

2017-1

Nitrate-Rich Beetroot Juice Selectively Lowers Ambulatory Pressures and LDL Cholesterol in Uncontrolled but not Controlled Hypertension: a Pilot Study.

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Recommended Citation

Kerley, C., Dolan, E., Cormican, L. (2017) Nitrate-rich beetroot juice selectively lowers ambulatory pressures and LDL cholesterol in uncontrolled but not controlled hypertension: a pilot study. *Irish journal of medical science*, 2017 Nov;186(4):895-902. doi: 10.1007/s11845-016-1551-2.

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Irish Journal of Medical Science (1971 -)

Nitrate-rich beetroot juice selectively lowers ambulatory pressures and LDL cholesterol in uncontrolled but not controlled hypertension: a pilot study.

--Manuscript Draft--

Manuscript Number:	IJMS-D-16-00495R1	
Full Title:	Nitrate-rich beetroot juice selectively lowers ambulatory pressures and LDL cholesterol in uncontrolled but not controlled hypertension: a pilot study.	
Article Type:	Original Article	
Keywords:	dietary nitrate, nitrite, nitric oxide, hypertension, blood pressure.	
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Funding Information:	Irish Heart Foundation (IE)	Dr. Conor Kerley
Abstract:	<p>Background Dietary nitrate has been shown to increase nitrate/nitrite levels in multiple populations, with potential blood pressure lowering effects. However, there are few reports among hypertensives.</p> <p>Aims We aimed to assess the effect of daily nitrate in subject with controlled hypertension vs. uncontrolled hypertension.</p> <p>Methods On day 0, hypertensives wore an ambulatory BP monitor (ABPM) for 24h and fasting blood was taken. Subjects then consumed concentrated beetroot juice (12.9mmol nitrate) for 14 consecutive days. On day 14 subjects consumed their last nitrate dose after fasting blood was drawn and again had an ABPM for 24h.</p> <p>Results According to baseline ABPM, 11 subjects had controlled BP while 8 had uncontrolled BP. There were similar, significant increases in serum nitrate/nitrite in both groups. We observed little change in BP variables among controlled hypertensives. However, there were reductions in BP variables in uncontrolled hypertensives where decreases in nighttime DBP ($-6 \pm 4.8\text{mmHg}$), arterial stiffness (-0.08 ± 0.03 ambulatory arterial stiffness index) and LDL ($-0.36 \pm 0.42\text{mmol/L}$) reached significance ($p=0.003$, 0.05 and 0.046 respectively).</p> <p>Conclusions Our results support the existing data suggesting an anti-hypertensive effect of nitrate-containing beetroot juice, but only among those with uncontrolled hypertension.</p>	

Abstract

Background

Dietary nitrate has been shown to increase nitrate/nitrite levels in multiple populations, with potential blood pressure lowering effects. However, there are few reports among hypertensives.

Aims

We aimed to assess the effect of daily nitrate in subject with controlled hypertension vs. uncontrolled hypertension.

Methods

On day 0, hypertensives wore an ambulatory BP monitor (ABPM) for 24h and fasting blood was taken. Subjects then consumed concentrated beetroot juice (12.9mmol nitrate) for 14 consecutive days. On day 14 subjects consumed their last nitrate dose after fasting blood was drawn and again had an ABPM for 24h.

Results

According to baseline ABPM, 11 subjects had controlled BP while 8 had uncontrolled BP. There were similar, significant increases in serum nitrate/nitrite in both groups. We observed little change in BP variables among controlled hypertensives. However, there were reductions in BP variables in uncontrolled hypertensives where decreases in nighttime DBP ($-6 \pm 4.8\text{mmHg}$), arterial stiffness (-0.08 ± 0.03 ambulatory arterial stiffness index) and LDL ($-0.36 \pm 0.42\text{mmol/L}$) reached significance ($p=0.003$, 0.05 and 0.046 respectively).

Conclusions

Our results support the existing data suggesting an anti-hypertensive effect of nitrate-containing beetroot juice, but only among those with uncontrolled hypertension.

