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RESEARCH CORRESPONDENCE

Dietary nitrate increases exercise tolerance in patients with non-ischemic, dilated cardiomyopathy—a double-blind, randomized, placebo-controlled, crossover trial

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Non-ischemic dilated cardiomyopathy (NIDCM) is defined as left ventricular dysfunction in the absence of causative coronary artery disease. It typically presents with impaired effort tolerance, which is a major prognostic factor.[1]

Nitric oxide (NO), a potent systemic vasodilator, is vital for skeletal muscle contraction and contributes to matching of blood flow and oxygen delivery necessary in active skeletal muscle. NIDCM is associated with dysregulated NO production, including decreased endothelial NOS expression and NO release. Furthermore, NO therapy has been shown to improve systemic circulation in NIDCM by reducing right ventricular after-load.

There are 2 pathways facilitating NO synthesis in vivo. The L-arginine–NO synthase pathway is well characterized and, until recently, was considered the sole source of endogenous NO. A second, NO synthase–independent pathway has recently been discovered and was found to be involved in simple reduction of dietary, inorganic nitrate to nitrite and NO.[2,3] Acute nitrate consumption has been shown to increase blood nitrate/nitrite levels and exercise performance in healthy, athletic, pulmonary vascular disease (PVD),[4] chronic obstructive pulmonary disease (COPD)[5] and heart failure (HF) subjects.[2] We hypothesized that dietary nitrate supplementation could acutely improve exercise capacity in NIDCM.

We recruited a group of ambulatory outpatients with NIDCM (New York Heart Association Functional Class II or III) who were on optimized therapy. Exclusion criteria were use of organic nitrate therapy, and diabetes or conditions affecting mobility. Our study received local institutional review board approval.

Subjects were tested on 2 separate days, 7 days apart, in a crossover fashion after consuming a light, self-selected, low-nitrate breakfast and taking usual medications. On both days, in an identical manner and at the same time of day, we performed resting blood pressure (BP) measures, blood draws and incremental shuttle-walk tests (ISWTs) before and 3 hours after beverage consumption, as this time-line has been shown to correspond to near peak blood nitrite concentrations after nitrate consumption.[4]

Upon completion of Day 1 pre-supplementation assessments, subjects were randomized in a double-blind, crossover trial design to consume 140 ml of either nitrate-rich beet-root juice (NO_3^-; 12.9 mmol nitrate) or nitrate-depleted beet-root juice (PL; <0.5 mmol nitrate). The PL preparation is identical in taste and appearance but has had the nitrate removed by anion exchange, as described in a previous study.[6] At each visit, subjects underwent baseline assessments followed by consumption of 140 ml of beverage (NO_3^- or PL) and rested quietly for 150 $\pm$ 10 minutes, when all measures were repeated. Thus, subjects underwent assessments on 4 occasions separated by a 7-day washout period (Figure 1), during which they were advised not to change behaviors that would influence NO pharmacokinetics or exercise capacity, specifically diet, exercise and medication.

Immediately before and after each of the 4 ISWTs, subjects rated their dyspnea and leg fatigue on the Borg scale and had oxygen saturation (SpO_2, in percent) and heart rate (HR) measured. BP was measured at the brachial artery using a manual sphygmomanometer and stethoscope before all 4 ISWTs.

Measurement of NO derivatives (nitrate/nitrite) in biologic fluids reflects NO bioavailability and was analyzed using the current "gold standard," ozone-based chemiluminescence analysis, on an NO analyzer (NOA280i; Sievers).

