Heart failure (HF) decreases both quantity and quality of life. A primary factor in the reduced quality of life is exercise intolerance. This impaired exercise capacity is partially because of reduced O2 delivery to, and utilization by, skeletal muscle, resulting in a reduction in VO2 peak.1 Skeletal muscle strength, velocity, and power, however, are also markedly reduced in HF.2,3 This muscle dysfunction also plays an important role in the compromised exercise capacity and reduced quality of life in HF.4 Moreover, diminished muscle function is a powerful prognostic indicator of survival in HF, even more powerful than a reduction in VO2 peak.5

The mechanisms responsible for altered muscle contractile properties in HF have not been fully elucidated. Notably, patients with HF are weaker, slower, and less powerful even when compared with equally sedentary healthy subjects with comparable limb muscle mass,3 indicating that these changes are not solely because of physical inactivity or muscle atrophy. Rather, muscle dysfunction in HF seems to be due, at least in part, to derangements at the molecular level. Specifically, HF has been associated with a selective reduction in myosin protein content,6 a slowing of cross-bridge kinetics, 7 and an increase in the percentage of muscle fibers expressing multiple myosin isoforms.8 Other studies9,10 of HF have demonstrated abnormalities in skeletal muscle sarcoplasmic

Acute Dietary Nitrate Intake Improves Muscle Contractile Function in Patients With Heart Failure
A Double-Blind, Placebo-Controlled, Randomized Trial

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Background—Skeletal muscle strength, velocity, and power are markedly reduced in patients with heart failure, which contributes to their impaired exercise capacity and lower quality of life. This muscle dysfunction may be partially because of decreased nitric oxide (NO) bioavailability. We therefore sought to determine whether ingestion of inorganic nitrate (NO3−) would increase NO production and improve muscle function in patients with heart failure because of systolic dysfunction.

Methods and Results—Using a double-blind, placebo-controlled, randomized crossover design, we determined the effects of dietary NO3− in 9 patients with heart failure. After fasting overnight, subjects drank beetroot juice containing or devoid of 11.2 mmol of NO3−. Two hours later, muscle function was assessed using isokinetic dynamometry. Dietary NO3− increased (P<0.05–0.001) breath NO by 35% to 50%. This was accompanied by 9% (P=0.07) and 11% (P<0.05) increases in peak knee extensor power at the 2 highest movement velocities tested (ie, 4.71 and 6.28 rad/s). Maximal power (calculated by fitting peak power data with a parabola) was therefore greater (ie, 4.74±0.41 versus 4.20±0.33 W/kg; P<0.05) after dietary NO3− intake. Calculated maximal velocity of knee extension was also higher after NO3− ingestion (ie, 12.48±0.95 versus 11.11±0.53 rad/s; P<0.05). Blood pressure was unchanged, and no adverse clinical events occurred.

Conclusions—In this pilot study, acute dietary NO3− intake was well tolerated and enhanced NO bioavailability and muscle power in patients with systolic heart failure. Larger-scale studies should be conducted to determine whether the latter translates into an improved quality of life in this population.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01682356.

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Key Words: heart failure ■ muscle contraction ■ muscle strength ■ nitrates ■ nitric oxide ■ nutrition theory ■ quality of life

Heart failure (HF) decreases both quantity and quality of life. A primary factor in the reduced quality of life is exercise intolerance. This impaired exercise capacity is partially because of reduced O2 delivery to, and utilization by, skeletal muscle, resulting in a reduction in VO2 peak.1 Skeletal muscle strength, velocity, and power, however, are also markedly reduced in HF.2,3 This muscle dysfunction also plays an important role in the compromised exercise capacity and reduced quality of life in HF.4 Moreover, diminished muscle function is a powerful prognostic indicator of survival in HF, even more powerful than a reduction in VO2 peak.5

