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'Every beet you take' – lowering systolic blood pressure and improving vascular function/exercise capacity via the dietary nitrate-nitrite-NO pathway in patients with COPD

Running Title: 'Every beet you take'

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Fifteen years ago in the *European Respiratory Journal*, Mannino et al., identified an increased risk of developing hypertension in patients with COPD (GOLD stage 3/4) of 1.6-fold, and cardiovascular disease (CVD) 2.4-fold (1). Thus, hypertension prevalence in COPD is high: 35-55% (2). Hypertension/high systolic blood pressure (SBP) remains the single biggest risk-factor contributor to global mortality (and disability in >50-year-olds) accounting for >50% of CVD deaths, the single largest cause of mortality (3). Therefore, the finding of a significant reduction in SBP (primary outcome) with 90 days' dietary nitrate as beetroot juice in 81 patients with COPD by Alasmari et al., has important implications. With beneficial effects on vascular function and exercise capacity too, we will consider some of the related background (4).

A key pathophysiological link between COPD and hypertension/CVD is endothelial dysfunction associated with diminished nitric oxide (NO) availability, exacerbated by oxidative stress and inflammation (5). The deficit in NO is due largely to impaired production from dysfunctional endothelial NO synthase (eNOS), perturbed by e.g., oxidised low-density lipoprotein and lysophosphatidylcholine, inhibited by asymmetric dimethylarginine (ADMA) and 'uncoupled' from its cofactor tetrahydrobiopterin (BH4) or substrate L-arginine (removed by increased arginase). Thus, instead of generating vasculo-protective NO, eNOS produces reactive oxygen species (ROS), e.g., superoxide, which scavenge NO, decreasing its intravascular bioavailability.

Physiological testing can be used to interrogate NO responses. Brachial artery flow mediated dilatation (FMD), is a non-invasive measure of endothelium-dependent function. A meta-analysis of 8 observational studies found significantly lower FMD in 174 patients with COPD versus 148 controls ($P<0.001$), reflecting diminished NO production/availability (6). FMD impairment was also correlated with FEV_1, independently of hypertension, smoking habit, smoking pack-years, diabetes mellitus, obesity, hyperlipidaemia. From 20 studies, prognostically-important surrogate markers of subclinical atherosclerosis were worse in patients with COPD (n=2082) than 4844 controls: common carotid intima-media thickness (CIMT) ($P<0.001$) and carotid plaques ($P<0.0001$) associated also with GOLD-class severity) (7).

A major challenge has been restoring 'NO deficiency'. NO (gas), with its short half-life, is ineffective. Organic nitrates (isosorbide mononitrate, nitroglycerin) metabolised to NO/activating soluble guanylate cyclase (sGC)), become ineffective within hours (tolerance) and generate ROS, exacerbating vascular dysfunction. The sole endogenous source of NO was thought to be NOS (liable to dysfunction), with the oxidised product of NO, inorganic nitrite (NO_2^-), being physiologically inert. However, nitrite was discovered to represent an alternative source of NO, through chemical/enzymatic reduction via deoxyhaemoglobin and xanthine oxidoreductase, supported by hypoxic and ischaemic conditions (8, 9). Subsequently, inorganic nitrate (NO_3^-), as $NaNO_3$ was demonstrated to lower diastolic BP (DBP) after 3 days ingestion (10), and beetroot juice lowered both SBP and DBP acutely at 2.5-3h, coinciding with peak plasma [nitrite] following ingestion, alongside preservation of endothelial function (11). Hence the 'nitrate-nitrite-NO' pathway was realised (12).

Here, ~25% of ingested nitrate is concentrated in/secreted by the salivary glands (with ~66% excreted via the kidneys), and reduced to nitrite on the posterior surface of the tongue by strict anaerobes (e.g., *Veillonella species*) and facultative anaerobic bacteria (*Actinomyces odontolyticus* and *Rothia mucilaginosa*) (13, 14).

Mainly outside of COPD, several meta-analyses have confirmed BP-lowering effects of inorganic nitrate: with 1-15 days' intervention (16 studies, 254 participants) a significant SBP decrease, ~-4 mmHg (15), and 1-6 weeks' intervention (13 studies, 325 participants) for clinic BP measurements: SBP -4.1 mmHg (95% CI: -6.1, -2.2) and DBP -2.0 mmHg (95% CI: -3.0, -0.9), though not significant for ambulatory and/or home BP monitoring (16). Subsequent longer/larger studies include the 'Vasera' study, a double-blind, randomised controlled study in 126 patients with hypertension and with/at risk of, type 2 diabetes, using daily beetroot juice shots (active nitrate-containing versus identical nitrate-depleted placebo) for 6 months found that whilst nitrate had no effect on large artery (aortic) stiffness (primary outcome) (17), nitrate lowered central/aortic systolic BP by ~2.6 mmHg (18), decreased left-ventricular volumes (19), and decreased CIMT by ~8% (20). Sundqvist et al., randomised 231 patients with clinic SBP 130-159 mmHg to 5 weeks' of either low nitrate vegetables + placebo pills, low-nitrate vegetables + nitrate (300 mg) pills, or green leafy vegetables (300 mg nitrate) + placebo pills but found no effect on 24h ambulatory SBP (primary endpoint) (21).