We based the sample size on our recent study with an identical protocol among COPD subjects where a significant treatment effect of nitrate on ISWT was observed on a sample of 11 subjects.[5] ΔNO_3^- and ΔPL data were compared by using 2-tailed, paired *t*-tests for normally distributed data and Wilcoxon's test for non–normally distributed data. Correlations were assessed using Pearson's correlation coefficient. The presence of a carryover effect was assessed using 2-tailed, unpaired *t*-tests by comparing the observed ISWT difference among those who received NO_3^- followed

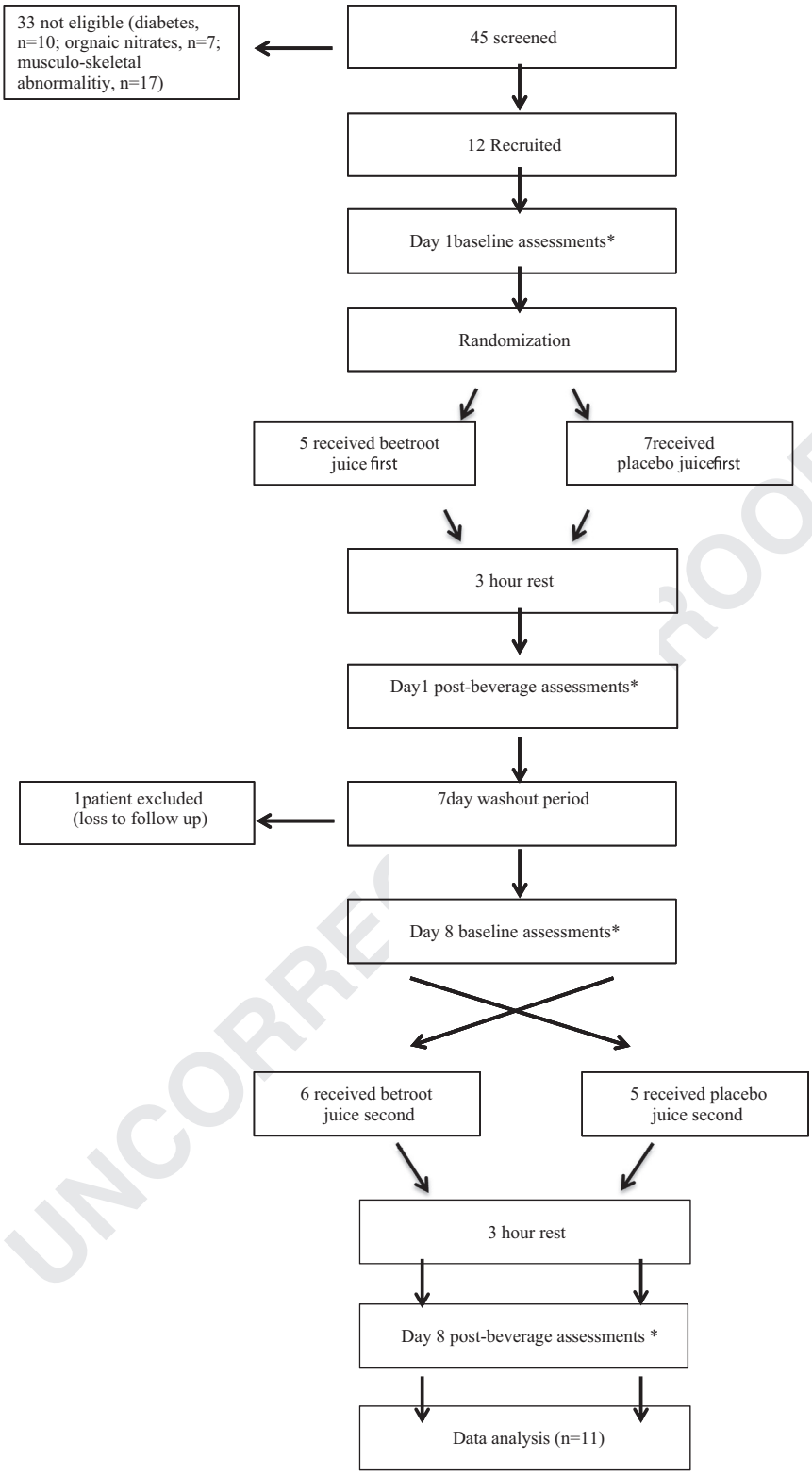

Figure 1 Trial design. Assessments included resting blood pressure, blood draw, resting, pre-ISWT dyspnea and arterial oxygen concentration, ISWT, post-ISWT dyspnea and arterial oxygen concentration. BRJ, beet-root juice; PL, placebo.

by PL with those who received the opposite. All statistical tests were conducted at the 2-sided 0.05 significance level using SPSS for Windows (version 15.0).