Clinical Perspective on p 920

The mechanisms responsible for altered muscle contractile properties in HF have not been fully elucidated. Notably, patients with HF are weaker, slower, and less powerful even when compared with equally sedentary healthy subjects with comparable limb muscle mass, indicating that these changes are not solely because of physical inactivity or muscle atrophy. Rather, muscle dysfunction in HF seems to be due, at least in part, to derangements at the molecular level. Specifically, HF has been associated with a selective reduction in myosin protein content, a slowing of cross-bridge kinetics, and an increase in the percentage of muscle fibers expressing multiple myosin isoforms. Other studies of HF have demonstrated abnormalities in skeletal muscle sarcoplasmic...
Dietary $\text{NO}_3^-$ Improves Muscle Function in HF

Methods

Subjects

We studied HF patients with systolic dysfunction, that is, an ejection fraction of <45%. Subjects had to be ≥18 years of age, on a stable HF treatment regimen, and without significant orthopedic limitations or other contraindications to exercise. All underwent a medical history, physical examination, and blood tests for fasting chemistries. Subjects were excluded if they had major organ system dysfunction other than HF or were pregnant or were unable to give informed consent. Other exclusion criteria included the use of antacids or proton pump or xanthine oxidase inhibitors, which can affect the reduction of $\text{NO}_3^-$ and $\text{NO}_2^-$ to $\text{NO}$. Individuals taking phosphodiesterase inhibitors (eg, Viagra) were also excluded, as these can potentiate NO effects. After screening of 37 subjects, 9 subjects were enrolled and completed the study (Figure 1). Subjects provided written informed consent, and the study was approved by the Human Research Protection Office at Washington University School of Medicine.

Experimental Design

Each subject was studied twice using the same experimental protocol. On one occasion, they were tested after ingesting 140 mL of a concentrated BRJ supplement (Beet It Sport; James White Drinks, Ipswich, UK) containing 11.2 mmol of $\text{NO}_3^-$, and on the other after ingesting the same volume of $\text{NO}_3^-$-depleted BRJ. There was a 1- to 2-week washout period between treatments. Because the half-life of the increase in plasma $\text{NO}_3^-$ after acute dietary intake is <8 hours, this length of washout period was considered more than sufficient to minimize any possible carryover effects. The placebo, which is prepared by the manufacturer by extracting $\text{NO}_3^-$ from BRJ using an ion exchange resin, is indistinguishable from the standard product in packaging, color, texture, taste, and smell, and it does not alter plasma $\text{NO}_3^-$ or $\text{NO}_2^-$ concentrations or NO levels. Both the subjects and the investigators were blinded to the order of treatment, which was randomized using http://www.randomization.com.

On questioning after completion of their second visit, subjects were unable to reliably identify the active trial. Subjects were instructed to avoid high $\text{NO}_3^-$ foods for 10 days before intervention and throughout the study. Food records were obtained during the washout period and reviewed for adherence to the diet. These measures were instituted to minimize variation in baseline $\text{NO}_3^-$, $\text{NO}_2^-$, and NO levels. Because chewing gum or antibacterial mouthwash can block conversion of $\text{NO}_3^-$ to $\text{NO}_2^-$ by bacteria in the oral cavity, subjects were also instructed to avoid these products on study days. Finally, subjects were instructed to avoid food, caffeine, alcohol, and exercise for 12 hours before study.