1 **Title Page**

2

3 **Title:** Nitrate-rich beetroot juice selectively lowers ambulatory pressures and LDL
4 cholesterol in uncontrolled but not controlled hypertension: a pilot study.

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6 **Brief title:** Beetroot juice lowers blood pressures and LDL.

7

8 **Abstract word count:** 183

9 **Word Count:** 2,830

10 **Tables:** 3

11 **Figures:** 0

12

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31 **Acknowledgements:**

32 This work was funded by the Irish Heart Foundation. The authors also wish to
33 acknowledge Dr. Jamie Blackwell (BSc) of the University of Exeter, who analyzed
34 serum samples for nitrate and nitrite.

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51 **Abbreviations:**

52 AASI = ambulatory arterial stiffness index;

53 ABPM = ambulatory blood pressure;

54 BMI = body mass index;

55 BP = blood pressure;

56 BRJ = beetroot juice;

57 FMD = flow mediated dilation;

58 HTN = hypertension;

59 NO = nitric oxide;

60 NOS = nitric oxide synthase.

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76 **Abstract**

77 **Background**

78 Dietary nitrate has been shown to increase nitrate/nitrite levels in multiple
79 populations, with potential blood pressure lowering effects. However, there are few
80 reports among hypertensives.

81

82 **Aims**

83 We aimed to assess the effect of daily nitrate in subject with controlled hypertension
84 vs. uncontrolled hypertension.

85

86 **Methods**

87 On day 0, hypertensives wore an ambulatory BP monitor (ABPM) for 24h and fasting
88 blood was taken. Subjects then consumed concentrated beetroot juice (12.9mmol
89 nitrate) for 14 consecutive days. On day 14 subjects consumed their last nitrate dose
90 after fasting blood was drawn and again had an ABPM for 24h.

91

92 **Results**

93 According to baseline ABPM, 11 subjects had controlled BP while 8 had uncontrolled
94 BP. There were similar, significant increases in serum nitrate/nitrite in both groups.

95 We observed little change in BP variables among controlled hypertensives. However,
96 there were reductions in BP variables in uncontrolled hypertensives where decreases
97 in nighttime DBP ($-6 \pm 4.8\text{mmHg}$), arterial stiffness (-0.08 ± 0.03 ambulatory arterial
98 stiffness index) and LDL ($-0.36 \pm 0.42\text{mmol/L}$) reached significance ($p=0.003$, 0.05 and
99 0.046 respectively).

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101 Conclusions

102 Our results support the existing data suggesting an anti-hypertensive effect of nitrate-
103 containing beetroot juice, but only among those with uncontrolled hypertension.

104 **Keywords:** dietary nitrate, nitrite, nitric oxide, hypertension, blood pressure.

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126 **Introduction**

1
2 127 Nitric oxide (NO) is a pluripotent molecule with diverse systemic effects, including
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4 128 systemic vasodilation [1] and blood pressure (BP) regulation [2]. NO bioavailability
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6 129 is reflected by levels of its metabolites; nitrate and nitrite [3]. Multiple studies have
7
8 130 shown that NO metabolites are significantly lower in hypertension (HTN) compared
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10 131 to matched controls [4-7]. Additionally, serum NO levels have been reported to
11
12 132 correlate negatively with both systolic and diastolic BP [7] and depend on dietary
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14 133 intake in HTN and ischemic stroke [8]. Therefore, dietary interventions to increase
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16 134 either the bioavailability or bioactivity of NO may have clinical utility in HTN.
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24 136 Until recently it was assumed that the only route for NO synthesis *in vivo* was via NO
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26 137 synthase (NOS) acting on its substrate, L-arginine. However, nitrite derived from
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28 138 dietary inorganic-nitrate has been shown to be a substrate for NOS-independent
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30 139 production of NO [9]. This involves both enzymatic and non-enzymatic reduction of
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32 140 nitrate to nitrite and to NO. Indeed, dietary nitrate has been shown to act as a
33
34 141 precursor, in a dose-dependent manner, to nitrite and hence NO (10). Dietary nitrate
35
36 142 has multiple cardioprotective effects as reviewed previously [11-13] and several
37
38 143 authors have suggested that dietary nitrate is the major component responsible for the
39
40 144 cardioprotective effect of vegetables [11, 13]. Further, a 2013 meta-analysis
41
42 145 concluded that dietary nitrate can reduce systolic BP by 4.4mmHg ($p<0.001$) and
43
44 146 diastolic BP by 1.1mmHg ($p=0.06$) among those without hypertension (14). There is a
45
46 147 lack of data regarding dietary nitrate supplementation among hypertensives. However,
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48 148 two recent randomized, double-blind, placebo-controlled trials of nitrate
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50 149 supplementation among hypertensives have emerged but 24h ABPM results were
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52 150 conflicting [15,16].
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152 In this proof of concept study, we wanted to assess the effect of 14d dietary nitrate on
153 ambulatory BP, arterial stiffness, serum nitrate/nitrite, lipids as well as renal and liver
154 indices among treated hypertensives. We further wanted to assess any differences
155 between controlled hypertensives and uncontrolled hypertensives as well as any
156 potential adverse outcomes.

