Grapefruit juice enhances the systolic blood pressure-lowering effects of dietary nitrate-containing beetroot juice

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Grapefruit juice enhances the systolic blood pressure-lowering effects of dietary nitrate-containing beetroot juice

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Aims: Dietary nitrate from sources such as beetroot juice lowers blood pressure (BP) via the nitrate–nitrite–nitric oxide (NO) pathway. However, NO and nitrite are inactivated via reoxidation to nitrate, potentially limiting their activity. Cytochrome P450-3A4 inhibition with troleandomycin prevents nitrite re-oxidation to nitrate in rodent liver. Grapefruit juice contains the CYP3A4 inhibitor furanocoumarin. We therefore hypothesized that grapefruit juice would enhance BP-lowering with beetroot juice by maintaining circulating [nitrite].

Methods: We performed a randomized, placebo-controlled, 7-hour crossover study in 11 healthy volunteers, attending on 3 occasions, receiving: a 70-mL shot of active beetroot juice (Beet-It) and either (i) 250 mL grapefruit juice (Active Beet+GFJ), or (ii) 250 mL water (Buxton, Active Beet+H_2O); or (iii) Placebo Beet+GFJ.

Results: The addition of grapefruit juice to active beetroot juice lowered systolic BP (SBP): Active Beet+GFJ *vs* Active Beet+H_2O (P = .02), and pulse pressure, PP (P = .0003). Peak mean differences in SBP and PP were seen at T = 5 hours: −3.3 mmHg (95% confidence interval [CI] −6.43 to −0.15) and at T = 2.5 hours: −4.2 mmHg (95% CI −0.3 to −8.2), respectively. Contrary to the hypothesis, plasma [nitrite] was lower with Active Beet+GFJ *vs* Active Beet+H_2O (P = .006), as was salivary nitrite production (P = .002) and saliva volume (−0.34 mL/min [95% CI −0.05 to −0.68]). The taste score of Beet+GFJ was 1.4/10 points higher than Beet+H_2O (P = .03).

Conclusion: Grapefruit juice enhanced beetroot juice's effect on lowering SBP and PP despite decreasing plasma [nitrite]. Besides suggesting more complex mechanisms, there is potential for maximising the clinical benefit of dietary nitrate and targeting isolated systolic hypertension.

KEYWORDS
blood pressure, cytochrome p450, nitric oxide

The authors confirm that the PI for this paper is Dr Andrew J. Webb and that he had direct clinical responsibility for patients.

1 | INTRODUCTION

Dietary inorganic nitrate (NO_3^-) is found in beetroot and green leafy vegetables. Beetroot juice has been shown to produce beneficial cardiovascular effects,[1] decreasing blood pressure (BP) in both healthy volunteers[2] and in patients with hypertension.[3–5] Furthermore, independent of peripheral BP, in hypertensive patients with or at risk of diabetes mellitus, chronic treatment with beetroot juice over 6 months results in beneficial changes in cardiac chamber volume.[6] Dietary nitrate also has additional benefits on platelets, endothelial function and mitochondrial efficiency.[7,8]

Much of the recent focus on the nitrate-nitrite- nitric oxide (NO) pathway has been on the mechanisms of bioactivation: nitrate reduction to nitrite (i.e. via the enterosalivary circulation and lingual bacterial nitrate reductases[9,10]), and nitrite reduction to NO (e.g. by deoxyhaemoglobin[11]). However, the redox nature of these metabolic processes permits re-oxidation of NO back to nitrite and then nitrate. Indeed, before the enterosalivary pathway was characterised, nitrate and nitrite were thought to be merely biologically inert by-products of NO oxidation, rather than key components of an important NO synthase-independent mechanism of NO production.

The cytochrome P450 (CYP) enzymes, particularly CYP3A, present in gut enterocytes and the liver[12] which play a major role in drug metabolism, may also be involved in nitrite oxidation. Using troleandomycin to inhibit CYP3A4, Curtis *et al*. recently demonstrated inhibition of nitrite re-oxidation back to nitrate in rat liver homogenates.[13] Grapefruit juice is recognised as interacting with many drugs via inhibition of CYP3A4,[14] attributed to furanocoumarins in the juice, particularly 6′,7′-dihydroxybergamottin.[15] Thus, it is plausible that the co-ingestion of grapefruit juice with beetroot juice would decrease CYP3A4-mediated oxidation of nitrite to nitrate, resulting in an increased plasma nitrite concentration.

We hypothesised that grapefruit juice, when co-ingested with beetroot juice, would potentiate beetroot juice's BP-lowering effect, via increased plasma nitrite concentration due to furanocoumarin-mediated CYP3A4 inhibition of nitrite oxidation.

What is already known about this subject

- Dietary nitrate (e.g. beetroot juice) lowers blood pressure via the nitrate–nitrite– nitric oxide pathway.
- Cytochrome P450-3A4 inhibition with troleandomycin prevents nitrite re-oxidation to nitrate in rodent liver.

What this study adds

- Grapefruit juice potentiated the systolic blood pressure-lowering effect of beetroot juice, despite decreasing plasma [nitrite] and salivary nitrite production.
- The haemodynamic mechanisms are unclear but may involve a synergistic dynamic effect and/or the production of nitric oxide species other than nitrite.

2 | METHODS

2.1 | Approvals

Ethical approval for this study was obtained from the South East London Research Ethics Committee (REC; 10/H0802/52). Written informed consent was obtained from all volunteers prior to commencing any protocol-related procedures.

2.2 | Participants

Participants were healthy volunteers aged 18–45 years, with normal BP (systolic BP [SBP] 90–140 mmHg and diastolic BP [DBP] <90 mmHg), a body mass index (BMI) of 18–40 kg/m^2 and without any recent illness or regular systemic medication (other than oral contraceptive pill).