Experimental Protocol

On the subject’s arrival at the Clinical Research Unit at 8 AM, an antecubital venous catheter was inserted and a blood sample was obtained for subsequent determination of plasma $\text{NO}_3^-$ and $\text{NO}_2^-$ levels using a commercial kit (Cayman Chemicals, Ann Arbor, MI). Heart rate and blood pressure were then measured, after which a portable analyzer (NIOX Mino; Aerocline Inc, Morrisville, NC) was used to measure breath NO as a biomarker of whole-body NO production. The subject then ingested the BRJ and rested quietly while it was digested/absorbed. The aforementioned measurements were repeated 1 hour and 2 hours after BRJ ingestion, after which exercise testing began. This timing was based on previous studies demonstrating that plasma NO $^{22–24}$ and breath NO $^{17,25}$ peak 2 hours after dietary NO intake. A 6-minute walk test was first conducted to determine the effects of dietary NO $^-$ on aerobic exercise performance and also to serve as a warm-up for the subsequent muscle function testing. The latter was performed using a Biodex 4 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). After adjustment of the dynamometer, subjects performed 3 to 4 maximal knee extensions at angular velocities of 0, 1.57, 3.14, 4.71, and 6.28 rad/s (0, 90, 180, 270, 360 deg/s)
Results

Subject characteristics are listed in Table 1. All subjects had a nonischemic cardiomyopathy; all were treated with β-blockers, and most were treated with an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker. No patient had a change in HF medication during the study.

As intended, ingestion of BRJ containing 11.2 mmol of NO$_3^-$ resulted in a large, that is, ≥20-fold, increase (P<0.0001) in plasma NO$_3^-$ concentration (Table 2). Consistent with the literature,21,22 this was accompanied by a much smaller, and in this study not statistically significant, elevation in plasma NO$_2^-$ compared with the placebo trial. Whole-body NO bioavailability, on the other hand, did increase significantly after dietary NO$_3^-$, as evidenced by a 35% to 50% increase (P<0.001–0.05) in breath NO over baseline (Table 2).

Hemodynamic data are shown in Table 3. Heart rate and blood pressure were not affected by NO$_3^-$ ingestion, and no significant adverse clinical events occurred. One subject, however, did report some abdominal cramping and loose stools.

Dietary NO$_3^-$ improved several measures of muscle contractile function. Specifically, NO$_3^-$ ingestion increased peak torque and hence peak power at the 2 highest angular velocities tested (ie, 4.71 and 6.28 rad/s) by 9% (P<0.05) and 11% (Figure 2), respectively. Consequently, calculated maximal knee extensor power was 13% higher (P<0.05) in the NO$_3^-$ trial (Figure 3, top). Calculated maximal knee extensor velocity was also 12% higher (P<0.05) after dietary NO$_3^-$ (Figure 3, bottom). On the other hand, no differences in muscle function were observed during the 50 contraction fatigue test conducted at 3.14 rad/s (Table 4). Six-minute walk distance (528±30 m [NO$_3^-$] versus 517±31 m [placebo]; P=0.29) and maximal isometric torque (1.99±0.10 Nm/kg [NO$_3^-$] versus 1.98±0.16 [Nm/kg] placebo; P=0.86) also did not change significantly with NO$_3^-$.

The latter is in keeping with previous studies that have also failed to observe any change in maximal voluntary isometric torque as a result of dietary NO$_3^-$ intake.17,26
Table 3. Effects of Dietary NO$_3^-$ on Heart Rate, Blood Pressure, and Rate Pressure Product in Patients With Heart Failure

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Trial</th>
<th>Pre Ingestion</th>
<th>1 h Post Ingestion</th>
<th>2 h Post Ingestion</th>
<th>10 min Post Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats per min</td>
<td>Placebo</td>
<td>63±3</td>
<td>67±5</td>
<td>64±4</td>
<td>69±4</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td>65±4</td>
<td>67±4</td>
<td>66±3</td>
<td>74±5</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>Placebo</td>
<td>102±5</td>
<td>102±5</td>
<td>101±5</td>
<td>106±6</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td>105±4</td>
<td>106±3</td>
<td>102±2</td>
<td>106±5</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>Placebo</td>
<td>66±3</td>
<td>66±4</td>
<td>65±3</td>
<td>68±3</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td>68±3</td>
<td>65±3</td>
<td>65±3</td>
<td>68±3</td>
</tr>
</tbody>
</table>

Values are mean±SE for n=9.