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176 **Materials and Methods**

177 **Study design**

178 During this uncontrolled, pilot study, subjects were tested on two separate occasions,
179 baseline (day 1) and endpoint (day 15) (Fig. 1). On both days, in an identical manner
180 and at the same time of day, fasting blood was drawn, the subject was fitted with an
181 ambulatory blood pressure monitor (ABPM) for 24h and demographics, including
182 body mass index as well as habitual dietary, exercise, smoking, alcohol and
183 medication habits were recorded.

184

185 **Study participants**

186 We conducted an uncontrolled, pilot study of daily nitrate supplementation in
187 clinically stable, Caucasian hypertensive outpatients, established on diverse
188 antihypertensive regimens. We excluded subjects with kidney disease or diabetes and
189 those on organic nitrates.

190

191 This study was conducted according to the guidelines laid down in the Declaration of
192 Helsinki and all procedures involving human subjects/patients were approved the
193 Human Research Ethics Committee of Connolly Hospital, Dublin. Informed consent
194 was obtained from all individual participants included in the study.

195

196 **Beetroot juice**

197 All subjects were asked to consume 140ml beetroot juice (BRJ) daily for 14
198 consecutive days. We selected this dose (12.9mmol nitrate) as the nitrate content is
199 attainable with a diet rich in vegetables [17]. During the trial all subjects were
200 provided with written and verbal instructions not to alter dietary, tobacco, alcohol,

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201 exercise or medication habits and not to use mouthwash, which is a known inhibitor
202 of dietary nitrate reduction to nitrate [18]. Subjects took their 14th and final dose of
203 BRJ after their second fasted blood sample, while wearing the ABPM for a second
204 occasion. Compliance with the BRJ was assessed with a daily diary.

205

206 **Outcome measures**

207 **Twenty four hour ambulatory blood pressure measurement**

208 24h ABPM was performed on day 1 and day 15 using the non-invasive ABPM
209 Spacelabs 90207 machine (Spacelabs Healthcare Ltd., Issaquah, WA, USA). For each
210 assessment, the ABPM was fitted on the upper left arm by a researcher (CPK) in the
211 morning after 10 minutes of quiet rest.

212

213 An initial BP reading was taken in the clinic and subjects were asked to return to the
214 clinic wearing the ABPM 24h later. The device was programmed to record BP every
215 30mins between the hours of 07.00 and 23.00 and every 60mins from 23.00 to 07.00.
216 Participants were advised that they could carry out their usual activities but to avoid
217 strenuous exercise. For each BP measurement, volunteers were asked to hang their
218 arm loosely down the side of their body while keeping still until the end of the
219 measurement. If active, the volunteers were instructed to stop and remain stationary
220 while the measurement was being recorded. Proprietary software was used to
221 download readings and produce 24h, daytime (0700–2300 hours) and nighttime
222 (23:00– 07:00 hours) mean BP readings (90256 ABP Report Management System;
223 Spacelabs Healthcare). We defined uncontrolled HTN as either baseline 24h SBP
224 >130mmHg or 24h DBP >80mmHg as recorded with APBM and controlled BP as
225 <130/80mmHg [19]. Ambulatory arterial stiffness index (AASI) was derived from

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226 individual 24h ABPM whereby, regression slope of diastolic BP on systolic BP was
227 computed. AASI was defined as 1 minus the regression slope, as previously described
228 [21].

