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Case Report

Dietary Nitrate Acutely and Markedly Increased Exhaled Nitric Oxide in a Cystic Fibrosis Case

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Abstract

Airway nitric oxide (NO) is a ubiquitous signaling molecule with bronchoprotective, anti-inflammatory and anti-infective roles. Cystic fibrosis (CF) is a chronic lung condition associated with deceased exhaled NO. Strategies to increase exhaled NO in CF have yielded inconsistent results. A potential new method of increasing systemic NO involves ingestion of dietary, inorganic nitrate which is reduced to nitrite and NO. We present the case of a 12 year-old, athletic male with CF who demonstrated acute but marked increases in exhaled NO following dietary nitrate consumption compared to placebo.

Keywords: Cystic fibrosis; Dietary nitrate; Nitrite; Exhaled nitric oxide

Introduction

Nitric oxide (NO) is a ubiquitous signaling molecule with multiple systemic and airway roles. Cystic fibrosis (CF) is a chronic lung condition which is associated with decreased fraction of exhaled NO (FeNO)[1-4].

The impact of decreased FeNO in CF is unclear. However, potentially of relevance to CF, NO has bronchoprotective, anti-inflammatory and anti-infective roles as well as effects on ion transport[5] and ciliary motility[6]. Interestingly, pulmonary function in CF subjects is positively correlated to airway NO[7] and sputum NO metabolites[8]. Further, NO deficiency contributes to impairment of airway relaxation in a murine model of CF[1]. Therefore, increasing NO levels may be of functional importance in CF. Strategies to increase FeNO in CF have included inhaled NO, L-arginine and PDE5 inhibitors (e.g. sildenafil ®) but have yielded inconsistent safety and efficacy profiles (table 3).

Until recently, it was thought that the only pathway for NO synthesis *in vivo* was via oxidation of L-arginine in the presence of oxygen catalysed by NO synthase (NOS) enzymes. However recently an alternative pathway for *in vivo* NO synthesis has been discovered whereby inorganic nitrate is reduced by oral bacteria to nitrite. This nitrite can be further reduced to NO enzymatically and non-enzymatically[9]. Dietary nitrate is now recognized as an additional and very significant source of NO, whereby a single serving of a nitrate rich vegetables (e.g. spinach) contains more nitrate than what is endogenously formed by the all three NOS isoforms combined in 24h[9]. Recent human intervention trials have demonstrated hypotensive and ergonomic effects

of dietary nitrate in conjunction with increased blood NO metabolites among multiple healthy and clinical groups.

Case report

We present a 12 year-old, non-smoking, athletic, male (BMI = 19 kg/m^2) with CF, normal lung function (FEV_1 = 105%) and pancreatic sufficiency. This case initially presented early in life with a history of recurrent chest infections and wheezy episodes. An initial sweat test chloride of 112 and confirmatory sweat test chloride of 106 in addition to genotype analysis (F508/c.262_263delTT) lead to a CF diagnosis at 9 months of age. Current investigations revealed a normal x-ray, DEXA, and glucose tolerance test with no recent hospital admissions or gastrointestinal symptoms. Further, the case had no other medical history of note, including no asthma or allergy (which could influence FeNO measurement). He generally requires antibiotics every 8-12 weeks but had no recent infection or antibiotic use and had no evidence of concurrent bacterial colonization. Further his medication regimen was stable and typical for a CF case (table 1). We utilized a double-blind, randomized, placebo-controlled, crossover design to assess the acute impact of dietary nitrate as 140ml beetroot juice (BRJ, 12.9mmol nitrate) or 140ml matched, nitrate-depleted beetroot juice (PL, <0.5mmol) on exhaled NO. This nitrate dose is obtainable with a diet rich in vegetables.

We conducted two clinic visits 14 days apart, where there were no relevant changes in factors known to influence NO (including diet, exercise and medication habits). On the morning of each study visit, the subject did not use any medications or complete chest physio and consumed an identical, low nitrate breakfast which was confirmed by a review with a dietitian (CPK). On both

days, in an identical manner and at the same time, FeNO (NiOx MINO; Aerocrine, Sweden) and pulmonary function were assessed before and 1.5h after the study beverage. We added water and blackcurrant cordial (sugar- and nitrate-free) to the juice in an identical manner on both days to make it more palatable at the request of the subject. The 1.5h delay between baseline and post-beverage testing was to facilitate *in-vivo* reduction of dietary nitrate to NO[9]. During this delay, the case rested quietly in a clinic room and did not exercise, eat, drink or take medication. This case report intervention complies with the Declaration of Helsinki and approval was granted by the research ethics committee of The National Children's Hospital. Written informed consent was provided by the mother for permission to conduct and publish this case report.