No significant treatment×order interaction effects were observed for any variable, verifying the absence of any carryover effects.

Discussion

In this study, we have shown for the first time that dietary NO$_3^-$ increases plasma NO$_3^-$ concentration and hence NO bioavailability (as indicated by an increase in breath NO levels) in patients with systolic HF. This was accompanied by a significant improvement in muscle contractile function, that is, in the maximal velocity and hence power of the knee extensor muscles. Just as importantly, in this pilot study, acute dietary NO$_3^-$ intake was well tolerated by patients with HF, and in particular, it did not lead to deleterious changes in blood pressure or other major untoward clinical effects.

As indicated above, dietary NO$_3^-$ ingestion resulted in a significant increase in breath NO. However, both the baseline value and the magnitude of the increase after NO$_3^-$ intake were slightly, but significantly (P<0.05), lower than those we found recently in a parallel study of healthy, younger men and women. The former is consistent with previous studies of patients with systolic HF.27,28 This reduction in breath NO has been shown to be predictive of reduced exercise capacity28 and increased mortality risk in this population.29 Presumably, lower baseline breath NO in HF is at least partially the result of decreased NO synthase–mediated NO synthesis, as evidenced by diminished urinary excretion of $^{15}$NO$_2^-$ after intravenous infusion of $^{15}$L-arginine.30 To our knowledge, however, the effect of dietary NO$_3^-$ on breath NO has not been previously assessed in this population. The somewhat lesser rise in breath NO after dietary NO$_3^-$ intake in patients with HF could be because of differences in the rate of NO$_3^-$ absorption or reduction, as suggested by the insignificant increase in plasma NO$_3^-$ that we observed. Alternatively (or in addition), however, it may also reflect more rapid destruction of NO as a result of increased oxidative stress in HF.11

Despite this apparently slightly blunted increase in NO bioavailability in patients with HF, acute dietary NO$_3^-$ intake resulted in considerable improvements in muscle velocity and power. In fact, compared with the healthy, younger subjects we studied previously,12 the patients with HF in this study seemed to benefit even more from dietary NO$_3^-$ supplementation. Specifically, in patients with HF, dietary NO$_3^-$ increased peak knee extensor power at 4.71 and 6.28 rad/s by 9% (P=0.07) and 11% (P<0.05), respectively, whereas in healthy subjects, we found no difference at the lower velocity and only a 4% (P<0.01) increase at the higher velocity. Correspondingly, in the patients with HF, $P_{max}$ increased by 13% (P<0.05) after dietary NO$_3^-$ ingestion versus the 6% (P<0.05) we observed in healthy subjects. In light of the slightly but significantly smaller increase in breath NO, these data indirectly support the hypothesis that the impaired skeletal muscle contractile performance of patients with HF is due in part to reduced NO-soluble guanylyl cyclase-cGMP signaling, as is thought to be true in cardiac muscle.11 More importantly, however, these data illustrate the efficacy of dietary NO$_3^-$ in enhancing muscle function in patients with HF. Indeed, on the basis of the study by Toth et al.,3 in which patients with HF were carefully matched with control subjects on the basis of age, sex, leg lean mass, habitual physical activity, and use of statins, ingestion of dietary NO$_3^-$ in this study seems to have acutely erased approximately one third of the deficit in muscle power typically resulting from HF. This is in stark contrast to the effects of commonly prescribed HF drugs, that is, β-blockers, angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, and aldosterone antagonists, which have proven ineffective in improving skeletal muscle contractile function in HF.31 The magnitude of the improvement in isokinetic muscle function that we observed is comparable with that resulting from 2 to 3 months of resistance exercise training in patients with HF,32,33 which has been shown to result in significant improvement in Minnesota Living with Heart Failure Questionnaire score.34 Longer-term, larger-scale clinical trials of dietary NO$_3^-$ ingestion in chronic HF are needed to confirm these preliminary findings.