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230 **Biochemical analysis**

231 On day 1 and 15, two fasting, venous blood samples were drawn into serum tubes
232 (Sarstedt Monovette Serum Z) which have a low nitrate/nitrite content before ABPM
233 set up. One tube was analyzed locally in our clinical laboratory for routine lipid
234 parameters (total cholesterol, LDL, HDL), liver (albumin, calcium, urea) and renal
235 (sodium, potassium, creatinine) parameters.

236

237 The second tube was centrifuged at 4,000RPM and 4°C for 10m immediately after
238 phlebotomy. Serum was subsequently extracted into Eppendorf tubes and frozen at
239 -80°C and later analyzed for NO metabolites (nitrate/nitrite), which reflect NO
240 bioavailability [3] as previously described [20].

241

242 **Statistical methods**

243 For this pilot study we did not perform a power calculation. Paired t-tests were used
244 to compare baseline and post BRJ values for each group while unpaired t-tests were
245 used to compare controlled and uncontrolled HTN. Results were expressed as mean \pm
246 standard deviation. All statistical tests were conducted at the two-sided 0.05
247 significance level using SAS (version 9.0).

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251 **Results**

252 **Study population**

253 Of the 55 subjects we screened, twenty subjects were recruited. There was a single
254 drop out (female, 61y, BMI=33.6kg/m²) due to acute complication of an underlying
255 pulmonary issue (unrelated to study). The baseline characteristics of the 19 subjects
256 who completed this pilot study are displayed in table 1. Both groups were composed
257 mainly of males. There was no difference in baseline BMI but the uncontrolled group
258 were significantly younger (49 vs. 60.9y; p=0.023). There was little difference in anti-
259 hypertensive use among the groups (1.5 agents per subject in controlled vs. 1.1 agents
260 per subject in uncontrolled). Throughout the study, there was no reported change in
261 prescribed medication, or habitual diet/exercise between visits.

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276 **Table 1: Baseline characteristics**

	Controlled (n 11)		Uncontrolled (n 8)		P-value
	Mean	SD	Mean	SD	
Age (years)	60.9	9.1	49.3	14.5	0.023
Male gender n (%)	10 (91)		7 (88)		-
BMI (kg/m ²)	30.8	2.4	29.5	6.0	0.3
Weight category*					
Healthy range n (%)	0 (0)		2 (25)		-
Overweight n (%)	3 (27)		3 (38)		-
Obese n (%)	8 (73)		3 (38)		-
Smoking status (n)					
Current smoker	0		2 (25)		-
Ex-smoker	7 (64)		4 (50)		-
LLNS	4 (36)		2 (25)		-
Baseline systolic BP	124	14.0	136	11.0	0.035
Baseline diastolic BP	73	9.0	89	8.0	0.0004
Average No. BP meds	1.5		1.1		-
Aspirin n	2		4		-
Statin n	4		4		-
Co-morbidities					
Cerebrovascular disease n	4		1		-
Coronary artery disease n	2		2		-
Hypercholesterolaemia n	4		2		-

277 BP, blood pressure; LLNS, life-long non-smoker

278

279 **Biochemistry**

280 Baseline serum nitrate was 116% higher in the uncontrolled group compared to the
281 controlled group but this was not significant (23.6 vs. 50.9µM; p=0.09). In contrast,
282 baseline serum nitrite was 14% lower in the in the uncontrolled group compared to
283 the controlled group. Again this was not significant (98.2 vs. 86.2nM; p=0.25) (table
284 2).

