate threshold, represents a progressive loss of skeletal muscle contractile efficiency and is associated with the fatigue process. They note that dietary nitrate supplementation can reduce the magnitude of the VO₂ slow component and reduce muscle fatigue development either by improving muscle oxidative capacity or by enhancing bulk muscle oxygen delivery. The increased oxygen delivery may enhance oxygen distribution in the exercising muscle. Kenjale and others (2011) reported that gastrocnemius tissue fractional oxygen extraction was lower during walking exercise following beetroot supplementation in individuals with peripheral arterial disease. One possibility is an increased oxygen delivery to the slow-twitch muscle fibers in the gastrocnemius, as contrasted to the fast-twitch fibers. Slow twitch muscle fibers can use oxygen more efficiently than fast twitch muscle fibers. Another possibility to reduce oxygen uptake during exercise is to reduce the amount consumed by the heart muscle. Drechsler-Parks (1995) found that inhaled air with
nitrite induced a decrease in cardiac output during exercise, which would reduce the work of the heart and oxygen consumption.

An improvement in muscle energy production efficiency during exercise could lead to a reduction in oxygen uptake. Although Lansley and others (2011B) reported no change in mitochondrial oxidative capacity during exercise following several days of dietary nitrate supplementation, Larsen and others (2011) reported improved oxidative phosphorylation efficiency in skeletal muscle mitochondria which correlated to the reduction in oxygen cost during exercise. They noted that after nitrate supplementation, skeletal muscle mitochondria displayed an improvement in oxidative phosphorylation efficiency (P/O ratio), which was correlated with the reduction of oxygen cost during exercise. This finding suggests a more efficient production of ATP for muscle contraction from a given amount of oxygen. They concluded that dietary nitrate has profound effects on basal mitochondrial function. However, although Bailey and others (2010B) indicated results from their study are not able to exclude the possibility that nitrate supplementation increases the P/O ratio, they suggest that the reduced oxygen cost of exercise is consequent to an improved coupling between ATP hydrolysis and skeletal muscle force production, which would result in a reduced ATP cost of muscle force production. The total ATP turnover rate was estimated to be less for both low-intensity and high-intensity exercise following dietary nitrate supplementation. Additionally, Vanhatalo and others (2011) noted that compared to the placebo trial under hypoxic conditions, nitrate supplementation had favorable effects on phosphocreatine recovery time and muscle pH, factors that could contribute to enhanced exercise performance. The authors noted that nitrate supplementation in hypoxia restored exercise tolerance and oxidative function to values observed in normoxia. Overall, these findings suggest dietary nitrate supplementation could enhance muscle energy efficiency during exercise, which could lead to a reduction in oxygen consumption.

Other factors may be involved as well. The central fatigue hypothesis suggests that neural factors, primarily the brain, are involved in fatigue. Presley and others (2011) measured cerebral perfusion in older adults and concluded that the results suggest dietary nitrate may be useful in improving regional brain perfusion in critical brain areas known to be involved in executive functioning. Such an effect may be involved in a reduction of central fatigue and an improvement in exercise performance.

Additional research is recommended to help ascertain the mechanism underlying the reduced oxygen cost of exercise following nitrate supplementation, particularly with beetroot juice. Bailey and others (2011B) note that beetroot juice is also rich in antioxidants and phenols and indicate it is possible that these compounds may act independently or synergistically with nitrate.

Considerations on Use of Nitrates for Performance Enhancement in Sport

Lundberg and others (2011) note that although the true performance-enhancing effects of nitrate are yet to be proven under actual competitive conditions, it is clear from internet
forums, articles, and discussions within the sports community that the use of nitrate supplementation currently is spreading rapidly among athletes. They, along with others, suggest caution in the use of various nitrate or nitrite preparations.

**Drugs and salts**

Lundberg and others (2011) note that drugs that contain organic nitrates and nitrites, such as nitroglycerine and amyl nitrite, are extremely potent vasodilators and unintentional overdosing may lead to fatal vascular collapse. At this time, they also advise athletes to refrain from the uncontrolled use of nitrate and nitrite salts as dietary supplements, indicating that while the acute toxicity of nitrate is very low or absent, any confusion leading to a large unintentional intake of nitrite or organic nitrates and nitrites is potentially life threatening. For example, consuming various doses of nitrite found in dietary supplements in conjunction with vasodilation erectile dysfunction drugs, such as Viagra and Cialis, may cause health problems (Allen, 2011). If you use any drugs, check with your physician prior to use of such dietary supplements. Individuals with various health problems, such as peripheral arterial disease, may benefit from nitrate or nitrite salt supplementation, but also should consult with their physician regarding such use with exercise.

**Dietary supplements**

As noted previously, most “nitric oxide” dietary supplements marketed to athletes contain L-arginine as the purported active ingredient, but there is little scientific evidence that L-arginine supplementation enhances exercise performance. Other supplements may contain different ingredients marketed to deliver "real nitric oxide" to the circulation, but research with such supplements is currently limited. One study with resistance-trained men reported a small, but statistically insignificant, effect of such a supplement on increasing circulating nitrate/nitrite within the 1-hour postigestion period, but there was no effect on various hemodynamic variables (Bloomer et al., 2010). Additional research is merited with such “nitric oxide” dietary supplements.