2.3 | Study design

A 3-visit randomised (for the 2 active beetroot juice visits), single-blind (with respect to active *vs* placebo beetroot juice), placebo-controlled crossover intervention design was used. The 3 different visits involved the consumption of either:

- Active beetroot juice (nitrate-containing: ∼0.4 g) and golden grapefruit juice (Active Beet+GFJ) i.e. 70 mL of nitrate-containing beetroot juice shot (Beet-It James White Drinks, UK) with 250 mL of grapefruit juice (Golden Grapefruit Juice, Tropicana UK Ltd, UK).
- Active beetroot juice and water (Active Beet+H_2O) i.e. 70 mL of nitrate-containing beetroot juice shot + 250 mL of low-nitrate water (<0.1 mg/L, Buxton Mineral Water, UK).
- Placebo beetroot juice (nitrate-depleted) and grapefruit juice (Placebo Beet+GFJ) i.e. 70 mL of nitrate-depleted beetroot juice shot (James White Drinks, UK) + 250 mL of grapefruit juice. The placebo beetroot juice is identical in appearance, taste, and smell to the active beetroot juice. This visit was performed once the volunteer had completed the 2 active beetroot juice visits.

Each of the visits lasted 7 hours. For the duration of the visit, participants sat in an examination chair with their feet resting on a stool (to avoid changes in posture affecting plasma [nitrite]).[16] Blood pressure was measured in triplicate every 15 minutes from 1 hour (T = −1) before ingestion of the juice intervention at (*T* = 0) and until 6 hours postingestion. The BP for an individual at a given time point was taken as the average of the 3 (triplicate) readings. The BP readings from timepoints T = −1 to T = 0 were averaged to produce a

baseline reading. Blood pressure measurements were taken according to guidelines, using an automated BP monitor (Intellisense 705IT, Omron, UK). To avoid the action of BP recording interfering with the measurement of plasma [nitrate]/[nitrite],[17,18] BP recordings were taken from the arm contralateral to that from which blood samples were taken. Recordings of heart rate were taken alongside BP recordings.

To ensure participants remained hydrated during the study visit, we adopted an *optimised hydration protocol* whereby at every hour following the juice intervention, participants consumed 250 mL of low-nitrate water (<0.1 mg/L, Buxton Mineral Water, UK). Participants were given 2 slices of toasted Hovis wholemeal thick brown bread just after T = 3 h.

Volunteers were asked to rate the taste of each intervention by giving it a score of between 1 and 10 (disgusting–delicious), immediately after drinking the intervention.

2.4 | Sample collection

Blood and saliva samples were taken immediately prior to ingestion of each of the juice interventions, then at 30-minute intervals for the first 3 hours, and hourly for the final 3 hours. At time points where BP and blood samples were taken, BP recordings were taken prior to blood sampling. Urine samples were collected every hour, and the pH and volume recorded. A urine dipstick was performed upon collection of the first urine sample to exclude the possibility of high nitrite concentrations due to the presence of a urinary tract infection.

At each blood draw, 6 mL of venous blood was drawn into a chilled syringe then transferred to a chilled green lithium heparin tube (Vacutainer, BD). Samples were immediately centrifuged at 4°C and 2000 *g* for 5 minutes (MIKRO 220R, Hettich, Germany), after which plasma was collected.

Prior to collection of saliva, volunteers were asked to avoid swallowing saliva for 2 minutes. They were then asked to drool all saliva into a collecting tube. The volume of saliva collected was recorded.

All plasma, saliva and urine samples were divided into 2 chilled 2-mL tubes and stored at −80°C.

2.5 | Sample analysis

Plasma and saliva samples were analysed for nitrate and nitrite concentrations using chemiluminescence, as described previously.[19] The quantification of nitrate and nitrite in plasma and saliva was performed by an investigator who was blinded to the treatment allocation.

2.6 | Data analysis

In this pilot study, data was analysed using GraphPad Prism 8.0 (GraphPad Software Inc.). All data are expressed as mean ± standard error of the mean unless otherwise stated (e.g. non-parametric statistics [median ± interquartile range] for non-normally distributed data). Data were compared by 2-way ANOVA and/or 1-way ANOVA as appropriate, with Fisher's LSD post-test. $P < .05$ was considered statistically significant.

3 | RESULTS

A total of 11 volunteers were enrolled in the study (of 11 potential volunteers screened, all 11 were eligible and randomised). Volunteer baseline demographics for the 11 participants are shown in Table 1. Of the 11 volunteers, 9 completed all 3 visits (the other 2 volunteers did not attend the Placebo Beet+GFJ visit). There were no adverse events attributed to study participation.

3.1 | Plasma

The addition of grapefruit juice to active beetroot juice (Active Beet +GFJ) had no effect on plasma [nitrate] compared to active beetroot juice with water (Active Beet+H_2O); P = .38 (Figure 1A). Plasma nitrate was not measured for the Placebo Beet+GFJ intervention.

As anticipated, both Active Beet+GFJ and Active Beet+H_2O significantly increased plasma [nitrite] compared to Placebo Beet+GFJ (both $P < .0001$). Comparison between the 2 active beet interventions found that the addition of grapefruit juice decreased mean plasma [nitrite] by ∼14%; P = .006 (Figure 1B).

3.2 | Saliva

Grapefruit juice decreased nitrate secretion into the mouth (measured as total salivary nitrate and nitrite production), which was lower in Active Beet+GFJ than Active Beet+H_2O (P = .02), with the peak difference occurring at T = 1.5 h: −437 μmol/h (95% confidence interval [CI] −844 to −30); see Figure 2A. Salivary nitrite production

TABLE 1 Clinical parameters of participants, taken at time of screening.

