



Technological University Dublin
ARROW@TU Dublin

Articles

School of Biological Sciences

2016-4

Dietary Nitrate in Decreased Blood Pressure in Obstructive Sleep Apnoea Syndrome: a Series of N-of-1 trials

Conor Kerley

Technological University Dublin, conor.kerley@gmail.com

Liam Cormican

Department of Respiratory and Sleep Diagnostics, Connolly Hospital, Dublin 15,

Eamon Dolan

Department of Medicine for the Elderly, Stroke and hypertension Unit, Connolly Hospital, Blanchardstown, Dublin 15

Follow this and additional works at: <https://arrow.tudublin.ie/scschbioart>



Part of the [Medical Immunology Commons](#)

Recommended Citation

Kerley, C., Dolan, E. and Cormican, L. (2016) Dietary Nitrate in Decreased Blood Pressure in Obstructive Sleep Apnoea Syndrome: a Series of N-of-1 trials. *Journal of hypertension, Apr. 34:4, 808-810, 2016* doi: 10.1097/HJH.0000000000000858

This Article is brought to you for free and open access by the School of Biological Sciences at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact yvonne.desmond@tudublin.ie, arrow.admin@tudublin.ie, brian.widdis@tudublin.ie.



This work is licensed under a [Creative Commons Attribution-Noncommercial-Share Alike 3.0 License](#)



Correspondence

Dietary nitrate in decreased blood pressure in obstructive sleep apnoea syndrome: a series of N-of-1 trials

AQ1 **Conor P. Kerley^{a,b}, Liam Cormican^a, and Eamon Dolan^c**

Obststructive sleep apnoea syndrome (OSAS) is a consequence of repetitive oropharyngeal airway narrowing/closure during sleep resulting in chronic intermittent hypoxaemia [1]. OSAS is regarded as an independent risk factor for hypertension (HTN) development [2] and is associated with decreased cerebral blood flow [3], leading to daytime neuropsychological sequelae [1]. Blood pressure (BP) follows a circadian rhythm termed dipping and the absence of nocturnal BP dipping is associated with target-organ damage, cerebrovascular disease, myocardial remodelling and increased cardiovascular events/mortality [4]. Abnormal BP in OSAS typically manifests as reduced nocturnal BP dipping [5]. Although continuous positive airway pressure therapy (CPAP) represents the current gold standard treatment of OSAS, its antihypertensive effect is limited [6].

AQ4 Nitrate/nitrite levels indicate the bioavailability of nitric oxide (NO) [7], which is a potent vasodilator. Decreased serum nitrate/nitrite has been reported in OSAS compared with controls [8–10], which could play a significant role in the vascular/neuropsychological consequences of OSAS. Supplementation with dietary nitrate, through reduction to nitrite, has emerged as a novel strategy to increase NO bioavailability and decreased BP in a number of populations [11–13]. We have recently shown an acute BP decrease in the hypoxic condition of COPD after nitrate consumption [14].

AQ5 The nitrate pathway is upregulated under hypoxic conditions [15]. As OSAS is associated with, neuropsychological consequences and elevated BP, dietary manipulation could represent a novel adjunctive method of treatment of OSAS associated with cardiovascular risk. Evidence regarding specific nutritional intervention in OSAS is lacking. We wanted to assess the effects of dietary nitrate in a preliminary but reliable manner using a series of n-of-1 trials before embarking on a more rigorous, double-blind, placebo-controlled trial.

We recruited three middle-aged (mean age = 53 years), obese (mean BMI = 38 kg/m²), Caucasian, CPAP naïve males with severe OSAS (mean AHI = 34) from the sleep clinics of Connolly Hospital (Dublin, Ireland) (Table 1). We excluded study participants who were clinically unstable or using CPAP, organic nitrates or antibiotics. However, two of the study participants were on antihypertensives and two of the study participants were on antidiabetic medications. This study was approved by the Human Research Ethics

Committee of Connolly Hospital, Dublin and written informed consent was obtained from all study participants.