There was no effect on pulmonary function after either beverage. However, 1.5h following BRJ, FeNO increased by 150% (30ppb) but decreased by 17% after PL (-4ppb) (figure 1 and table 2).

Discussion

To our knowledge, this is the first report of dietary nitrate in CF. The acute but marked FeNO incease following dietary nitrate compares favourably to other NO therapies in CF (table 3). Interestingly, the subject studied here did not have low baseline FeNO (20ppb). Dietary nitrate as a precursor to NO seems most effective in those with dysregulated NO and therefore it is possible that dietary nitrate may increase FeNO to a similar or greater extent in those with diminished baseline FeNO. Despite an increase in FeNO, there was no effect on lung functon. In this context, it is noteworthy that baseline lung function was not impaired (105%), perhaps minimising any potential benefit.

Dietary nitrate is water soluble and rapidly absorbed ~100% in the stomach and small intestine. There is no evidence of nitrate malabsorption in CF. Approximately 75% of ingested nitrate is excreted via the kidneys with the remainder concentrated in the oral gland and subsequently reduced to nitrite by tongue anaerobes. This nitrite is swallowed and then further reduced to NO under suitable conditions within the airway, stomach and endothelium. After nitrate ingestion, salivary/plasma nitrate levels increase rapidly (~15m) and peaking at 90-120m[13]. The levels remain high for several hours following ingestion after which they slowly decline, remaining elevated from baseline for ~24h[13]. This is why we measured FeNO before and 1.5h after each of 2 beverages. Further, we measured total exhaled NO. Future studies may utilize different doses of nitrate among larger groups as well employ serial measurements of NO, nitrate and nitrite to further our understanding of NO metabolism in response to exogenous nitrate in CF. Importantly, this CF case did not display several behaviors known to decrease the reduction of dietary nitrate to NO including use of tobacco[14] antibiotics[15] or mouthwash[16].

Although, our report is limited to a single male, CF case with mild disease, dietary nitrate is a component of certain vegetables, appears remarkably safe and increased FeNO to a greater extent than exisitng CF therapies.

Nitrate and nitrate, as precursors to NO, are known to have broad antimicrobial effects[10-12], including against common pathogens in CF such as Pseudomonas aeruginosa[10, 12] and Staphylococcus aureus[12]. Further, this antimicrobial activity has been demonstrated under cystic fibrosis airway conditions[10].

We recommend that future studies measuring FeNO are aware of potential confounding by dietary intake. Further the dietetic focus regarding CF typically emphasizes dietary fat with little focus on vegetable intake. We suggest the dietary modification of FeNO, including via vegetable consumption in CF is worthy of further exploration. We suggest the dietary modification of FeNO, including via vegetable consumption in CF is worthy of further exploration.

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Table 1: Current medication regimen

Bronchodilators	Ventolin Flixotide
PERT	Creon
Airway clearence aid	Nebulized pulmozyme
Nutritional supplements	Vitamin D Aquadex Probiotics
Chest physio	Twice daily with acapella® device.

PERT = pancreatic enzyme repalcement therapy.

Table 2: Pulmonary function tests

	Day 1	Day 2
Intervention	BRJ	PL
Baseline		
FeNO (ppb)	20	24
FVC *L (%)*	3.66 (106)	3.63 (105)
FEV_1 *L (%)*	3.07 (107)	3.03 (105)
Post beverage		
FeNO (ppb)	50	20
FVC *L (%)*	3.67 (106)	3.52 (101)
FEV_1 *L (%)*	3.06 (106)	2.9 (101)