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290 **Table 2: Biochemical indices before and after nitrate supplementation (BRJ)**

	Controlled				Uncontrolled				
	Pre-BRJ	Post-BRJ	Absolute change	* P-value	Pre-BRJ	Post-BRJ	Absolute change	* P-value	# P-value
Nitrate (μM)	23.6	181.6	158	0.0018	50.9	155.3	104.4	0.016	0.38
Nitrite (nM)	98.2	174.3	76.1	0.011	86.2	163.7	77.5	0.007	0.89

291 BRJ, beetroot juice

292 * p-values are derived from paired t-tests of the absolute change within each group

293 # p-values derived from unpaired t-tests of the absolute changes between groups.

294

295 Fasting serum nitrate increased by 770% and 310% in the controlled and uncontrolled

296 groups respectively. Similarly, serum nitrite increased 177% and 190% in the

297 controlled and uncontrolled groups respectively. However, these differences did not

298 reach statistical significance (table 2). Mean LDL concentrations decreased in 7 of 8

299 subjects in the uncontrolled group (and this reduction was significant compared to the

300 controlled group (3.35 ± 0.47 to 2.99 ± 0.64 vs 2.14 ± 0.67 to 2.47 ± 0.87 ; $p=0.023$).

301 There were no significant differences in any other lipid, liver or renal parameters

302 within or between groups (data not shown).

303

304 Ambulatory blood pressure

305 Average ABPM wear time was 23.3h at baseline and 22.8 at endpoint, with a mean of

306 34.5 successful readings taken per subject at both baseline and endpoint. Mean group

307 values for 24h, day and night systolic and diastolic BP as well as ambulatory arterial

308 stiffness index (AASI) are displayed in table 3. Consumption of dietary nitrate was

309 not associated with decreased 24h, day or night BP in the controlled hypertensives. In

310 contrast after nitrate consumption, there were reductions in night BP and arterial

311 stiffness in the uncontrolled hypertensives (table 3).

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314 **Table 3: Ambulatory blood pressure monitor results**

1 2 3 4	Controlled				Uncontrolled				# P-value
	Pre-BRJ	Post-BRJ	Absolute change	* P-value	Pre-BRJ	Post-BRJ	Absolute change	* P-value	
24h SBP	123.7 ± 13.7	126.8 ± 16.6	3.1	0.2	135.7	133.1	-2.6	0.29	0.09
24h DBP	72.3 ± 8.7	75 ± 10.7	2.7	0.078	88.8	86.1	-2.7	0.15	0.026
1 Day SBP	126.2 ± 13.7	127.5 ± 17.6	1.3	0.42	136.4	136.1	-0.3	0.48	0.33
1 Day DBP	74.5 ± 9.4	75.7 ± 10	1.2	0.39	89.8	88.9	-1	0.42	0.2
Night SBP	118.2 ± 15.4	121.1 ± 15.8	2.9	0.37	125.8	119	-6.8	0.11	0.07
Night DBP	66.5 ± 9.1	72.1 ± 12.8	5.6	0.08	80.3	74.3	-6	0.03	0.0058
Night SBP max	134.8 ± 20	139.6 ± 19	4.8	0.37	145.3 ± 9.6	135 ± 11.6	-10.3	0.1	0.064
Night DBP max	79.5 ± 20.4	85.4 ± 17	5.9	0.18	94.5 ± 6.3	88.1 ± 13.3	-6.4	0.26	0.083
AASI	0.43 ± 0.2	0.48 ± 0.18	0.05	0.13	0.42 ± 0.13	0.34 ± 0.11	-0.08	0.05	0.1
%SBP Dipping	6.2 ± 9.1	4 ± 11.1	-2.2	0.64	9.2 ± 4	13.7 ± 5.3	4.5	0.13	0.21
%DBP Dipping	11 ± 8.1	5.1 ± 12.8	-5.9	0.21	11.7 ± 6.6	17.5 ± 8	5.8	0.09	0.04

315 AASI, ambulatory arterial stiffness index; BRJ, beetroot juice; DBP, diastolic blood pressure; SBP, systolic blood pressure.

316 * p-values are derived from paired t-tests of the absolute change within each group.

317 # p-values derived from unpaired t-tests of the absolute changes between groups.