During this randomized, controlled, single-blind, crossover, study participants were tested on three occasions, 14 days apart. On the morning of testing, study participants consumed an identical, light, self-selected breakfast and refrained from alcohol and tobacco. On test days, in an identical manner at the same time of day, study participants had blood drawn for nitrate analysis with the widely used and validated Sievers Nitric Oxide Analyzer [12] and completed the following validated questionnaires: Epworth Sleepiness scale, Pittsburgh Sleep Quality Index, Fatigue Severity Scale and Beck Depression Inventory as well as completing trail making forms A and B, which assess visual attention and task switching by timing the participants speed of completion [16]. After these assessments, study participants wore the noninvasive ambulatory blood monitor (ABPM) Spacelabs 90207 device (Spacelabs Healthcare Ltd., Issaquah, Washington, USA) for 24 h, which has previously been validated by the British Hypertension Society.

Following completion of baseline assessments, the three study participants were randomized to drink either 140 ml concentrated, nitrate-rich beetroot juice (BRJ) (12.9 mmol nitrate) or 140 ml water (<0.5 mmol nitrate) 1 h prior to bedtime for 14 days followed by the crossover condition. The standardized dose of nitrate is attainable with a diet rich in vegetables [17]. We did not include a washout period since the half-life of dietary nitrate is ~6 h and the bioactivity of nitrate returns to baseline 24 h after a bolus dose [18]. Instructions not to alter dietary, tobacco, alcohol, exercise and medication habits were provided to all study participants and advice to avoid use of mouthwash, which is known to decrease nitrite and NO formation from dietary nitrate [19].

For this series of N-of-1 trials, we did not perform a sample size calculation, rather we were interested in the acceptability of our trial design and to assess if dietary nitrate had any effect on BP profiles and several secondary measures. Data was normally distributed and we used 1-tailed, paired *t*-tests as recommended for use in N-of-1 trials [20] to compare the changes after BRJ to the changes after water. We selected nocturnal SBP as the primary outcome because existing literature suggests dietary nitrate has hypotensive effects [11–13] and OSAS is associated with HTN [2,5], which is difficult to treat [6].

The three study participants completed all measures and had 100% compliance with the beverages and reported no lifestyle of medical changes during the protocol. Table 1 displays results for measures of sleep, fatigue, mood, neuropsychological function, BP and serum nitrate. Further, there was a significant negative correlation between serum nitrate and nocturnal SBP values from pooled analysis from the study participants at the 3 time-points ($r = -0.6$; $P = 0.043$).

OSAS is highly prevalent and independently associated with elevated BP [2]. OSAS related HTN is predominantly nocturnal, manifesting as lack of nocturnal BP dipping [5]

Correspondence

TABLE 1. Mean values for baseline post-beetroot juice and post-water assessments

	Baseline	BRJ*	Water*	P value
ESS (0–24)	13	5 (–8)	11 (–2)	0.1
PSQI (0–21)	6	4 (–2)	6 (0)	0.1
FSS (0–60)	45	28 (–17)	38 (–7)	0.018
BDI (0–63)	13	3 (–10)	10 (–3)	0.13
Trail making A (s)	30	27 (–3)	34 (+4)	0.02
Trail making B (s)	51	40 (–11)	61 (+10)	0.056
Mean 24-h SBP (mmHg)	117	114 (–3)	125 (+12)	0.12
Mean 24-h DBP (mmHg)	73	73 (0)	76 (+3)	0.24
Mean nocturnal SBP (mmHg)	118	106 (–12)	123 (+5)	0.08
Mean nocturnal DBP (mmHg)	71	67 (–4)	74 (+3)	0.1
% nocturnal SBP readings above limits	41.8	11.1	56.9	0.08
% nocturnal DBP readings above limits	62.9	42.3	49.7	0.12
% dipping SBP	–1.3	7.5 (+8.8)	2.7 (+4)	0.09
% dipping DBP	4.3	10.4 (+6.1)	4.3 (0)	0.07
NO ₃ [–] (μmol/l)	40	181 (+141)	43 (+3)	0.024

BDI, Beck Depression Inventory; ESS, Epworth Sleepiness scale; FSS, Fatigue Severity Scale; NO₃[–], serum nitrate; PSQI, Pittsburgh Sleep Quality Index.

AQ6

AQ7

and is difficult to treat. CPAP represents the gold standard treatment for OSAS but according to a recent, comprehensive meta-analysis, the antihypertensive effect size is small (2.6 and 2 mmHg drop in SBP and DBP, respectively) [6].