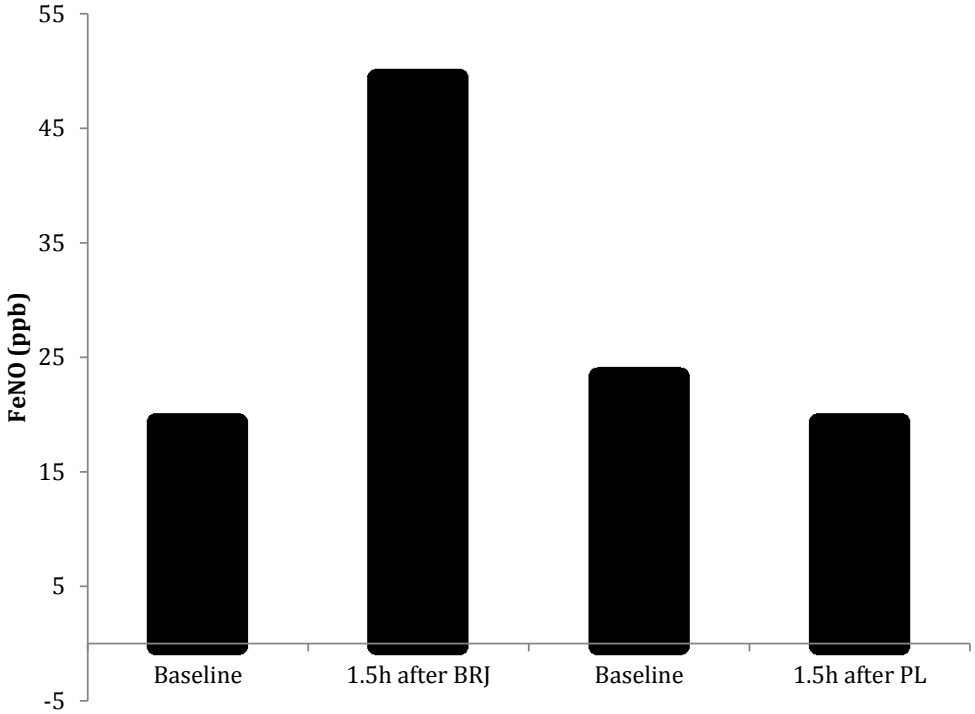
Abbreviations:
BRJ = nitrate-rich neetroot juice; FeNO = fraction exhaled nitric oxide; FEV_1 = forced expiratory volume; FVC = forced vital capacity; ppb = parts per billion; PL = placebo - nitrate-depleted beetroot juice.

Table 3: Comparison of FeNO response to oral nitrate and L-arginine in CF

Sample	Intervention	Intervention period	Baseline FeNO	End FeNO	Effect on FeNO	Comment	References
6 children with (3 male, aged 6-16y)	Oral L-arginine at a dose of 150 mg/kg/day	4 weeks	Not reported	Not reported	No significant impact on FeNO	Plasma L-arginine increased	Everard & Donnelly 2005[2]
8 subjects (3 male, aged range 14–37y)	Oral L-arginine at a a dose of 200 mg/kg	Acute	5.4±2.1ppb	Increased to 6.5ppb at 1h (p=0.06) and 9.2ppb at 3h (p=0.02)	Increased by 1.1ppb (+20%) at 1h and by 3.8ppb (+70%) at 3h	Significant increases in plasma arginine and products of L-arginine metabolism by arginase (ornithine and urea) but not citrulline (the product of L-arginine conversion by NOS)	Grasemann et al 2005[3]
11 subjects (6 male, aged 15-41y) after 2 weeks of antibiotic therapy	Oral L-arginine (600 mg/kg/day) followed by placebo or vice versa	6 weeks	11.4±6ppb	9.7ppb	Decreased by 1.7ppb (-18%)	FeNO decreased after 6w placebo by 6.3ppb which was significantly lower than FeNO at baseline (p=0.001) and after L-arginine (p=0.003)	Grasemann et al 2005[3]
15 CF cases	Ivacaftor	4 weeks	8.5±5.0	16.2±15.5ppb	Increased by 7.7ppb (+90%)	-	Grasemann et al 2015[4]
A single case (male, aged 12)	Dietary nitrate (12.9mmol) vs. placebo (0.5mmol)	Acute	20ppb	50ppb	Increased by 30ppb (+150%)	FeNO decreased after PL (-4ppb, 17%)	Current case report

Abbreviations: FeNO = fraction of exhaled nitric oxide; PL = placebo; ppb = parts per billion.

Figure 1: FeNO response to BRJ and PL



Abbeviations:
BRJ = nitrate-rich; PL = placebo, nitrate-depleted beetroot juice.