Twelve subjects were recruited. There was 1 drop-out due to loss to follow-up. Thus, 11 subjects completed the study (Table 1). After NO_3^- consumption, mean ISWT distance increased by 65 ± 41 meters (431 to 496 meters, 15%), whereas there was a decrease of 5 ± 35 meters (437 to 433 meters, 1%) after PL ($p = 0.0056$). The mean and individual ISWT distances after NO_3^- and PL are shown in Figure 2a.

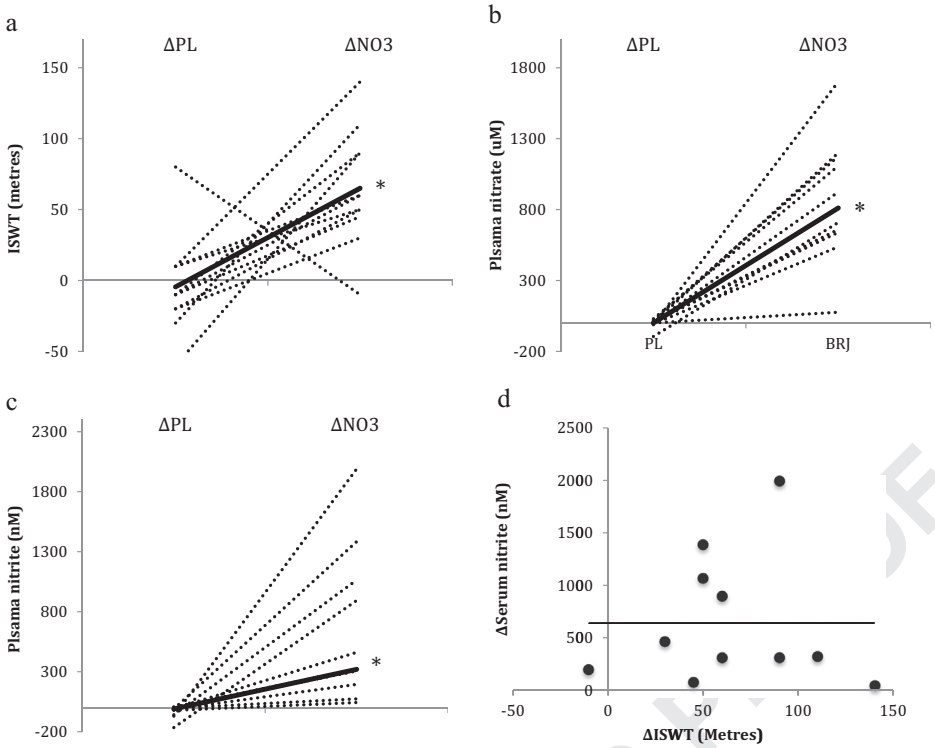

Figure 2 Absolute changes in ISWT, plasma nitrate, plasma nitrate. Dotted lines represent each individual; bold line represents the group mean (a) or median (b and c). (a) Incremental shuttle-walk test distance. (b) Plasma nitrate. (c) Plasma nitrite. (d) Correlation between absolute change in plasma nitrite and ISWT distance ($r = 0.0007$, $^{*}p < 0.005$).

Ten of the 11 subjects walked further after NO_3^- (30 to 140 meters), whereas 1 had a decreased exercise capacity (–10 meters). However, after PL, only 4 subjects had increased walking capacity (10 to 80 meters), but 7 walked shorter distances (–10 to –60 meters). There was no significant difference regarding SpO_2, HR, dyspnea or leg fatigue score (data not shown). Also, there was no difference in ISWT distance, regardless of treatment sequence ($p = 0.24$), suggesting no carryover effect of the intervention. Table 2

The median and individual plasma nitrate and nitrite concentrations pre- and post-beverage (NO_3^- and PL) are shown in Figure 2b and c. After NO_3^-, median plasma nitrate increased by 811.1 (range 649.5 to 1,142.2) μmol/liter, with little change after PL ($p = 0.003$). Similarly, median plasma nitrite increased by 319.7 nmol/liter (250.6 to 978.2) after NO_3^-, whereas there was a small decrease after PL (–10 nmol/liter; $p = 0.003$). There was no correlation between absolute change in plasma nitrite and absolute change in ISWT distance ($r = 0.0007$, $p = 0.99$; Figure 2d).