Parameter	Value
Number of participants (*n*)	11
Sex (*n* male)	8
Age (y)	23 ± 4
Weight (kg)	60.9 ± 9.8
BMI (kg/m^2)	21.3 ± 1.9
HR (beats/min)	80 ± 14
SBP (mmHg)	122 ± 8
DBP (mmHg)	75 ± 8

Data are mean ± standard deviation
BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure

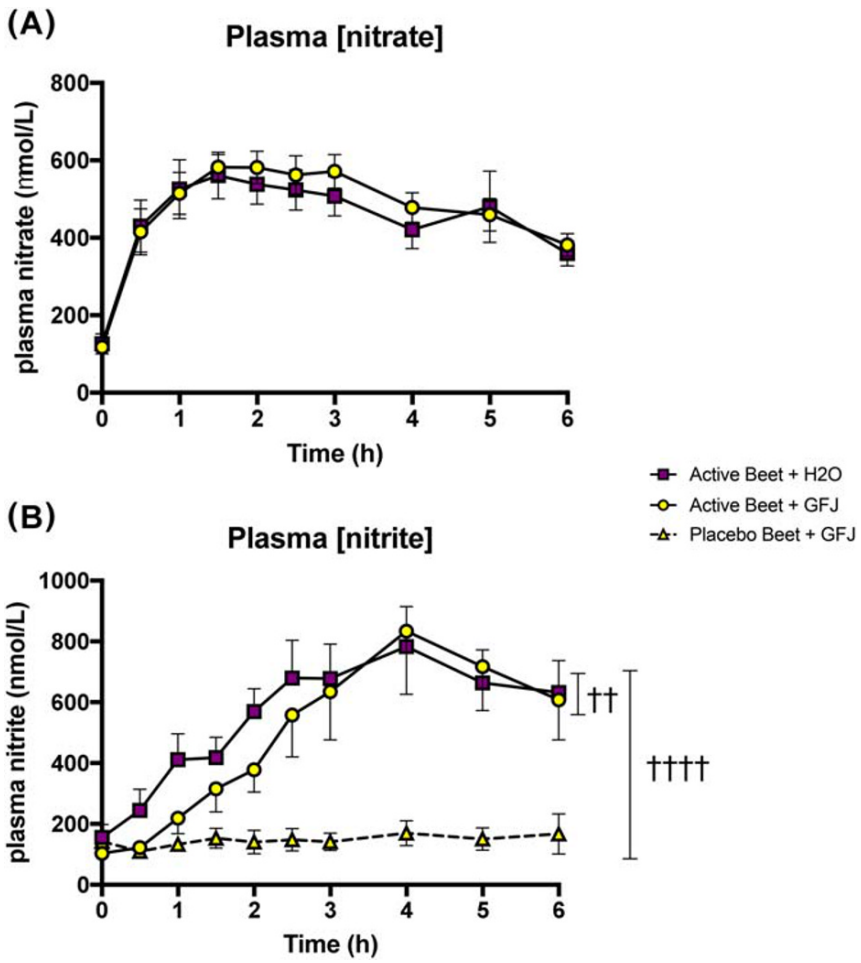

FIGURE 1 The effect of grapefruit juice (GFJ) and beetroot juice (given at T = 0 h) on plasma [nitrate] and [nitrite]. (A) Plasma [nitrate], n = 11, (note: Not measured for the Placebo Beet+GFJ intervention) (B) Plasma [nitrite] n = 11 for Active Beet+GFJ and Active Beet +H_2O, n = 9 for Placebo Beet+GFJ. Data shown as mean ± standard error of the mean. Statistical significance shown as, $^{††}P < .01$, $^{††††}P < .0001$ as analysed by 2-way ANOVA between the curves

was markedly decreased by grapefruit juice: i.e. lower with Active Beet+GFJ than Active Beet+H_2O (P = .002); see Figure 2B. Salivary [nitrate]:[nitrite] was higher with Active Beet+GFJ *vs* Active Beet +H_2O (P = .01), with the peak difference occurring at T = 0.5 h: +7.4 (95% CI +1.5 to +13.3); see Figure 2C.

Dietary nitrate (Active Beet+H_2O) increased salivary flow from baseline, P = .02 overall, and by 0.38 mL/min at 4 h (95% CI 0.16 to 0.61); see Figure 3A. Whilst grapefruit juice (Placebo Beet+GFJ) did not significantly decrease salivary flow relative to baseline, P = .7, grapefruit juice appeared to exert an astringent effect compared to Active Beet+H_2O: salivary volume was decreased with Placebo Beet +GFJ (P < .0001) and Active Beet+GFJ (P = .04) with the peak difference occurring at T = 4 h: −0.34 mL/min (95% CI −0.05 to −0.68); see Figure 3A.

Grapefruit juice significantly increased the salivary pH when combined with dietary nitrate (P = .005) for Active Beet+GFJ *vs* Active Beet+H_2O (Figure 3B). Dietary nitrate also increased salivary pH compared to grapefruit juice: Active Beet+H_2O *vs* Placebo Beet+GFJ (P < .0001) with the peak difference occurring at T = 3 h: ΔpH 0.37 (95% CI 0.16 to 0.58). Grapefruit juice without dietary nitrate resulted in decreased salivary pH: Placebo Beet+GFJ *vs* Active Beet+GFJ (P < .0001) with the peak difference occurring at T = 3 h: ΔpH 0.53 (95% CI 0.26 to 0.80).

3.3 | Blood pressure

Baseline BP data are presented in Table 2.

The addition of grapefruit juice to active beetroot juice resulted in a lower SBP: Active Beet+GFJ *vs* Active Beet+H_2O (P = .02), with the peak mean difference in SBP seen at T = 5 hours: −3.3 mmHg (95% CI −6.43 to −0.15); see Figure 4A. As expected, active nitrate-containing beetroot juice (Active Beet+GFJ) also lowered SBP *vs* Placebo Beet+GFJ (P = .0005). However, no difference in SBP was seen between Active Beet+H_2O and Placebo Beet+GFJ. Relative to baseline, SBP was decreased by the active nitrate-containing beetroot juice combinations: Active Beet+GFJ (P = .02) and Active Beet+H_2O (P < .01) but not by Placebo+GFJ (P = .09).

In contrast to SBP, grapefruit juice tended to increase DBP, i.e. Active Beet+GFJ *vs* Active Beet+H_2O (P = .04); see Figure 4B. Moreover, Placebo Beet+GFJ resulted in a significant decrease in DBP *vs* Active Beet+GFJ (P = .002) but not Active Beet+H_2O (P = .1). Relative to baseline, DBP was not decreased by any of the interventions (all P = .2).