In patients with COPD, a recent meta-analysis of dietary nitrate included eleven randomised controlled trials (RCTs: ten beetroot juice, seven with blinded placebo, one $NaNO_3$ versus NaCl). Most studies had small numbers: n=8-21 (nine crossover-studies) and n=25, n=122 (2 parallel-design studies), most with short interventions: 1-14 days (ten studies), except one study: 56 days (22). Nitrate marginally reduced SBP −3.39 mm Hg and DBP –2.20 mm Hg (both p=0.05). None of these studies had BP as their primary outcome.

Of the studies in the meta-analysis, the largest was ON-EPIC: multicentre in 165 patients with COPD (GOLD stage 2-4), randomising 78 to nitrate-rich beetroot juice and 87 to placebo, alongside a twice-weekly 56-day pulmonary rehabilitation programme (23). The primary outcome, exercise capacity: incremental shuttle walk test (ISWT) distance, was significantly increased by 30 m by nitrate. Nitrate also lowered clinic BP by 7/7 mmHg (p<0.0005) versus placebo (no home BP measurements were taken in that study). Only ON-EPIC assessed endothelial function: nitrate enhanced FMD (P=0.046) in a sub-study (n=10 active, n=10 placebo juice).

Two studies of dietary nitrate in COPD assessing BP followed. EDEN-OX enrolled 20 patients with COPD on long-term oxygen therapy. Nitrate-containing beetroot juice acutely increased endurance shuttle walk-test time (primary outcome) by ~62 s (P<0.0001) and enhanced FMD by ~12% (P=0.0003) (24). Such large effects could reflect the hypoxic COPD population studied, with baseline resting oxygen saturation FiO_2 0.21 of ~92%, in whom greater reduction of nitrite to NO would be expected, with greater vasodilatation. However, no effect on BP (at 3h) was found; some of nitrite's haemodynamic effects may be normoxia-dependent/inhibited by hypoxia (25).

The current paper by Alasmari et al., is a single-centre study, but with a 50% longer intervention period than ON-EPIC (90 days instead of 8 weeks), though with a smaller number of patients (81), with 40 and 41 patients (average age ~63.5 years) randomised per intervention arm (nitrate-rich beetroot juice (NR-BRJ) and placebo nitrate-depleted beetroot juice (Pl-BRJ), respectively) (4). The patients were selected for a baseline home SBP ≥130 mmHg (Pl-BRJ ~136, NR-BRJ ~135 mmHg), stratifying for antihypertensive use (up to 2 antihypertensives allowed: 15 and 17 to NR-BRJ and Pl-BRJ, respectively). The primary outcome was change in home SBP assessed using a validated device and with a comprehensive measurement schedule: 2 readings at each sitting, three times daily over four days at baseline and then again during the last week of the study, using the mean of all measures recorded on days 2 to 4. The result was a highly significant lowering of SBP by NR-BRJ v Pl-BRJ -4.5mmHg (95% CI, -3.0 to -5.9], p<0.001); see Figure. This highlights the fundamental need, given the intrinsic variability and liability to external confounders of BP, for multiple repeated and carefully performed BP measurements to reduce the noise-to-signal ratio (26, 27). The effect was seen despite potential attenuation by current smoking status (28) in ~14% and antiseptic mouthwash use (n=11) (29), which lacked interaction with the treatment effect. Furthermore, there was no evidence of tolerance/loss of effect over 90 days' intervention, a major limitation of organic nitrates.

The magnitude of SBP-lowering (4.5 mmHg), if sustained, would result in a major impact on cardiovascular events. For example, a recent individual participant-level meta-analysis using data from 358,707 participants from 51 RCTs revealed that pharmacologically lowering SBP by 5 mmHg (median follow-up 3.7-4·5 years) significantly reduced major cardiovascular events by ~18% and ~9% in individuals <55 years and those aged 55–84 years, respectively, an effect seen with a SBP to <120 mmHg (30). The average SBP reduction was 6·3 mmHg. These are similar age/SBP ranges and changes as this study (4).