Figure 2. Effect of ingestion of beetroot juice containing either or devoid of 11.2 mmol of NO$_3^-$ on peak knee extensor power at varying angular velocities in patients with heart failure. Peak power significantly higher in NO$_3^-$ trial vs placebo trial at those velocities: *P=0.07 and †P<0.05.
Importantly, although dietary NO$_3^-$ resulted in significant increases in muscle velocity and power, it did not reduce either systolic or diastolic blood pressure in our small cohort of subjects. This is in contrast to the results of some, but not all, previous studies of the effects of dietary NO$_3^-$ on blood pressure in other subject groups. Our data, however, are in keeping with the results of Kapil et al, who found that the magnitude of the reduction in systolic or diastolic blood pressure in response to dietary NO$_3^-$ was inversely related to baseline blood pressure, especially in men (in post hoc analyses, we found no sex-related differences in this study). It is also consistent with the results of a recent study by Zamani et al of patients with HF with preserved ejection fraction, in whom dietary NO$_3^-$ supplementation also did not reduce blood pressure.

Precisely why dietary NO$_3^-$ lowers blood pressure in some subject groups but not, apparently, in HF patients with reduced (or preserved) ejection fraction is difficult to determine. It is possible that the endothelial dysfunction characteristic of HF is related to the contractile machinery of muscle itself rather than via other mechanisms, as discussed below. Before its precise chemical nature was known, NO was referred to simply as endothelium-derived relaxing factor; indeed, the physiological importance of NO was first recognized based on its ability to stimulate soluble guanyl cyclase and, hence, causes relaxation of smooth muscle. It may therefore be tempting to speculate that our results are because of vasodilation and hence an increase in muscle blood flow. It is unclear, however, how such an effect could influence force and hence power during isolated muscle contractions lasting only a few hundred milliseconds. On the other hand, NO stimulates soluble guanyl cyclase and thus increases cGMP levels in numerous tissues, including skeletal muscle, in which activation of this pathway has been shown to increase maximal shortening velocity and hence maximal power, especially in fast-twitch fibers. Thus, our results seem to be most likely the result of this latter mechanism and not an increase in blood flow. Nonetheless, it remains possible that dietary NO$_3^-$ supplementation may enhance other aspects of physical function in patients with HF by increasing blood flow or via additional mechanisms, for example, by reducing O$_2$ demand or improving muscle energetics during exercise.

Studies should therefore be conducted to determine whether this dietary NO$_3^-$–induced increase in muscle power enhances habitual physical activity and quality of life (or possibly even survival) in patients with HF.

In contrast to the improvements in peak muscle power at 4.71 and 6.28 rad/s, and hence in calculated maximal power and velocity, described above, no differences were observed during the 50 contraction fatigue test conducted at 3.14 rad/s. This is in keeping with our previous study of healthy subjects, in whom acute dietary NO$_3^-$ intake did not influence performance during repeated maximal knee extensions at this velocity. Somewhat along the same lines, dietary NO$_3^-$ intake also did not significantly improve the distance the HF patients were able to walk in 6 minutes. The latter may simply reflect a type II error, as the effect size was moderate (ie, Cohen $d$=0.55), and post hoc analyses indicated that 28 subjects would have been needed to provide adequate statistical power to detect differences in this secondary outcome. Further research will therefore be required to definitively determine whether acute dietary NO$_3^-$ ingestion can improve 6-minute walk distance in patients with HF. Nonetheless, in a relative sense, the nonsignificant increase in 6-minute walk distance we observed was small compared with the significant changes found in muscle power (ie, ≈2% versus ≈10%). This, along with the lack of changes during the fatigue test, implies that acute dietary NO$_3^-$ intake improves physical function in patients with HF primarily by acting on the contractile machinery of muscle itself rather than via other mechanisms, as discussed below.
Dietary NO\textsuperscript{3} Improves Muscle Function in HF

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Disclosures

None.

References


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