Pulse pressure (PP) was decreased by Active Beet+GFJ *vs* Active Beet+H_2O (P = .0003), with the peak mean difference in PP seen at T = 2.5 hours: −4.2 mmHg (95% CI −0.3 to −8.2); see Figure 4C.

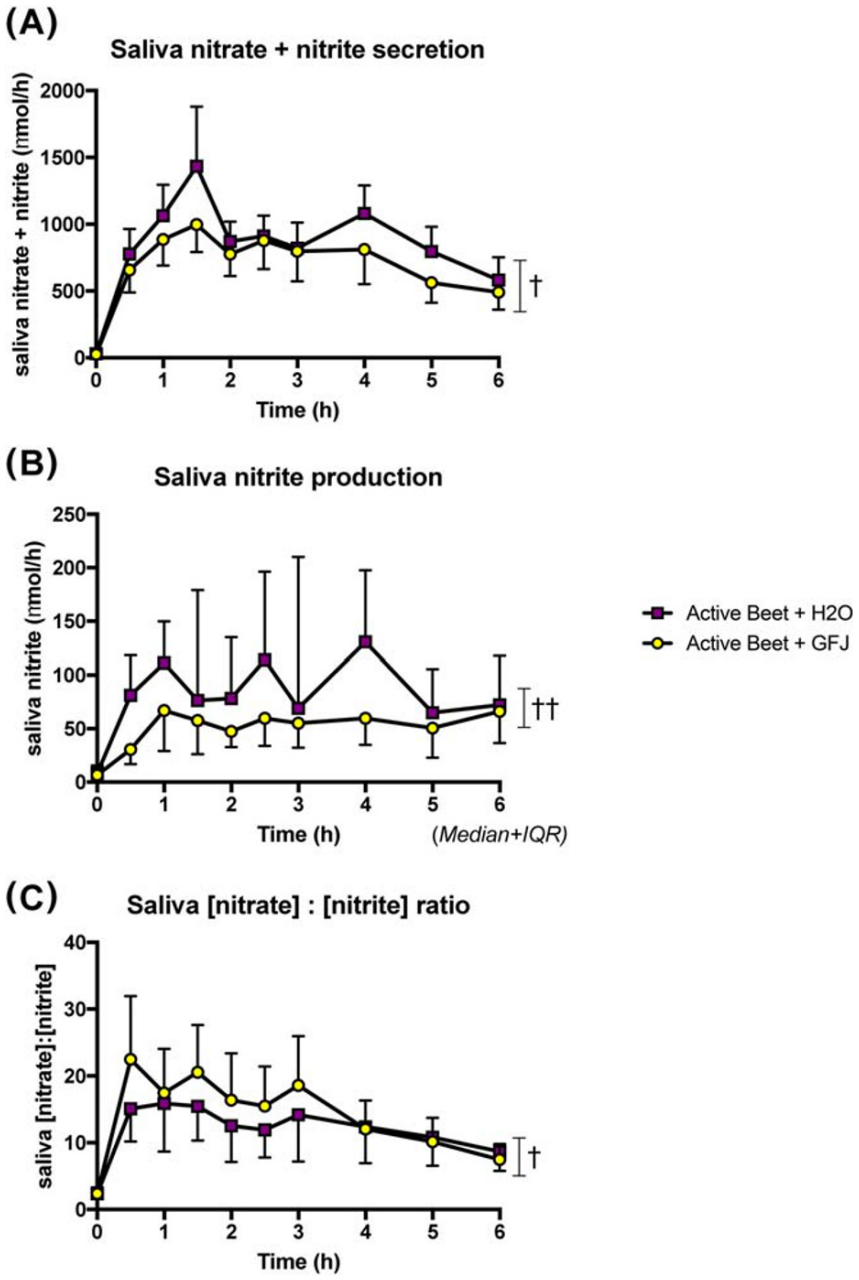

FIGURE 2 The effect of grapefruit juice (GFJ) and beetroot juice (given at T = 0 h) on salivary nitrate secretion and metabolism. (A) Total salivary nitrate secretion (i.e. amount of salivary nitrate and amount of salivary nitrite), n = 9. Data shown as mean ± standard error of the mean. (B) Salivary nitrite production, n = 11. Data shown as median ± interquartile range. (C) Saliva [nitrate]:[nitrite] ratio, n = 9. Data shown as mean ± standard error of the mean. Statistical significance shown as $^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$ as analysed by 2-way ANOVA between the curves (Placebo Beet+GFJ data not presented)

Active Beet+GFJ decreased PP *vs* Placebo Beet+GFJ ($P < .0001$), with a peak mean difference in PP seen at T = 4 hours: −6.6 mmHg (95% CI −2.0 to −11.2). Similarly, Active Beet+H_2O decreased PP *vs* Placebo Beet+GFJ ($P = .006$), with a peak mean difference in PP seen at T = 6 hours: −4.2 mmHg (95% CI −0.4 to −8.0).

Relative to baseline, PP was decreased by the active nitrate-containing beetroot juice combinations: Active Beet+GFJ ($P = .04$) and Active Beet+H_2O (both $P = .03$).

Mean arterial pressure (MAP, calculated as DBP + 0.4 × PP) was not significantly changed by Active Beet+GFJ *vs* either Active Beet +H_2O ($P = .6$) or *vs* Placebo Beet+GFJ ($P = .7$; data not shown). Relative to baseline, MAP was significantly decreased by Active Beet +GFJ ($P = .04$), but not by Active Beet+H_2O ($P = .1$) or Placebo Beet +GFJ ($P = .3$; data not shown).

PP index (PPi, the ratio of the pulse pressure to MAP[20]) was significantly decreased by Active Beet+GFJ *vs* both Active Beet+H_2O ($P = .001$) and Placebo Beet+GFJ ($P < .0001$). Active Beet+H_2O decreased PPi *vs* Placebo Beet+GFJ ($P = .01$). Relative to baseline, PPi was decreased by Active Beet+GFJ ($P = .03$) and Active Beet +H_2O ($P = .01$) and increased by Placebo Beet+GFJ ($P = .01$).