Whilst there was no reduction in DBP (-1.1mmHg (95% CI -3.1 to 1.0) p=0.31), this represents a numerical decrease of ~3.4 mmHg in pulse pressure (a prognostic marker for adverse cardiovascular events (31). Overall adherence was confirmed by significant increases in plasma [nitrate + nitrite], 'NOx' and FeNO, with nitrate, supported by diary card returns (Pl-BRJ 96% vs NR-BRJ 97%). The beetroot juice was well tolerated and there were no serious adverse events.

As with ON-EPIC and EDEN-OX, endothelial function was assessed but instead of FMD, finger plethysmography peripheral arterial tonometry (EndoPAT2000®) was used: dietary nitrate enhanced reactive hyperaemia index (RHI) (P=0.03) and lowered augmentation index, corrected for heart rate 75 bpm (AIx75) (P=0.026). Again, nitrate-derived NO is likely to be the main explanation for these improvements. Endopat® is a convenient method, here as a secondary outcome (32). AIx is a surrogate measure of arterial stiffness (AS) but is affected by multiple factors including left ventricular ejection, pulse wave velocity (PWV), timing of reflection, arterial tone, structure at peripheral reflecting sites, BP, and heart rate (33). These factors augment the systolic pressure: this augmented pressure (AP), when expressed as a percentage of the pulse pressure, gives the AIx. Whilst PWV is the gold standard of AS, (central) AIx has also been associated with total mortality independently of BP (34).

Six-minute walk test (6MWT) distance was improved by ~30 m by NR-BRJ (n=24) versus Pl-BRJ (n=20) p<0.001, similar to ISWT in ON-EPIC.

Dietary nitrate is a well-tolerated non-pharmacological approach that avoids contributing to side effects/interactions related to polypharmacy and worsening adherence, prevalent in multi-morbid populations. Also, nitrate directly targets underlying 'NO deficiency', related to poor intake of nitrate-rich vegetables (35), and the other mechanisms of endothelial dysfunction. The range of beneficial effects (SBP-lowering, exercise-capacity, endothelial function) means that dietary nitrate is an option for individual patients with COPD. However, nitrate's acceptance into common practice/guidelines will likely require more evidence from larger/longer studies.

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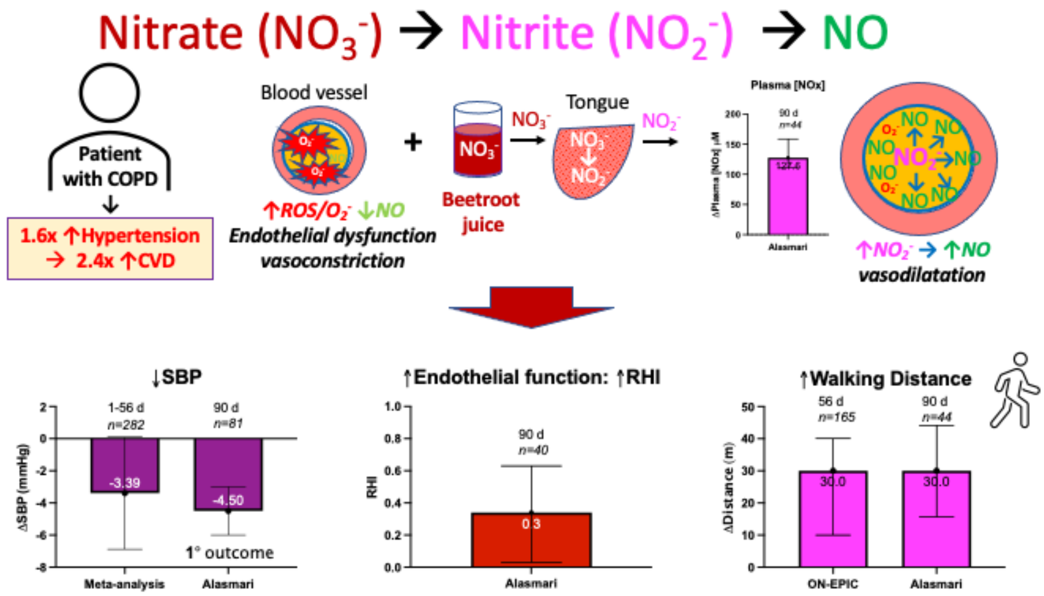

Figure. The 'Nitrate-Nitrite-NO Pathway' in COPD: ingestion of dietary nitrate (beetroot juice) increases circulating plasma [NOx] (NOx = [nitrate] + [nitrite]), lowering systolic blood pressure (SBP), improving vascular/endothelial function: reactive hyperaemia index (RHI), and walking distance. (Other abbreviations: CVD - cardiovascular disease; ROS – reactive oxygen species). Data shown as mean or median ± 95% confidence intervals.

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