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320 **Adverse events**

321 There were no reported adverse events. The BRJ was well tolerated and as reported

322 previously [16], 8 subjects (38%) reported transient, red/pink urine (beeturia).

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328 **Discussion**

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2 329 In this proof of concept study, we demonstrate that daily, nitrate-rich beetroot juice
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5 330 for 14d led to increased NO bioavailability in both controlled and uncontrolled HTN
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7 331 and reductions night BP, AASI as well as LDL cholesterol in uncontrolled
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9 332 hypertensives only. Further, the intervention was well-tolerated, safe and did not lead
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11 333 to excessive BP lowering in controlled HTN.
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17 335 Nitrate-rich beetroot juice was associated with significant increases in both serum
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19 336 nitrate and nitrite in controlled and uncontrolled hypertensives. These increases were
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21 337 less than observed in previous, acute studies utilizing the same dose of nitrate [16,17].
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24 338 In this context it is important to note that we collected fasting blood samples and
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26 339 therefore the BRJ would have been consumed 12-24h prior to blood collection.
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29 340 Considering the peak increase in plasma nitrate and nitrite due to exogenous nitrate
30
31 341 occurs 2-3h following ingestion [22] it is understandable that the increases in serum
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33 342 nitrate and nitrite we observed were blunted compared to previous studies. It is
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35 343 noteworthy that all subjects had fasting blood samples taken on both day 1 and 15 and
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37 344 were then fitted with the ABPM before consuming BRJ – therefore ABPM recordings
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39 345 were conducted while nitrate was bioactive.
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46 347 Here, we observed decreases in BP profiles in uncontrolled hypertensives only. The
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48 348 effects were most apparent in nighttime DBP (p=0.03), DBP dipping (p=0.09).
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51 349 Although, other variables did not reach statistical significance, this proof of concept
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53 350 study included a small sample and was not powered to detect statistically significant
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55 351 results. In this context, our observations are clinically significant. Since the discovery
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57 352 that dietary nitrate can increase NO bioavailability, much research has focused on its
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353 anti-hypertensive potential. A 2013 meta-analysis concluded that dietary nitrate can
354 reduce systolic BP by 4.4mmHg ($p<0.001$) and diastolic BP by 1.1mmHg ($p=0.06$) in
355 those without hypertension [14]. Despite much recent interest in the anti-hypertensive
356 effect of dietary nitrate, most of the trials in this meta-analysis were of short duration
357 (2h to 15d) and assessed young, healthy adults. There is a lack of interventional data
358 among hypertensives. A 2013 pilot study, provided evidence that dietary nitrate was a
359 plausible antihypertensive agent. In a small cohort of untreated, stage 1 hypertensives
360 exogenous nitrate increased plasma nitrite 150% and this was associated with
361 decreases in mean 24h in systolic- (-11.2mmHg; $p<0.001$) and diastolic-blood
362 pressure (-9.6mmHg; $p<0.001$) [23]. Two recent, well-conducted trials provided
363 conflicting evidence, one demonstrating benefit [15] and another, no effect [16].
364 These differences may be due to differing dosing regimens, intervention periods and
365 patient demographics including BMI, age and medications. Our results suggest that
366 any reduction of BP in treated hypertensives may be greatest among those with higher
367 initial BP and after ≥ 7 d of nitrate dosing. This observation is consistent with previous
368 studies [15, 16, 24].
369
370 Plasma nitrite reflects flow mediated dilation (FMD) [25] and through its
371 bioactivation to NO is recognized to be a critical pathway regulating basal vascular
372 tone, arterial stiffness and BP [25, 26]. Therefore altering plasma nitrite, including by
373 dietary means, has potential to affect endothelial function and arterial stiffness. In
374 addition to BP reduction, we also observed a significant reduction in arterial stiffness
375 ($p=0.05$). Previous randomized, controlled crossover trials have demonstrated that
376 dietary nitrate can significantly decrease arterial stiffness and significantly improve
377 endothelial function in healthy subjects acutely (2-6 hours) in conjunction with