3.4 | Heart rate

The addition of grapefruit juice to Active beetroot juice resulted in a higher heart rate: Active Beet+GFJ *vs* Active Beet+H_2O ($P = .0009$), but without any significant change at individual timepoints (Figure 5). Both Active Beet+GFJ and Active Beet+H_2O increased heart rate compared to Placebo Beet+GFJ ($P < .0001$ and $P = .0006$

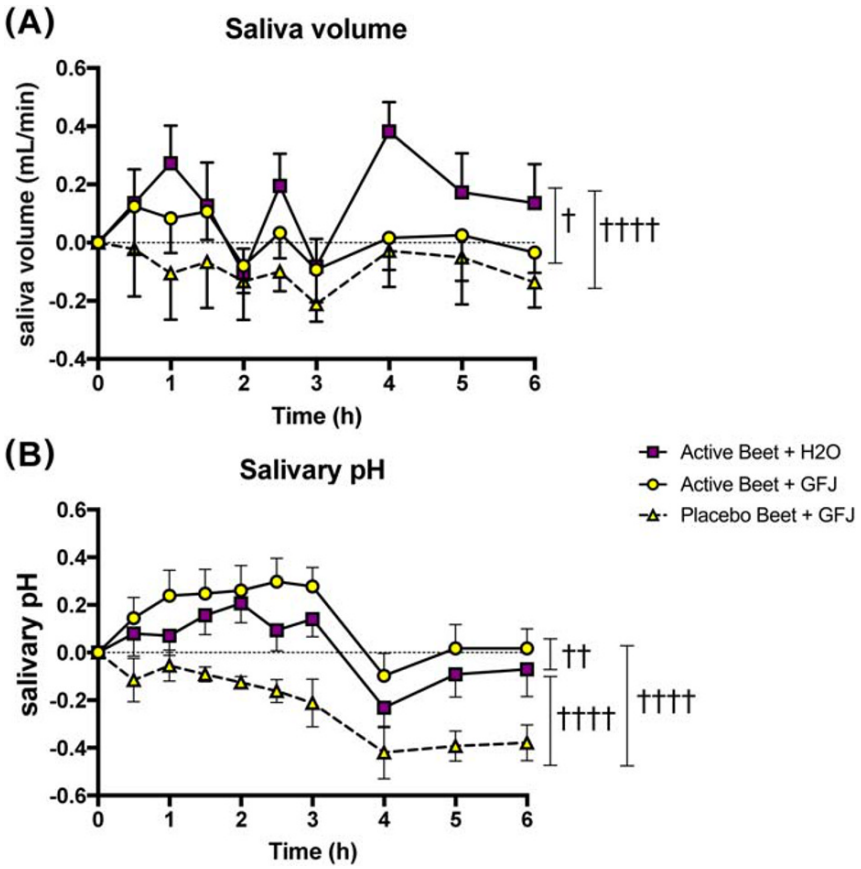

FIGURE 3 The effect of grapefruit juice (GFJ) and beetroot juice (given at T = 0 h) on saliva volume and pH. (A) Salivary volume, n = 11 for active beet+GFJ and active beet H_2O, n = 9 for Placebo Beet+GFJ. (B) Salivary pH values, n = 11 for Active Beet+GFJ and Active Beet+H_2O, n = 7 for Placebo Beet+GFJ. Data shown as mean ± standard error of the mean. Statistical significance shown as, $^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$, $^{\dagger\dagger\dagger\dagger}P < .0001$ as analysed by 2-way ANOVA between the curves

TABLE 2 Baseline blood pressure (BP) parameters (taken as an average of BP readings T = -1 to T = 0)

	Active beet +GFJ	Active beet +H_2O	Placebo beet +GFJ
SBP (mmHg)	108.1 ± 7.5	107.7 ± 8.2	106.5 ± 8.7
DBP (mmHg)	67.6 ± 5.6	66.2 ± 6.2	67.2 ± 6.1
MAP (mmHg)	83.8 ± 6.1	82.8 ± 6.2	83.0 ± 6.9
PP (mmHg)	40.5 ± 4.0	41.5 ± 7.0	39.3 ± 4.8
PPi	0.48 ± 0.04	0.50 ± 0.09	0.47 ± 0.05

GFJ, grapefruit juice; SBP, systolic BP; DBP, diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; PPi, pulse pressure index. Data are mean ± standard deviation

respectively). Relative to baseline, heart rate was decreased by Active Beet+H_2O (P = .03) and Placebo Beet+GFJ (P = .03), but not by Active Beet+GFJ (P = .09).

3.5 | Urine

Grapefruit juice as Placebo Beet+GFJ increased urinary pH *vs* Active Beet+H_2O (P < .0003) but not *vs* Active Beet+GFJ (P = .1; Figure 6).

3.6 | Taste score

The addition of grapefruit juice to active beetroot juice resulted in a significantly higher taste score, suggesting a greater palatability compared to beetroot juice and water: Active Beet+GFJ *vs* Active Beet+H_2O: +1.4 (95% CI 0.15 to 2.58), P = .03, and Placebo Beet+GFJ *vs* Active Beet+H_2O: +1.56 (95% CI 0.16 to 2.95), P = .03. There was no significant difference in taste score between the 2 grapefruit juice-containing interventions (P = .7; Figure 7).

4 | DISCUSSION

We have demonstrated that co-ingestion of grapefruit juice with dietary nitrate results in a potentiation of dietary nitrate's effect in lowering SBP and PP, albeit without the hypothesised increase in plasma [nitrite]. Therefore, the effect on SBP was unlikely to have been via furanocoumarin-mediated CYP3A4 inhibition of nitrite oxidation. Indeed, plasma [nitrite] was lower with Active Beet+GFJ compared to the Active Beet+H_2O (though DBP was slightly higher with Active Beet+GFJ *vs* Active Beet+H_2O).