Mean systolic and diastolic BP and mean arterial pressure (MAP) decreased after NO_3^- (–2.7, –1.4 and –2 mm Hg, respectively), but increased after PL (4.5, 2.7 and 3.3 mm Hg, respectively). Changes did not reach statistical significance.

We found that acute consumption of NO_3^- (12.9 mmol) significantly increased plasma nitrate/nitrite and significantly increased exercise capacity in NIDCM patients. Our findings are supported by previous reports of increased exercise capacity in selected clinical groups, including subjects with

We also observed an 18% increase in ISWT distance with NO_3^- (–1.5% to 39%), whereas PL led to a 2% decrease (–17.6% to 11.6%). Similar to the NO response, there was marked variation regarding ISWT response to NO_3^-. It is probable that differences in baseline fitness/NO bioavailability, medications and NIDCM severity contribute to this variation. Nevertheless, 10 of 11 subjects had increased ISWT distance post-NO_3^-.

Exercise capacity is regarded as a principal variable for the evaluation of new therapeutic approaches in NIDCM. Further, we have demonstrated that exercise capacity represents an important prognostic factor related to future mortality in NIDCM.[1] Controversy remains regarding the optimal test to assess exercise capacity in NIDCM. Herein we have we utilized the ISWT as our primary end-point, which represents a standardized procedure and has been shown to strongly predict maximal aerobic capacity and future outcome in HF.

Our observations are supported by recent trials in HF patients. One trial used constant-intensity cycle cardiopulmonary exercise testing to assess 17 HF patients with preserved ejection fraction. Although there was no change in exercise efficiency (primary end-point), there were significant increases in exercise oxygen consumption, total work performed, exercise duration and plasma NO metabolites.[2] A second trial used isokinetic dynamometry to assess 9 HF patients with NIDCM and showed acutely increased peak knee extensor power.[3] It is of interest to

Table 1 Baseline Characteristics of Overall Chronic Obstructive Disease Patients ($n = 11$)

Characteristic	Value
Age (years)	56 ± 11 (43–80)
Males [*n* (%)]	7 (64)
BMI (kg/m^2)	32.5 ± 4.6 (25.7–40.8)
Obese [*n* (%)]	7 (64)
Smoking status [*n* (%)]	
Current smoker	4 (36)
Ex-smoker	1 (9)
Life long non-smoker	6 (55)
NYHA functional class	
II	9 (82)
III	2 (18)
MLwHFQ	13 ±7 (9–29)
LVEF (Simpson's rule, %)	35 ± 13 (15–50)
LVIDd (cm)	6.3 ± 0.7 (5.4–7.5)
Left atrial area (cm)	20.4 ± 4.4 (15.9–27.1)
Resting reart rate (BPM)	73 ± 18
Resting SBP (mm Hg)	118 ± 25
Resting DBP (mm Hg)	71 ± 12
BNP (ng/liter) (median ± IQR)	295 ± 228
Creatinine (reference range 45−110 μmol/liter)	93 ± 31
Sodium (reference range 135−145 mEq/liter)	141 ± 1
Pharmacotherapy	
ACE-I	9 (82)
Aspirin	6 (55)
β-blockers	10 (91)
Diuretics	10 (91)
Sprionolactone	5 (45)
Digoxin	1 (9)
HTN	4 (36)
ECG	
Normal QRS duration [*n* (%)]	7 (64)
Normal sinus rhythm [*n* (%)]	10 (91)
Atrial fibrillation [*n* (%)]	1 (9)

Data expressed as as mean ± standard deviation, unless noted otherwise. ACE-I, angiotensin-converting enzyme inhibitor; BMI, body mass index; BNP, brain-type natriuretic peptide; DBP, diastolic blood pressure; HTN, hypertension; IQR, interquartile range; LVEF, left ventricular ejection fraction; MLwHFQ, Minnesota Living with Heart Failure Questionnaire; NYHA, New York Heart Association; SBP, systolic blood pressure.