To our knowledge, this is the first report of dietary nitrate in OSAS study participants. In this single-blind, crossover study of CPAP naïve, severe, OSAS study participants, we demonstrate that 14 days of nocturnal BRJ significantly increased serum nitrate ($P=0.024$) and improved symptomatology, particularly fatigue as well as visual attention ($P=0.018$ and 0.02 , respectively). We noted improvements in BP profiles including a nocturnal BP decrease with BRJ consumption (–12/–4 mmHg) compared to water control (+5/+3 mmHg) (Table 1). Although, there was no statistical difference in our primary end point (nocturnal SBP), the size of the effect was clinically significant. For example, it has been estimated that a SBP reduction of 5 mmHg could decrease the risk of mortality because of stroke and CVD by 14 and 9% and 14% [13]. Our findings support previous findings of decreased BP in healthy volunteers [11], study participants with COPD [12] and hypertensive study participants [21] following nitrate consumption. The BP lowering effect we observed (–12/–4 mmHg) was greater than that previously observed in clinical populations with ABPM, including among hypertensives (–7.7/–5.2 mmHg). It is possible that dietary nitrate will have a greater hypotensive role in hypoxic conditions, such as OSAS, because of upregulated reduction of nitrate to NO [15]. Additionally, we observed a significant, inverse correlation between pooled serum nitrate values and nocturnal SBP values. Previously, negative correlations have been demonstrated between nitrate levels and OSAS severity [8–10] as well as SBP in OSAS [8], raising the intriguing possibility that the pathogenesis of HTN in OSAS may be related to the NO bioavailability, and that NO manipulation may have therapeutic potential.

Our pilot study is important for several reasons. It is the first report of dietary nitrate provision in OSAS – a common syndrome associated with neuropsychological sequelae and difficult to treat HTN. The study participants were

phenotypically similar (male, severe OSAS and Caucasian) and typical of the OSAS population attending sleep medicine clinics (obese, sedentary, comorbidities present). Interestingly, a recent trial demonstrated a lack of benefit of dietary nitrate in type 2 diabetes [22]. The large BP reductions observed here may be because of hypoxic upregulation of the nitrate-NO pathway [5]. Two of the three study participants were on antihypertensive medications and baseline ABPM values revealed well controlled SBP in all 3 study participants (122, 122 and 125 mmHg respectively). Nevertheless, nocturnal BRJ supplementation was associated with decreased 24 h and particularly nocturnal BP as well as increased SBP and DBP dipping.

We included stable OSAS outpatients with no medical changes throughout the study. Further, nitrate has a short half-life (~6 h) [18] and rapidly measurable biochemical/physiological effects. These characteristics are essential for n-of-1 trials [23,24]. Although we included homogenous study participants in whom medication and CPAP exposure did not change, our pilot study is limited by a small sample size and the lack of a true placebo. However, values for all parameters were similar at baseline and after water control suggesting that the dietary nitrate was responsible for the effects. Our preliminary findings suggest a potential benefit of dietary nitrate in OSAS, at least in Caucasian males with severe OSAS.

Nocturnal CPAP represents the current gold standard treatment of OSAS. However, the antihypertensive effect size is small [6]. Although the lack of effect may be because of noncompliance, there are few therapeutic alternatives to CPAP and scope exists for the development of adjunctive therapeutic modalities directed towards the attenuation of the hypertensive burden of OSAS.

Nocturnal dietary nitrate may improve NO bioavailability in OSAS, resulting in direct benefits to BP profiles, particularly nocturnal BP. Further work with a larger sample and a true placebo is required to evaluate this therapeutic concept. Our group are currently conducting a double-blind, placebo-controlled crossover trial among OSAS study participants to determine the effect, if any, of dietary nitrate in OSAS.

ACKNOWLEDGEMENTS

Information about previous presentations: subsets of the data have been presented by Conor Kerley (BSc, PhD candidate) at the European Society of Hypertension Summer School (Schloss Hernstein, Austria, September 2015) and at the Irish Cardiac Society meeting in Killarney, Ireland, in October 2015.