The potential mechanism(s) by which grapefruit juice (in the Beet+GFJ intervention) decreases plasma [nitrite] *vs* Beet+H_2O will be considered by the kinetic processes (absorption, distribution, metabolism and secretion/excretion) at different anatomical sites.

In the oral cavity, grapefruit juice appears to have inhibited the metabolic conversion of nitrate to nitrite, as indicated by the increased salivary [nitrate]:[nitrite] ratio. This was associated with an unexpected increase in salivary pH with grapefruit juice. Whilst previous studies have suggested the relationship between salivary pH

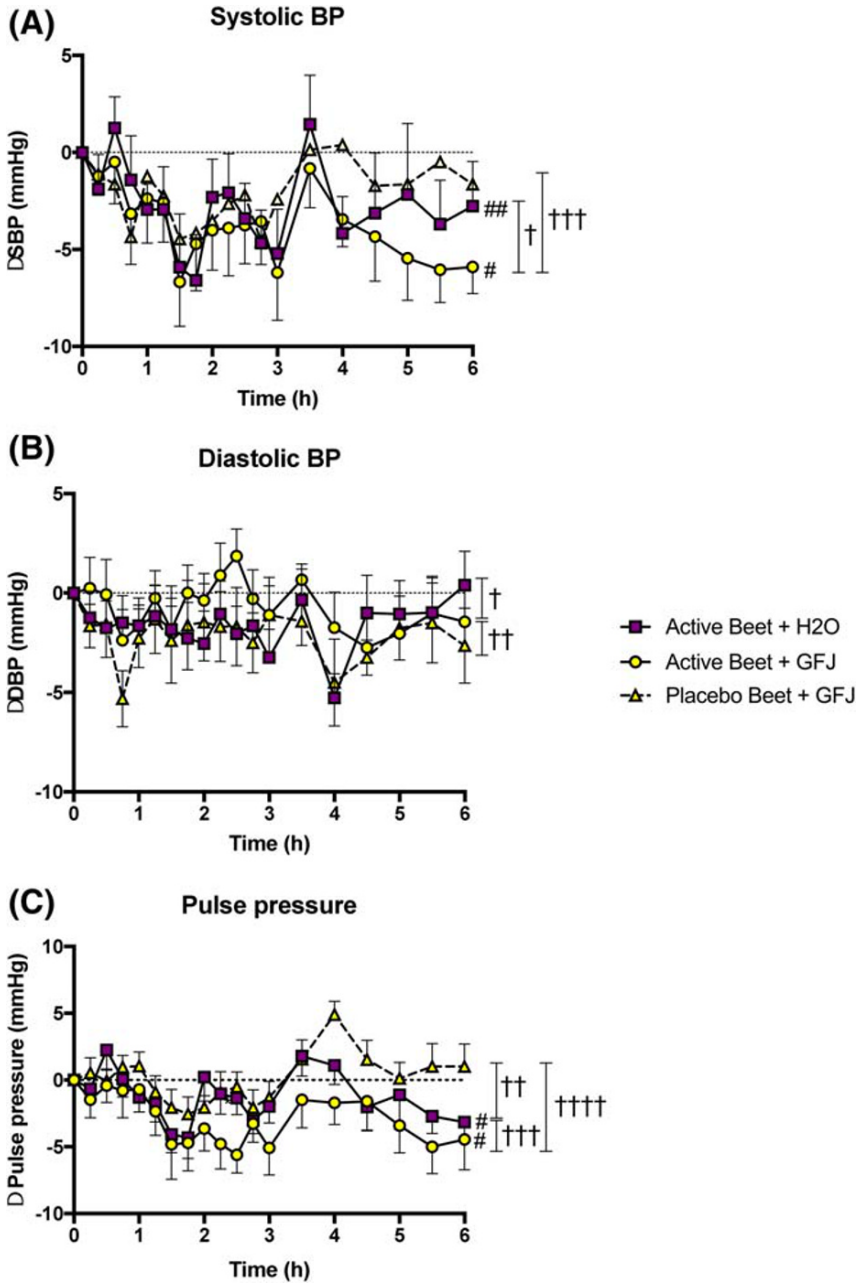

FIGURE 4 The effect of grapefruit juice (GFJ) and beetroot juice (given at T = 0 h) on blood pressure (BP). (A) Systolic BP. (B) Diastolic BP. (C) Pulse pressure. *n* = 9 for all interventions and parameters assessed. Statistical significance shown as $^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$, $^{\dagger\dagger\dagger}P < .001$, $^{\dagger\dagger\dagger\dagger}P < .0001$ as analysed by 2-way ANOVA between the curves and $^{\#}P < .05$, $^{\#\#}P < .01$ by 1-way ANOVA *vs* baseline

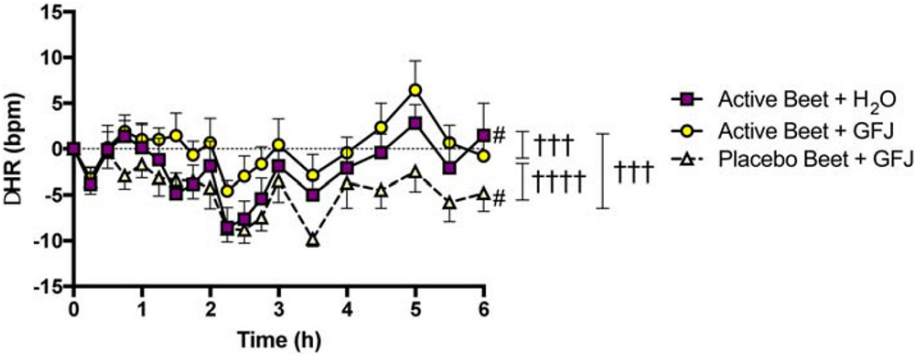

FIGURE 5 The effect of grapefruit juice (GFJ) and beetroot juice (given at T = 0 h) on heart rate. *n* = 9 for all interventions and parameters assessed. Statistical significance shown as $^{\dagger\dagger\dagger}P < .001$, $^{\dagger\dagger\dagger\dagger}P < .0001$ as analysed by 2-way ANOVA between the curves and $^{\#}P < .05$ by 1-way ANOVA *vs* baseline

