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

Conor P. Kerley, BSc; Emma Kilbride, BSc; Peter Grealley, MD; and Basil Elnazir, MD

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 decreased 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 boy with CF who demonstrated acute but marked increases in exhaled NO following dietary nitrate consumption compared to placebo

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

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

The impact of decreased FeNO in CF is unclear. However, potentially relevant to CF, NO has bronchoprotective, anti-inflammatory, and anti-infective roles as well as effects on ion transport⁵ and ciliary motility.⁶ Interestingly, pulmonary function in CF patients is positively correlated to airway NO⁷ 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 (eg, sildenafil), but these have yielded inconsistent safety and efficacy profiles (Table 1).

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 catalyzed 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 vegetable (eg, spinach) contains more nitrate than what is endogenously formed by 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 non-smoking, athletic (body mass index 19 kg/m²) boy, aged 12 years with CF. The patient had normal lung function (FEV1 = 105%) and pancreatic sufficiency. This patient initially presented early in life with a history of recurrent chest infections and wheezy episodes. An initial sweat test chloride of 112 mmol/L and confirmatory sweat test chloride of 106 mmol/L in addition to genotype analysis (F508/c.262_263delTT) lead to a CF diagnosis at 9 months of age. Current investigations revealed a normal radiograph, dual-energy x-ray absorptiometry (DEXA), and glucose tolerance test with no recent hospital admissions or gastrointestinal symptoms. Further, the patient 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 no evidence of concurrent bacterial colonization. Further, his medication regimen was stable and typical for a patient with CF (Table 2). We used a double-blind, randomized, placebo-controlled, crossover design to assess the acute impact of dietary nitrate as 140 ml beetroot juice (BRJ, 12.9 mmol nitrate) or 140 ml matched,

Corresponding Author: Conor P. Kerley, BSc, Paediatric Respiratory Department
National Children's Hospital, Tallaght, Dublin 24 IRELAND
Email: conorkerley@gmail.com

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Table 1. 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 (3 male, age range 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, age range 14-37y)	Oral L-arginine at 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

CF, cystic fibrosis; FeNO = fraction of exhaled nitric oxide; PL = placebo; ppb = parts per billion.

Table 2: Current medication regimen

Bronchodilators	Ventolin; Flixotide
PERT	Creon
Airway clearance aid	Nebulized pulmozyme
Nutritional supplements	Vitamin D; Aquadex; probiotics
Chest therapy	Twice daily with Acapella® device
PERT, pancreatic enzyme replacement therapy	

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 patient did not use any medications or complete chest therapy, and he consumed an identical, low nitrate breakfast that 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 1.5 hours 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 patient. The 1.5 hour delay between baseline and post-beverage testing was to facilitate in-vivo reduction of

dietary nitrate to NO.⁹ During this delay, the patient rested quietly in a clinic room and did not exercise, eat, drink, or take medication.

There was no effect on pulmonary function after either beverage. However, 1.5 hours following BRJ, FeNO increased by 150% (30 ppb) but decreased by 17% after PL (-4 ppb) (Figure 1 and Table 3).

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.

Discussion

To our knowledge, this is the first report of dietary nitrate in CF. The acute but marked FeNO increase following dietary nitrate compares favorably to other NO therapies in CF (Table 1). Interestingly, the patient 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 function. In this context, it is noteworthy that baseline lung function was not impaired (105%), perhaps minimizing any potential benefit.

Figure 1. FeNO response to BRJ and PL (BRJ = nitrate rich; PL = placebo, nitrate-depleted beetroot juice).

Table 3: 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)

BRJ, nitrate-rich beetroot juice; FeNO, fraction exhaled nitric oxide; FEV₁, forced expiratory volume; FVC, forced vital capacity; ppb, parts per billion; PL, placebo - nitrate-depleted beetroot juice.

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 (~15 m), peaking at 90-120 m.¹³ The levels remain high for several hours following ingestion, after which they slowly decline, remaining elevated from baseline for ~24 hours.¹³ This is why we measured FeNO before and 1.5 hours after each of two 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 patient with CF 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 patient with mild CF, dietary nitrate is a component of certain vegetables, appears remarkably safe, and increased FeNO to a greater extent than existing 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 CF 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 vegetable consumption in CF, is worthy of further exploration.