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378 significantly increased NO metabolites [22-31] These studies utilized nitrate doses
379 varying from 1.1-22.5mmol. Further, 20 healthy overweight/ slightly obese men were
380 randomized to a high fat meal with nitrate-rich BRJ (8.1mmol nitrate) or nitrate-
381 depleted BRJ. Postprandial impairment in FMD was improved after the nitrate-rich
382 BRJ compared with placebo (-0.37% vs -1.56%; p=0.03) [31]. Further, the effect
383 appears to be maintained as evidenced by longer trials (7d) [30]. However, 7d of a
384 high-nitrate diet (4.84mmol nitrate/day from green leafy vegetables) compared to a
385 low-nitrate diet did not affect multiple BP variables or arterial stiffness among 38
386 middle aged adults with high-normal BP (SBP=120-139mmHg) [32]. Nevertheless, a
387 double-blind, randomized, controlled trial of drug-treated (n=34) and drug-naïve
388 (n=34) hypertensives demonstrated that 28d of 6.4mmol nitrate improved endothelial
389 function (FMD) by ~20% (P<0.001) and reduced arterial stiffness (as assessed by
390 pulse wave velocity) by 0.59 m/s (0.24-0.93; P<0.01) [15].

391
392 We also demonstrate for the first time that 14d dietary nitrate significantly decreased
393 serum LDL among 8 subjects with uncontrolled HTN. According to the Third Report
394 of The National Cholesterol Education Program [33], baseline LDL levels in the
395 uncontrolled hypertensives were in the ‘borderline high’ category. After 14d dietary
396 nitrate, LDL levels were ‘near optimal/above optimal’ category (p=0.046). This
397 reduction was specific to the uncontrolled HTN group and was significant compared
398 to controlled hypertensives (p=0.023). This observation is interesting, particularly in
399 light of our small sample size, short intervention period and considering there was no
400 change in diet, exercise or medications, including antihyperlipidaemic medications.
401 We cannot however, rule out a type one error, particularly because we are not aware
402 of any human intervention study involving provision of dietary nitrate which reported

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403 cholesterol or its subfractions. In this context, it is interesting that decreased basal
404 plasma nitrate/nitrite level has been reported in hypercholesterolemic subjects with
405 suspected coronary artery disease but not in normocholesterolemic subjects [34].
406 Hypercholesterolemia may reduce the NO bioavailability and several explanations
407 have been offered for this, including decreased availability of L-arginine, the substrate
408 for NOS [35]; decreased synthesis of NO through degeneration of endothelial G-
409 protein or G-protein-dependent pathways [36] and reduced expression of endothelial
410 NOS [37, 38]. Further, plasma nitrate/nitrite levels have previously been reported to
411 correlate negatively with both total cholesterol ($r = -0.40$, $p < 0.01$) and LDL
412 cholesterol levels ($r = -0.37$, $p < 0.003$) [34]. Interestingly statins, widely prescribed for
413 their cholesterol lowering properties activate endothelial NOS [39]. Further, there is
414 evidence that 8 weeks of dietary nitrate (100 mg/L in drinking water) reduced LDL
415 cholesterol in normal (1.12 to 0.75mmol/L; $p < 0.05$) and diabetic rats (1.12 to
416 0.46mmol/L; $p < 0.05$) compared to normal and diabetic rats without nitrate [40].
417 Although, our study cannot provide mechanistic insight for the LDL reductions, our
418 data provide preliminary evidence for the first time that dietary nitrate reduces LDL
419 levels in uncontrolled hypertensive patients. This observation is consistent with
420 preliminary research suggesting that nitrate targets a novel pathway to enhance fat
421 metabolism and/or energy utilization [41] and decreases lipid levels in animal models
422 [40-42].
423
424 NO has multiple roles in cardio-metabolic regulation. Several comprehensive reviews
425 have highlighted the diverse cardioprotective effects of dietary nitrate [11-13]. Some
426 authors have even suggested that dietary nitrate is the major component responsible
427 for the cardioprotective effect of vegetables [11, 12]. It has been demonstrated that

