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# Randomized Trial of Nitrate-Replete Beetroot Juice on Blood Pressure in Hypertensive Adults with ADPKD



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## INTRODUCTION

Reduced circulating levels of nitric oxide metabolites are hypothesized, in part, to drive hypertension in autosomal dominant polycystic kidney disease (ADPKD).[1] Beetroot juice (BRJ), a rich dietary source of nitrate, increases nitric oxide metabolites via the entero-salivary nitrate-nitrite-NO pathway, and lowers blood pressure (BP).[2] In the largest randomized controlled trial of 68 hypertensive patients, nitrate-replete BRJ reduced systolic BP by a mean of 9.0 ± 7.8 mm Hg after 4-weeks compared to 0.1 ± 8.0 mm Hg in the nitrate-deplete BRJ group.[3] In this study, we tested the hypothesis that oral nitrate supplementation with BRJ lowers BP in ADPKD. We undertook a 4-week double-blind 2-parallel group randomized placebo-controlled trial and compared a daily shot of either nitrate-replete BRJ (400 mg/d) or nitrate-deplete BRJ on clinic BP (primary end point), home BP, urine albumin-to-creatinine ratio and adverse effects (secondary end points) (Supplementary Methods).

## RESULTS

### Recruitment, Follow-Up and Baseline Characteristics

Three hundred seventy-six participants were screened for eligibility, 313 were excluded, and 3 were screen failures leaving 60 participants who were randomized (Supplementary Figure S1). One participant in the nitrate-replete group stopped using BRJ due to pregnancy but continued follow-up; 2 in the nitrate-deplete group stopped using BRJ (1 lost to follow-up; 1 due to side effects but continued follow-up). The baseline characteristics did not differ between the BRJ groups (Table 1 and Supplementary Tables S1 and S2), and the majority of visits occurred in the morning (Supplementary Table S3). Antihypertensives drug dosage was unaltered during the study.

### Effect of BRJ on Nitric Oxide Metabolites and Asymmetric Dimethylarginine

Serum nitrate/nitrite increased 3.6-fold in the nitrate-replete group at 4 weeks compared to the nitrate-deplete group ($P < 0.05$, Supplementary Figure S2). The mean change from baseline in the nitrate-replete BRJ group was 20.08 mm ($P < 0.01$) compared to 0.97 mm ($P = 0.30$) in the nitrate-deplete BRJ group. Serum asymmetric dimethylarginine levels were similar in both groups at baseline and after 4 weeks of intervention (Supplementary Table S4).

### Primary End Point

There were no intergroup differences between the nitrate-replete and nitrate-deplete BRJ groups (systolic BP, $P = 0.51$; diastolic BP, $P = 0.67$) or when adjusted for age, estimated glomerular filtration rate, and baseline serum nitrate. At 4 weeks, in the nitrate-replete BRJ group, the mean systolic and diastolic clinic BP

**Table 1.** Baseline characteristics

| Characteristic | Nitrate-replete BRJ group ($n$ = 30) | Nitrate-deplete BRJ group ($n$ = 30) |
|---|---|---|
| Age | 50 (14) | 49 (12) |
| Females | 43.3% (13) | 46.7% (14) |
| Self-reported ethnicity | | |
| Caucasian | 67% (20) | 73% (22) |
| Asian | 27% (8) | 23% (7) |
| Other | 7% (2) | 3% (1) |
| Height, cm | 174.4 (10.5) | 171.7 (9.1) |
| Weight, kg | 84.6 (16) | 85.9 (18.7) |
| BMI | 28 (5) | 29 (6) |
| Age at diagnosis of ADPKD, yr | 31 (13) | 33 (14) |
| Family history of ADPKD | | |
| Yes | 73% (22) | 77% (23) |
| No | 27% (8) | 13% (4) |
| Unknown | 0.00% (0) | 10% (3) |
| Age at diagnosis of hypertension | 36 (12) | 35 (11) |
| Years since hypertension diagnosis[a] | 10 (5–20) | 11 (5–20) |
| Number of antihypertensives[a] | 1 (1–2) | 1 (1–2) |
| Antihypertensive drug class | | |
| ACEi/ARB | 93% (28) | 93% (28) |
| Diuretic | 10% (3) | 17% (5) |
| CCB | 30% (9) | 43% (13) |
| Beta-blocker | 23% (7) | 30% (9) |
| Alpha-blocker | 10% (3) | 3% (1) |
| Combination therapy | 10% (3) | 17% (5) |
| Comorbidities | | |
| Type 2 Diabetes Mellitus | 0.00% (0) | 3% (1) |
| IHD | 0.00% (0) | 0.00% (0) |
| Stroke | 3% (1) | 3% (1) |
| Hypercholesterolemia | 17% (5) | 30% (9) |
| No. prescribed medications[a] | 2 (1–3) | 3 (2–5) |
| No. total supplements[a] | 4 (3–7) | 4 (2–6) |
| Spot urine alb:Cr ratio | 2.2 (0.4, 5.4) | 3.9 (1.7, 9) |
| Creatinine (μmol/l)[a] | 97 (85–148) | 105 (85–135) |
| eGFR (ml/min per 1.73 $m^2$)[a] | 63 (44–87) | 61.5 (44–82) |
| Total cholesterol (mmol/l) | 4.93 (0.91) | 4.90 (0.88) |
| Triglycerides (mmol/l) | 1.10 (0.95–1.70) | 1.50 (0.90–2.20) |
| Fasting glucose (mmol/l) | 4.8 (0.5) | 5.0 (0.6) |