References

- Mhanna MJ, Ferkol T, Martin RJ, Dreshaj IA, van Heeckeren AM, Kelley TJ, Haxhiu MA. Nitric oxide deficiency contributes to impairment of airway relaxation in cystic fibrosis mice. *Am J Respir Cell Mol Biol*. 2001;24:621-626. doi:10.1165/ajrcmb.24.5.4313.
- Everard ML, Donnelly D. A pilot study of oral L-arginine in cystic fibrosis. *J Cyst Fibros*. 2005;4:67-69. doi:10.1016/j.jcf.2004.10.001.
- Grasemann H, Grasemann C, Kurtz F, Tietze-Schillings G, Vester U, Ratjen F. Oral L-arginine supplementation in cystic fibrosis patients: a placebo-controlled study. *Eur Respir J*. 2005;25:62-68. doi:10.1183/09031936.04.00086104.
- Grasemann H, Gonska T, Avolio J, Klingel M, Tullis E, Ratjen F. Effect of ivacaftor therapy on exhaled nitric oxide in patients with cystic fibrosis. *J Cyst Fibros*. 2015;14:727-732. doi:10.1016/j.jcf.2015.07.001.
- Kamosinska B, Radomski MW, Duszyk M, Radomski A, Man SF. Nitric oxide activates chloride currents in human lung epithelial cells. *Am J Physiol*. 1997;272:L1098-L1104.
- Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax*. 1993;48:1034-1043. doi:10.1136/thx.48.10.1034.
- Grasemann H, Michler E, Wallot M, Ratjen F. Decreased concentration of exhaled nitric oxide (NO) in patients with cystic fibrosis. *Pediatr Pulmonol*. 1997;24:173-177. doi:10.1002/(SICI)1099-0496(199709)24:3<173::AID-PPUL2>3.0.CO;2-O.
- Grasemann H, Ioannidis I, Tomkiewicz RP, de Groot H, Rubin BK, Ratjen F. Nitric oxide metabolites in cystic fibrosis lung disease. *Arch Dis Child*. 1998;78:49-53. doi:10.1136/ad.78.1.49.
- Lundberg JO, Gladwin MT, Ahluwalia A, Benjamin N, Bryan NS, Butler A, Cabrales P, Fago A, Feelisch M, Ford PC, Freeman BA, Frenneaux M, Friedman J, Kelm M, Kevil CG, Kim-Shapiro DB, Kozlov AV, Lancaster JR Jr, Lefer DJ, McColl K, McCurry K, Patel RP, Petersson J, Rassaf T, Reutov VP, Richter-Addo GB, Schechter A, Shiva S, Tsuchiya K, van Faassen EE, Webb AJ, Zuckerbraun BS, Zweier JL, Weitzberg E. Nitrate and nitrite in biology, nutrition and therapeutics. *Nat Chem Biol*. 2009;5:865-869. doi:10.1038/nchembio.260.
- Yoon SS, Coakley R, Lau GW, Lymar SV, Gaston B, Karabulut AC, Hennigan RF, Hwang SH, Buettner G, Schurr MJ, Mortensen JE, Burns JL, Speert D, Boucher RC, Has-sett DJ. Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite derivatives under cystic fibrosis airway conditions. *J Clin Invest*. 2006;116:436-446. doi:10.1172/JCI24684.
- Xia DS, Liu Y, Zhang CM, Yang SH, Wang SL. Antimicrobial effect of acidified nitrate and nitrite on six common oral pathogens in vitro. *Chin Med J (Engl)*. 2006;119:1904-1909.
- Major TA, Panmanee W, Mortensen JE, Gray LD, Hoglen N, Hassett DJ. Sodium nitrite-mediated killing of the major cystic fibrosis pathogens *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* under anaerobic planktonic and biofilm conditions. *Antimicrob Agents Chemother*. 2010;54:4671-4677. doi:10.1128/AAC.00379-10.
- Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, Ahluwalia A. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension*. 2008;51:784-790. doi:10.1161/HYPERTENSIONAHA.107.103523.

14. Tsuchiya M, Asada A, Kasahara E, Sato EF, Shindo M, Inoue M. Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation*. 2002;105:1155-1157. doi:10.1161/hc1002.105935.
15. Dougall HT, Smith L, Duncan C, Benjamin N. The effect of amoxycillin on salivary nitrite concentrations: an important mechanism of adverse reactions? *Br J Clin Pharmacol*. 1995;39:460-462. doi:10.1111/j.1365-2125.1995.tb04479.x.
16. Petersson J, Carlström M, Schreiber O, Phillipson M, Christofferson G, Jägar A, Roos S, Jansson EA, Persson AE, Lundberg JO, Holm L. Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. *Free Radic Biol Med*. 2009;46:1068-1075. doi:10.1016/j.freeradbiomed.2009.01.011.

Author Affiliations:

Conor P. Kerley, BSc,†; Emma Kilbride, BSc*; Peter Greally, MD*; Basil Elnazir, MD**

**Paediatric Respiratory Department, National Children's Hospital, Dublin 24, Ireland*

†School of Medicine and Medical Sciences, University College Dublin, Belfield, Dublin 4, Ireland