dietary nitrate delivered in the same fashion in a crossover design. It is difficult to compare our results directly. We noted an 18% increase in ISWT distance, whereas Zamani et al described a comparable 14% increase with constant-intensity cardiopulmonary exercise testing.[2] Our use of ISWT does not allow detailed physiologic data to be gathered. However, we believe that the increased exercise capacity we observed post-NO_3^- is probably related to the same mechanistic pathways uncovered in those earlier HF trials, namely increased cardiac output, muscular oxygenation and post-occlusive hyperemic flow, reduced aortic augmentation index[2] and increased skeletal muscle

Table 2 Hemodynamic, Biochemical and Physical Parameters Before and After Supplementation With Placebo (PL) or Nitrate-rich Beet-root Juice (NO_3)

	Before PL	3 hours After PL	ΔPL	Before NO_3^-	3 hours after NO_3^-	ΔNO_3^-	*p*-value
ISWT (ms)	437 ± 157	433 ± 173	−5 ± 35	431 ± 155	496 ± 141	65 ± 41	0.0056
Plasma nitrate (μmol/liter)	26.5 (17.8–43.7)	27.3 (21.8–39.9)	0.8 (−4.2–9.5)	30.2 (23.6–26.4)	841.5 (670.7–1,174.7)	811.1 (649.5–1,142.2)	0.003
Plasma nitrite (nmol/liter)	73.1 (59.8–106.3)	62.9 (53.1–73.4)	−10 (−40.4–1.7)	67.5 (57.8–93.9)	374 (305.6–1,092.5)	319.7 (250.6–978.2)	0.003

Normally distributed data are displayed as mean ± standard deviation, and *p*-values are derived from paired *t*-tests. Non−non-normally distributed data are displayed as median (Q1 to Q3), and *p*-values are derived from Wilcoxon's tests. NO_3^- and ΔPL derived from differences between baseline and post-beverage values, and *p*-values are derived by comparing these Δ values. BPM, beats per minute; DBP, diastolic blood pressure; HR, heart rate; ISWT, incremental shuttle-walk test; SBP, systolic blood pressure; SpO_2, oxyhemoglobin saturation.

As expected, NO_3^- supplementation led to a significant increase in plasma nitrite (>8.5-fold) and particularly nitrate (>28-fold). Similar to our COPD trial,[5] we observed marked variation in response to exogenous nitrate despite an identical dose and time interval (Figure 2b and c). We cannot explain these variations, but it is likely that differences in oral bacteria and stomach acidity, age and medication are contributing factors. Further, we recently reviewed data demonstrating a variation in metabolism of exogenously administered nitrate when measured at a single time-point (e.g., 3 hours), yet the extent of nitrite/nitrate production (across 24 hours) was found to be largely similar.[7] The importance of our placebo-controlled design is illustrated by our earlier demonstration of intra-individual variability with repeat doses of NO_3^-.[7]

Pharmacologic preparations of organic nitrate are used, with varied success, to treat HF and NIDCM. It should be noted that, despite their similar physiologic effects, organic and inorganic nitrate (such as dietary nitrate) possess different chemical structures and pharmacokinetics. The potency of inorganic nitrate is much lower than that of organic nitrate. However, organic nitrate may result in tolerance and, when discontinued, rebound effects are often evoked. In contrast, inorganic, dietary nitrates do not show any signs of tolerance, and physiologic effects may be potentiated with long-term ingestion.

Although our trial has some major limitations, including the acute nature of the assessments and the small sample size, we utilized a robust trial design among a cohort of well-characterized NIDCM subjects. Dietary nitrate has potential as a novel, therapeutic strategy to increase exercise tolerance in NIDCM. Our preliminary results require confirmation among larger samples in the long-term setting.

Disclosure statement

The authors have no conflicts of interest to disclose. This study was financially supported by the Irish Heart Foundation (to C.K.) and, in part, by the HeartBeat Trust.

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