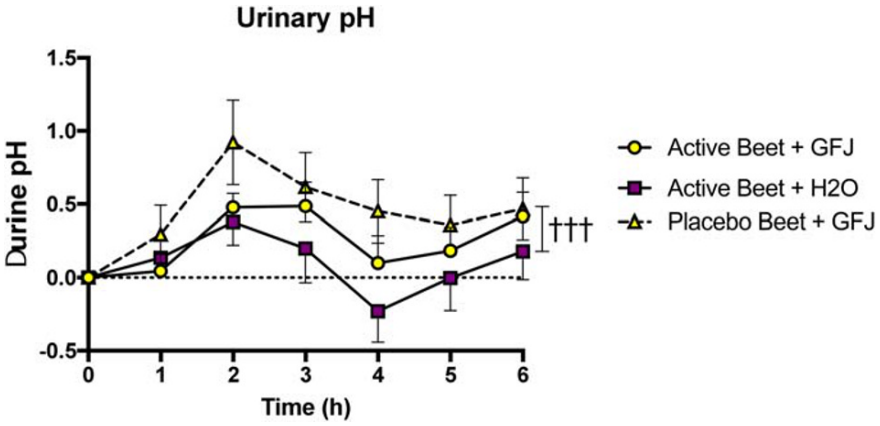

FIGURE 6 The effect of grapefruit juice (GFJ) and beetroot juice (given at T = 0 h) on urinary pH, *n* = 11 for Active Beet+GFJ and Active Beet+H_2O, *n* = 9 for Placebo Beet +GFJ. Statistical significance shown as $^{†††}P < .001$ as analysed by 2-way ANOVA between the curves

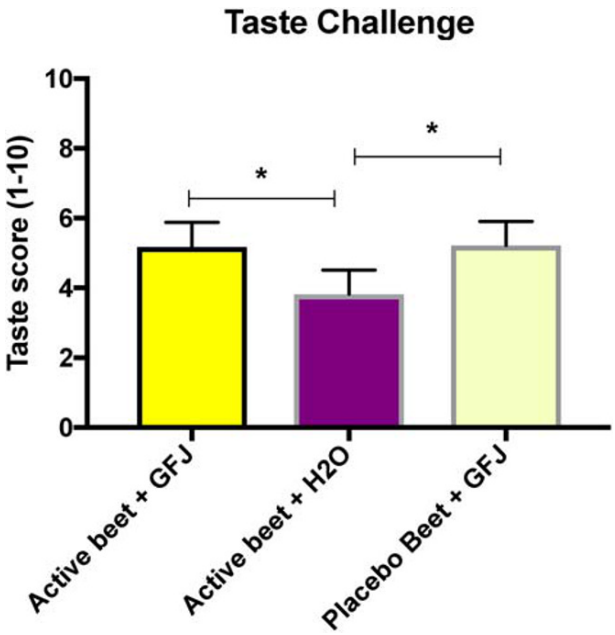

FIGURE 7 Taste challenge: Mean taste scores for each cocktail containing grapefruit (GFJ) and/or beetroot juice, *n* = 11 for Active Beet+GFJ and Active Beet+H_2O, *n* = 9 for Placebo Beet+GFJ. Data expressed as mean ± standard error of the mean. Statistical significance shown as $^{*}P < .05$

and nitrate reduction is in the opposite direction,[21,22] these are models, or only checked at a single timepoint of 30 minutes, rather than full physiological/kinetic studies. Our finding requires confirmation in further detailed mechanistic studies.

A potential additional contributing factor is the lower total salivary volume with grapefruit juice. Whilst the astringent effect of grapefruit (juice) may account for the *absolute* decrease in the total salivary nitrate secretion (measured as the total amount of salivary nitrate and nitrite: a secretory/excretory kinetic mechanism), this would not account for the change in rate of conversion of nitrate to nitrite (a metabolic mechanism) as indicated by the *relative* salivary [nitrate]:[nitrite] ratio. It is therefore likely that both kinetic processes—decreased metabolism and secretion—contribute to the decreased *absolute* salivary nitrite production.

Grapefruit juice *per se* decreased salivary pH (Placebo Beet+GFJ) from baseline and compared to the other 2 interventions. Dietary nitrate tended to increase salivary pH, which has been reported previously[23] and in combination with grapefruit juice resulted in a further increase in salivary pH. The mechanism for this interaction is not currently clear, although it could relate to **carbonic anhydrase**: **quercetin**, a flavonol found in grapefruit juice, has been demonstrated to inhibit human carbonic anhydrase in vitro.[24] Grapefruit juice also acted to increase the urinary pH, further supporting an effect on carbonic anhydrase.

In the stomach, grapefruit juice might impact gastric pH, modifying the chemical reactions contributing to a lower plasma [nitrite]. Despite being a citrus fruit, ingestion of a similar volume of grapefruit juice as our study (180 mL) has been found to more than double gastric pH (from 1.39 ± 0.4 to 3.20 ± 0.3; $P < .05$), albeit in the presence of indinavir.[25] Nitrite is reduced to NO and other NO species by acid disproportionation (with the remaining nitrite being absorbed into the systemic circulation) in the stomach: accordingly gastric NO production is inhibited by proton-pump inhibitors.[10] In rats, increasing gastric pH with a proton-pump inhibitor diminished the BP-lowering effect of orally-ingested nitrite independently of plasma [nitrite].[26] Similar results with a dissociation between plasma [nitrite] and BP following proton-pump inhibitor administration have been found in the study by Montenegro and colleagues in humans.[27] In their study, the plasma [nitrite] at 60 min following oral sodium nitrite ingestion, was significantly greater with the proton-pump inhibitor **esomeprazole** compared to placebo. However, the nitrite-induced BP decrease with placebo was blocked by esomeprazole. Therefore, the relationship between plasma nitrite and BP was comparable to our data. These data have important implications: stomach pH appears to be an important modulator of the enterosalivary pathway. However, dietary nitrate's BP-lowering effect is not fully explained by an increase in plasma [nitrite] alone. Such uncoupling of the plasma [nitrite] from the clinical response suggests that other NO species may be involved which were not measured in this study e.g. **thiocyanates** in beetroot juice interacting to form S-nitrosothiols (increasing gastric pH with esomeprazole did not decrease S-nitrosothiol levels in the study of Montenegro *et al.*[27]). Altered gastric NO species may also have an effect on splanchnic blood flow, which may in turn impact systemic BP.