ACEi, angiotensin converting enzyme inhibitor; ADPKD, autosomal dominant polycystic kidney disease; alb, albumin; ARB, angiotensin receptor blocker; BMI, body mass index; BRJ, beet root juice; CCB, calcium channel blocker; Cr, creatinine; eGFR, estimated glomerular filtration rate; IHD, ischemic heart disease; IQR, interquartile range.
[a]Continuous variables sufficiently approximated by normal distribution are summarized by sample mean and SD, and those that are not sufficiently approximated by a normal distribution were summarized by sample median and IQR. Categorical variables are summarized by sample proportions and count.

decreased from baseline (systolic BP, −5.36 ± 12.69; diastolic BP, −3.55 ± 7.08 mm Hg; $P < 0.05$) (Figure 1). In the nitrate-deplete BRJ group, only systolic BP decreased (−7.65 ± 14.07 mm Hg; $P = 0.01$; diastolic BP, −2.73 ± 7.81 mm Hg, $P = 0.07$). As shown in Supplementary Figure S3, 1 participant in each group had high baseline BP that declined during the follow-up period.

## Secondary End Points

Home BP was not different between the groups (systolic BP, $P = 0.95$; diastolic BP, $P = 0.59$) or when adjusted for age, estimated glomerular filtration rate, and baseline serum nitrate. Home BP decreased in both the nitrate-replete group (systolic BP, −0.51 [−1.40 to 0.38]; diastolic BP, −0.24 [−0.85 to 0.38] mm Hg/wk) and the nitrate-deplete BRJ group (systolic BP, −0.55 [−1.42 to 0.31]; diastolic BP, −0.47 [−1.07 to 0.13] mm Hg/wk) (Supplementary Figure S4). There are no intergroup differences ($P = 0.91$) in albuminuria after 4 weeks in both groups (0.20 mg/mmol; and −0.40 mg/mmol, respectively) (Supplementary Table S5), or when adjusted for age, estimated glomerular filtration rate, and baseline nitrate levels.

## Treatment Adherence

The number of returned empty BRJ bottles did not differ between the 2 groups (100%, interquartile range: 96.67–100 in both groups). The majority of participants consumed BRJ in the morning (87% in the nitrate-replete group; 97% in the nitrate-deplete group) and similar in both groups ($P = 0.32$; Supplementary Table S6). Adherence with daily home BP measurements, which was calculated by response to text messages, was similar in both groups (100%, interquartile range: 100–100 in the nitrate-replete group; 100%, interquartile range: 92.83–100 in the nitrate-deplete group).

## Adverse Effects

There were no intergroup differences in the frequency of adverse effects (Supplementary Table S7). Gastrointestinal symptoms (change in bowel habit, bloating, and gastritis) were the most frequent side effects occurring in 38% (23/60) of participants. Beeturia and beet-colored feces occurred in 37% (22/60) and 48% (29/60), respectively. Of note, 20% (12/60) perceived that side effects (improved bowel habits 13%; increased energy in 5%) were beneficial. Three percent (2/60) also reported positive symptoms of intentional weight loss due to increased satiety.