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428 dietary nitrate acts as a precursor to NO in a dose-dependent manner where by a single
429 serving of a nitrate-rich vegetables contains more nitrate than what is formed
430 endogenously by the all three NOS isoforms combined in 24h [9]. Dietary nitrate
431 increases vasodilation as well as inhibiting production of mitochondrial reactive
432 oxygen species and platelet aggregation. Despite the complex nature of NO and the
433 multiple contributors to NO bioavailability (e.g. underlying pathology, medication
434 use, serum lipids, tobacco exposure, exercise, alcohol intake), diet has been shown to
435 be the major influencer of serum NO in patients with HTN and ischemic stroke [8].
436 In this context, our results and those of others should not be considering surprising.
437
438 This trial has several key strengths. The use of 24h ABPM provides a robust, reliable
439 method of determining BP. We asked subjects to maintain their typical dietary,
440 exercise, alcohol, tobacco and medication habits throughout this study. Therefore our
441 observations closely reflect that effect of supplementary nitrate to the everyday lives
442 of hypertensives. The increases in serum nitrate and nitrite confirmed compliance
443 with the intervention.
444
445 This pilot study did not include a control arm or a placebo. Therefore, it is possible
446 that any observed effect was simply regression to the mean. It is also possible that any
447 effect observed here may be due to non-nitrate components of beetroot juice.
448 However, emerging trials utilizing nitrate-rich beetroot juice and identical, nitrate-
449 depleted beetroot juice have demonstrated no physiological effect of nitrate-depleted
450 beetroot juice. Further, our results are consistent with a recent meta-analysis [14] and
451 a double-blind, randomized, placebo-controlled trial [15]. Therefore we suggest that
452 our observations are due to dietary nitrate. The number of participants in our study (n

1 453 = 19) may be considered small. However, many previous studies investigating

2 454 chronic nitrate intake on BP had similar numbers [16, 24].

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7 456 In this pilot study, we observed significant decreases in night DBP, AASI and LDL

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9 457 cholesterol in conjunction with increased serum NO metabolites. These effects were

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11 458 confined to subjects with uncontrolled BP, suggesting that the physiological effects of

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13 459 exogenous nitrate may be greatest in these patients. Considering the conflicting data

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15 460 in the area, our pilot results should be confirmed with well-designed trials,

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17 461 particularly regarding LDL.

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478 **Ethical approval:**

479 “All procedures performed in studies involving human participants were in
480 accordance with the ethical standards of the institutional and/or national research
481 committee and with the 1964 Helsinki declaration and its later amendments or
482 comparable ethical standards.”

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Authorship Form

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- I have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;
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ICMJE Form for Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

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This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

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This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

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4. Are you the corresponding author?

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Nitrate-rich beetroot juice selectively lower ambulatory pressures and LDL cholesterol in uncontrolled but not controlled hypertension.

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Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? Yes No



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Dr. Dolan has nothing to disclose.

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

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Non-Financial Support: Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.

Other: Anything not covered under the previous three boxes

Pending: The patent has been filed but not issued

Issued: The patent has been issued by the agency

Licensed: The patent has been licensed to an entity, whether earning royalties or not

Royalties: Funds are coming in to you or your institution due to your patent



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1. Given Name (First Name)
Liam

2. Surname (Last Name)
Cormican

3. Date
12-October-2016

4. Are you the corresponding author?

Yes No

Corresponding Author's Name
Conor Kerley

5. Manuscript Title

Nitrate-rich beetroot juice selectively lower ambulatory pressures and LDL cholesterol in uncontrolled but not controlled hypertension.

6. Manuscript Identifying Number (if you know it)

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Are there any relevant conflicts of interest? Yes No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? Yes No



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Section 1. Identifying Information

1. Given Name (First Name)
Conor

2. Surname (Last Name)
Kerley

3. Date
12-October-2016

4. Are you the corresponding author? Yes No

5. Manuscript Title
Nitrate-rich beetroot juice selectively lower ambulatory pressures and LDL cholesterol in uncontrolled but not controlled hypertension.

6. Manuscript Identifying Number (if you know it)

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Are there any relevant conflicts of interest? Yes No

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Are there any relevant conflicts of interest? Yes No

Section 4. Intellectual Property -- Patents & Copyrights

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