This study was designed to assess the effect of grapefruit juice, with the mechanism postulated to be mediated by furanocoumarins in the juice. However, grapefruit juice also contains a number of other substances that may play a role through mechanisms other than CYP3A4. For example, grapefruit juice is abundant in compounds which inhibit organic anion-transporting peptide[28] and therefore potentially inhibits the uptake of organic anions; whether absorption of inorganic ions (such as nitrite) would be affected is not known. In addition to organic anion-transporting peptide, p-glycoprotein represents another possible mechanism by which grapefruit juice may affect gut absorption.[29]

Grapefruit juice also contains reducing agents, such as ascorbic acid, which could reduce nitrite to NO in the gut, diminishing nitrite absorption into the plasma (however the preparation of beetroot juice used also contains 2% lemon juice, which itself will contain ascorbic acid). Other potential reducing agents found in grapefruit juice are flavonoids, naringin, kaempferol and quercetin. In the stomach, nitrite and quercetin react to form NO,[30,31] a process that is favoured by acidic conditions. Whilst, the short half-life of NO would suggest that NO produced in the stomach would have only direct local effects, this has the potential to indirectly affect systemic BP via splanchnic blood flow as suggested above. Currently there is no evidence of flavonols reducing nitrite systemically.

The enhanced SBP-lowering effects of dietary nitrate and grapefruit juice co-consumption could represent a synergistic dynamic effect. Placebo Beet+GFJ was associated with a trend to decrease SBP (P = .09). It is therefore possible that grapefruit juice has its own independent BP-lowering effect; however, this study was not designed to measure such an effect. Combining the BP-lowering effect of beetroot juice with grapefruit juice would therefore be expected to result in an enhanced BP-lowering effect compared to beetroot juice alone. Indeed, grapefruit juice contains several vasoactive compounds including naringin and quercetin.[32] Naringin, when given daily for 6 weeks as grapefruit juice, has been shown to have a beneficial effect on arterial stiffness compared to treatment with a naringin-free grapefruit juice control,[33] and a single dose of quercetin has been shown to increase brachial artery diameter.[34] Actions of these vasoactive compounds might therefore explain the decreased PP observed for Active Beet+GFJ *vs* Active Beet+H_2O.

The results of this study have interesting potential clinical implications. The combination of beetroot juice with grapefruit juice decreased SBP and had a small but significant effect on increasing DBP. Therefore, the combination of the juices reduced PP (and PPi) but not MAP. Raised PP is highly prognostic for adverse cardiovascular events.[35] Furthermore, given the adverse effect of a low DBP in patients with isolated systolic hypertension,[36] an intervention which decreases SBP without affecting DBP has potential clinical utility.

5 | LIMITATIONS

This study had several limitations. This was a pilot study, involving a small number of participants, who were healthy volunteers. Larger studies of longer duration are required to confirm the findings reported in this paper and their applicability to patients with hypertension. In addition, in this study only the volunteers were blinded (to the Active *vs* Placebo Beet juice cocktails), therefore introducing the risk of bias. Future studies should be double-blinded to reduce this risk.

In this study BP was measured as peripheral BP. Given that inorganic nitrite is known to be an arterial dilator with selectivity for medium and large arteries over smaller resistance arterioles[37,38] the effects on central BP may have been more marked than those on peripheral BP[37] (as seen in the VaSera study, where 6 months treatments with beetroot juice was shown to decrease central, but not peripheral BP in hypertensive patients with/at risk of type 2 diabetes[39,40]). It would therefore have been preferable to have recorded measures of central BP in addition to peripheral BP.

A further limitation of the study design is that both juices contain bioactive substances other than those being studied, including ascorbic acid and flavonoids in the grapefruit juice, and thiocyanates in the beetroot juice, therefore limiting the study's ability to describe mechanistic aspects of the enterosalivary circulation over and above the clinical end-point of BP decrease as a result of combining the 2 juices. Paradoxically, although the complex mix of bioactive juices is a limitation, so too is the simplicity of this study as it is unclear whether the clinical effects seen in this tightly controlled study are reproducible when added to a standard, mixed diet.

6 | CONCLUSION

In summary, our study found that grapefruit juice potentiated the SBP-lowering effect of beetroot juice, despite a decrease in plasma [nitrite]. Grapefruit juice appears to have inhibited the metabolic conversion of nitrate to nitrite, as indicated by the increased salivary [nitrate]:[nitrite], associated with an increase in salivary pH with grapefruit juice. The BP-lowering effect may have been due to other NO species not measured in this study, such as S-nitrosothiols. These findings have implications for maximising the clinical benefit of dietary nitrate and also in further exploring mechanisms of dietary nitrate bioactivation. Given that the taste was improved by grapefruit juice, this combination has potential for use as a dietary approach to improve BP.

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COMPETING INTERESTS

A.J.W. holds shares in HeartBeet Ltd, which receives a royalty from James White Drinks Ltd, which manufactures the beetroot juice used in this study. The other authors have no competing interests to declare.

CONTRIBUTORS

A.J.W. and C.E.M. conceived of and designed the study. A.J.W., K.O.G., S.B.C., C.H., A.A.-S., F.S. and K.M. performed data collection and data analysis. K.O.G., A.J.W. and C.F. were involved in data interpretation, drafting (and revision) of the manuscript. All authors have given final approval of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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