# DISCUSSION

In this 4-week double blind randomized placebo-controlled trial, we have shown for the first time to our knowledge that home BP, albuminuria, treatment adherence, discontinuations, and adverse effects was similar in hypertensive patients with ADPKD receiving either nitrate replete or nitrate deplete BRJ. These data suggest that oral nitrate supplementation does not influence hypertension in ADPKD.

The increase in nitrate and effect size of BP reduction with BRJ in this study was consistent with previous studies.[2,3] These data suggest that the antihypertensive effect of BRJ in ADPKD does not require nitrate and is secondary to multiple other nonnitrate components of BRJ (such as betalains,

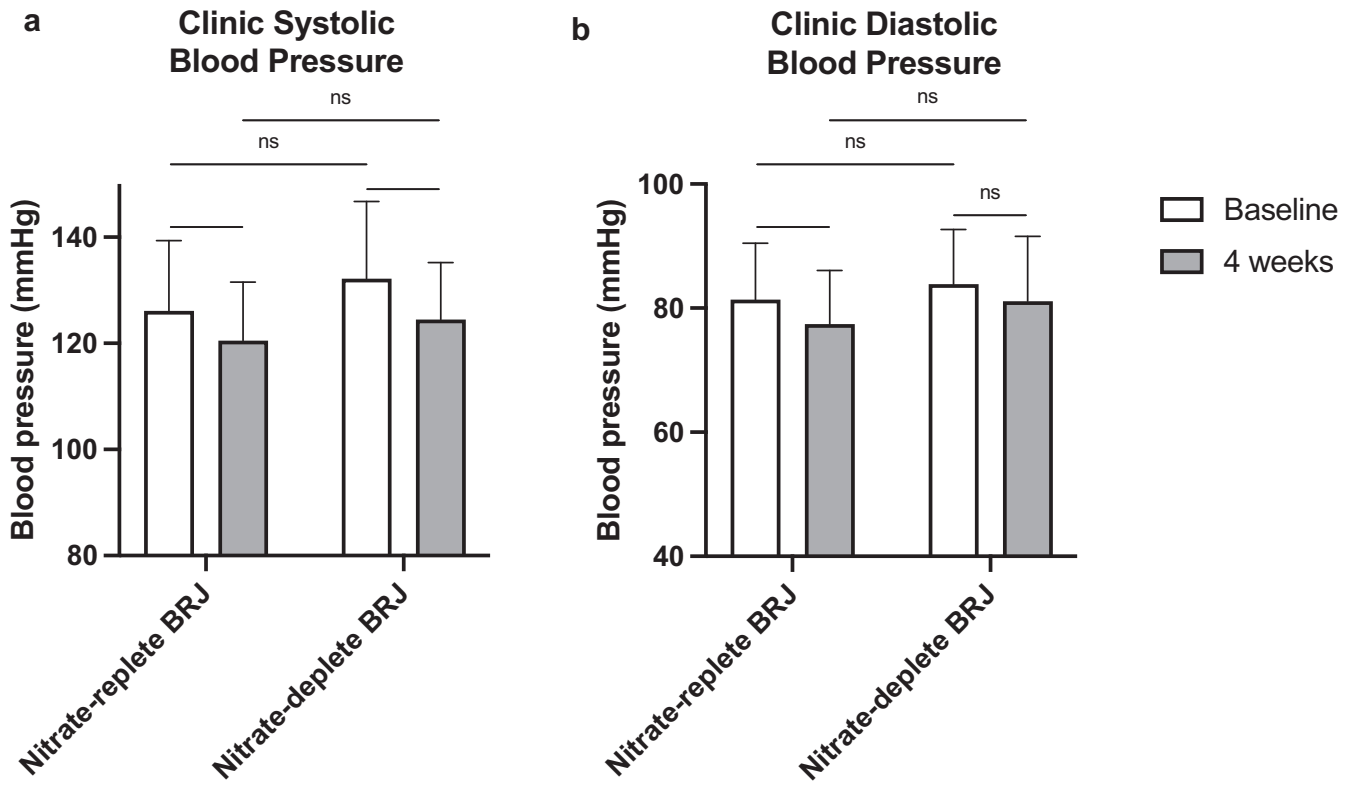


**Figure 1.** Mean clinic systolic (panel A) and diastolic (panel B) blood pressure levels in nitrate-replete and nitrate-deplete BRJ groups at baseline and after 4 weeks of treatment. Clinic systolic blood pressure at 4 weeks decreased in both groups and clinic diastolic blood pressure decreased in the nitrate replete BRJ group compared to baseline. Data expressed as mean ± SD; $^*P < 0.05$. BRJ, beet root juice; ns, not significant.