Funding information: this research was supported by the Irish Heart Foundation. The funding bodies had no involvement in study design, data collection, analysis or interpretation.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Bucks RS, Olaihe M, Eastwood P. Neurocognitive function in obstructive sleep apnoea: a meta-review. *Respirology* 2013; 18:61–70.
- Grote L, Hedner J, Peter JH. Sleep-related breathing disorder is an independent risk factor for uncontrolled hypertension. *J Hypertens* 2000; 18:679–685.
- Joo EY, Tae WS, Han SJ, Cho JW, Hong SB. Reduced cerebral blood flow during wakefulness in obstructive sleep apnea-hypopnea syndrome. *Sleep* 2007; 30:1515–1520.
- Palatini P, Reboldi G, Beilin LJ, Casiglia E, Eguchi K, Imai Y, *et al*. Added predictive value of night-time blood pressure variability for cardiovascular events and mortality: the Ambulatory Blood Pressure-International Study. *Hypertension* 2014; 64:487–493.
- Wolf J, Hering D, Narkiewicz K. Nondipping pattern of hypertension and obstructive sleep apnea syndrome. *Hypertens Res* 2010; 33:867–871.
- Fava C, Dorighi S, Dalle Vedove F, Danese E, Montagnana M, Guidi GC, *et al*. Effect of CPAP on blood pressure in patients with OSA/hypopnea a systematic review and meta-analysis. *Chest* 2014; 145:762–771.
- Kleinbongard P, Dejam A, Lauer T, Jax T, Kerber S, Gharini P, *et al*. Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. *Free Radic Biol Med* 2006; 40:295–302.
- Ip MS, Lam B, Chan LY, Zheng L, Tsang KW, Fung PC, Lam WK. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *Am J Respir Crit Care Med* 2000; 162:2166–2171.
- De Lima AM, Franco CM, De Castro CM, Bezerra Ade A, Ataíde L Jr, Halpern A. Effects of nasal continuous positive airway pressure treatment on oxidative stress and adiponectin levels in obese patients with obstructive sleep apnea. *Respiration* 2010; 79:370–376.
- Franco CM, Lima AM, Ataíde L Jr, Lins OG, Castro CM, Bezerra AA, *et al*. Obstructive sleep apnea severity correlates with cellular and plasma oxidative stress parameters and affective symptoms. *J Mol Neurosci* 2012; 47:300–310.
- Siervo M, Lara J, Oghonmwan I, Mathers JC. Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *J Nutr* 2013; 143:818–826.
- Kerley CP, Cahill K, Bolger K, McGowan A, Burke C, Faul J, Cormican L. Dietary nitrate supplementation in COPD: an acute, double-blind, randomized, placebo-controlled, crossover trial. *Nitric Oxide* 2015; 44:105–111.
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, *et al*. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; 42:1206–1252.
- Presley TD, Morgan AR, Bechtold E, Clodfelter W, Dove RW, Jennings JM, *et al*. Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide* 2011; 24:34–42.
- Lundberg JO, Weitzberg E. Nitrite reduction to nitric oxide in the vasculature. *Am J Physiol Heart Circ Physiol* 2008; 295:H477–H478.
- Bowie CR, Harvey PD. Administration and interpretation of the Trail Making Test. *Nat Protoc* 2006; 1:2277–2281.
- Sobko T, Marcus C, Govoni M, Kamiya S. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide* 2010; 22:136–140.
- Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, *et al*. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 2008; 51:784–790.
- Govoni M, Jansson EA, Weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 2008; 19:333–337.
- Chen X, Chen P. A comparison of four methods for the analysis of N-of-1 trials. *PLoS One* 2014; 9:e87752.
- Kapil V, Khambata RS, Robertson A, Caulfield MJ, Ahluwalia A. Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebo-controlled study. *Hypertension* 2015; 65:320–327.
- Gilchrist M, Winyard PG, Aizawa K, Anning C, Shore A, Benjamin N. Effect of dietary nitrate on blood pressure, endothelial function, and insulin sensitivity in type 2 diabetes. *Free Radic Biol Med* 2013; 60:89–97.
- Nikles J, Mitchell GK, Schluter P, Good P, Hardy J, Rowett D, *et al*. Aggregating single patient (n-of-1) trials in populations where recruitment and retention was difficult: the case of palliative care. *J Clin Epidemiol* 2011; 64:471–480.
- Hart A, Sutton CJ. n-of-1 trials and their combination: suitable approaches for CAM research? *Complement Ther Med* 2003; 11:213–214.

Journal of Hypertension 2016, 34:000–000

^aDepartment of Respiratory and Sleep Diagnostics, Connolly Hospital, Dublin 15, ^bSchool of Medicine, University College Dublin 4 and ^cDepartment of Medicine for the Elderly, Stroke and hypertension Unit, Connolly Hospital, Blanchardstown, Dublin 15, Ireland

Correspondence to Conor P. Kerley, BSc, Connolly Hospital, Blanchardstown, Dublin, Ireland Tel: +00353831458796; e-mail: conorkerley@gmail.com

DOI: 10.1097/HJH.0000000000000858