**Food sources of nitrate**

In general, most investigators indicate that there is very little harm, and possibly some health benefits, associated with consumption of healthful foods, particularly vegetables and vegetable juices, rich in nitrates (Allen, 2011; Lundberg et al., 2011; Machha and Schechter, 2011). One key research group notes that the dose of nitrate that reduces oxygen cost efficiently is in the range 300–500 milligrams and there is no evidence that higher doses would increase the effects further (Lundberg et al., 2011). However, these investigators indicate a potential risk exists with nitrate-containing vegetable juice if stored inappropriately. Contamination of the beverage by nitrate-reducing bacteria may then occur, leading to substantial nitrite accumulation over time.

**Possible contraindications of nitrate supplementation**

Although hypothetical at this point, dietary nitrate supplementation may be related to several concerns for athletes. Low serum iron levels, even to the level of iron deficiency anemia, appear to be more prevalent in athletes than in nonathletes, especially younger female
athletes; although it is likely that dietary choices explain much of a negative iron balance, evidence also exists for increased rates of red cell iron and whole-body iron turnover (Beard and Tobin, 2000). Increased production of nitric oxide also may be involved. For example, individuals who live at high altitude have a 10-fold-higher circulating concentration of bioactive nitric oxide products than their sea-level controls, but their red blood cells contain lower concentrations of iron complexes (Erzurum et al., 2007). In a study with rats engaged in exercise training over the course of 12 months, Xiao and Qian (2000) reported that strenuous exercise may lead to an increase in plasma nitric oxide concentrations as well as a low iron level, and suggested the possibility that the increased nitric oxide production might be associated with the development of the lower iron status in exercise. A long-term study with humans may be of interest.

An increase in nitric oxide from dietary nitrate may be particularly important in conditions of low oxygen availability (Jones, 2011). Thus, as nitrate supplementation may be beneficial under conditions of hypoxia, such use may be important to athletes training and competing at altitude. However, caution may be advised. In a case study, an elite mountaineer reported severe acute mountain sickness and ataxia during exercise at high altitude. The mountaineer was using transdermal nitroglycerin patches aimed to prevent frostbite. Use of nitroglycerin for this purpose is off-label, and its safety has not been assessed. The authors noted that a relation between nitrate-induced cerebral vasodilation and high altitude cerebral edema is theoretically possible on a pathophysiological basis (Mazzuero et al., 2008). This incident occurred at an altitude of 8,000 meters, which is not a usual venue for major sports competition, and it involved the use of a drug, not a food supplement. Nevertheless, caution in the use of nitrates by athletes at altitude may be advised.

Practical advice

Andrew M. Jones, an expert in nitrate supplementation research, offers some practical advice for athletes, and here is a summarization of some of his key points (Jones, 2011).

• The available evidence suggests that dietary supplementation with approximately 300-450 milligrams of nitrate results in a significant increase in plasma nitrite concentration and associated physiological effects.

• This dose can be achieved through the consumption of 0.5 liter of beetroot juice, or an equivalent high-nitrate foodstuff.

• Following ingestion, plasma nitrite concentration typically peaks within 2-3 hours and remains elevated for a further 6-9 hours before declining towards baseline. Thus, athletes should consume the nitrate 3 to 9 hours prior to training or competition.

• A daily dose of a high-nitrate supplement is required if plasma nitrite concentration is to remain elevated, but the effects of sustained dietary nitrate supplementation on adaptations to training are not clear.
• There is the possibility that uncontrolled high doses of nitrate salts might be harmful to health.

• Natural sources of nitrate are likely to promote health.

• Athletes wishing to explore the possible ergogenic effects of nitrate supplementation are recommended to employ a natural, rather than pharmacological, approach.

Sources of dietary nitrate
As noted in table 1, several vegetables are rich sources of dietary nitrate. Beetroot juice, in regular or concentrated form, has been used in most studies. Finding local sources of beetroot juice, or red beet juice, in the United States may be difficult. However, such products may be found on the internet at various sites, such as Amazon. You may also Google the term beetroot juice. Prices vary. For example, a 16.9-ounce bottle of Biotta beetroot juice may cost about $5-$7, whereas a 32-ounce bottle of Vitacost beetroot juice costs about $8.50. Beetroot powder and tablets are available, but no research evaluating their effects has been uncovered.

One possibility is to make your own juice from red beets. Use a blender to mix fresh red beets, and dilute with carrot and/or celery juice; modify to your taste. Blended drinks with other nitrate-rich vegetables may contribute rich sources. In the February 5, 2012 issue of Parade, Dr. Mehmet Oz provided a formula for a drink rich in fiber, antioxidants, and vitamins, and low in calories; it also would be rich in nitrates. Blend the following ingredients to make 3-4 servings. You could experiment with adding red beets as well.

- 2 cups spinach
- 2 cups peeled cucumber
- 6 stalks celery
- 1 bunch parsley
- 1 teaspoon ginger
- 2 peeled apples
- Juice of 1 lime
- Juice of ½ lemon

Future Research
The current research findings support an ergogenic effect of dietary nitrate supplementation. Laboratory studies clearly support an increase in nitric oxide and a decrease in the oxygen cost of exercise, as well as improved performance in various exercise tests. Although the true performance-enhancing effects of nitrate are yet to be proven under actual competitive conditions (Lundberg and others, 2011), the two studies involving simulated sport competitive performance (Cermak et al., 2012; Lansley et al., 2011A) did find beneficial effects on performance in trained cyclists. However, additional research with athletes, both endurance- and resistance-trained, is needed to support these preliminary findings.
Several investigators (Allen, 2011; Bescós et al., 2012; Jones et al., 2011) note that future studies may hone the supplementation protocol to maximize the improvement in sports performance in athletes, as well as exercise tolerance in females and the elderly, in whom nitric oxide metabolism is impaired by estrogen status and/or age, and in patient populations, such as individuals with various health problems.

References


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