flavonoids, polyphenols, saponins, and vitamin C which cause vasodilation).[2,3] In this regard, higher serum betaine was associated with lower BP in the Guangzhou Nutrition and Health prospective observational cohort study consisting of 1339 patients.[4] A second explanation is that the BP reduction with BRJ was due to a placebo-associated effect.[5] In a meta-analysis of 52 hypertension trials in non-ADPKD patients ($n = 7451$), Patel *et al*.[5] found that the systolic BP decreased by mean of 5.92 mm Hg in nonresistant hypertensive placebo groups.[5] Multiple factors could explain BP improvements with placebo, including better in-trial compliance, regression to the mean and/or unintentional bias.[5] In our study, adherence was similar in both groups; multiple home BP measurements were performed; participants were only included if their hypertension was stable; and the trial design was double-blind, thereby reducing bias. Nevertheless, the possibility of a placebo-associated effect, having some role, cannot be entirely excluded.

In previous observational cohort studies, serum levels of nitric oxide metabolites are reduced by approximately 50% in ADPKD, and *in vitro* intracellular nitric oxide is decreased by approximately 90% in human ADPKD-mutated kidney cell lines.[1,6] The mechanisms for the reduction in nitric oxide have been hypothesized to be due to impaired nitric oxide synthase activity mediated by polycystin dysfunction together with increased asymmetric dimethyl arginine.[1,6,7] The results of this clinical trial suggest that oral nitrate does not have a major antihypertensive effect in ADPKD, and is consistent with previous animal studies of polycystic kidney disease.[6]

Nearly 40% of participants reported gastrointestinal side effects, which is higher than in previous BRJ trials.[3] This could be due to mass effects of polycystic kidneys, which leads to increased sensitivity to gastrointestinal symptoms.[8] Of note, some participants perceived improvement in frequency and consistency of stools as being positive. BRJ also contains oxalic acid (300–525 mg/l in juice),[2] and in animal models of polycystic kidney disease, oxalate consumption predisposes to microtubular crystal formation.[9] In the current study, no episodes of renal colic occurred; however, the duration was insufficient to evaluate this completely.

The main strengths of this study are the double-blind and pragmatic trial design, which reduced bias and participant burden (as reflected by excellent engagement and adherence). The limitations were that a non-BRJ group was not included; the duration was 4 weeks and included only 2 time points; changes in diet

during the trial were not evaluated; NO was measured indirectly by serum nitrate/nitrite; and long-term safety of BRJ has not been assessed.

In conclusion, daily BRJ lowered BP in ADPKD independent of nitrate levels. The antihypertensive effects of BRJ could be due to biologically active nonnitrate components.

## DISCLOSURE

GR is a member of the scientific advisory board of PKD Australia. All the other authors declared no competing interests.

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## DATA AVAILABILITY STATEMENT

Deidentified individual participant data that underlie the results reported in this article will be available to researchers in a public repository 12 months following publication of this article. Requests for the repository location and data should be directed to the corresponding author.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Supplementary Methods.**

**Supplementary References.**

**Figure S1.** Flowchart of participant recruitment, randomization, and completion.

**Figure S2.** Serum nitrate/nitrite levels at baseline and week 4.

**Figure S3.** Individual values for blood pressure in the study groups.

**Figure S4.** Mean daily home blood pressure measurements.

**Table S1.** Additional baseline characteristics.

**Table S2.** Additional baseline characteristics (extrarenal manifestations).

**Table S3.** Time of clinic visits and blood pressure measurements.

**Table S4.** Serum ADMA levels in the treatment groups.

**Table S5.** Median spot urine albumin to creatinine ratio in the groups.

**Table S6.** Self-reported time of BRJ intake in participants.

**Table S7.** Adverse events.

**CONSORT Statement.**

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