The effects of inorganic nitrate and inulin co-ingestion on circulating metabolites and blood pressure in young adults: A pilot double-blind randomised crossover trial

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Abstract:

Dietary patterns enriched in fermentable fibre (such as inulin) and inorganic nitrate are linked to cardiovascular benefits, possibly mediated by gut microbiota-derived bioactive compounds including short-chain fatty acids (SCFAs) and nitric oxide (NO). However, the potential synergistic effects remain unclear. To address this knowledge gap, we conducted a randomised, double-blind, crossover study to investigate the acute effects of inulin (15 g; INU), nitrate (400 mg; NO_3^-), and their combination (INU+NO_3^-), on plasma nitrate and nitrite levels, SCFAs, and blood pressure (BP) in 20 normotensive participants. Plasma nitrate and nitrite were significantly elevated following INU+NO_3^- and NO_3^- compared to INU ($p < 0.001$). Plasma SCFAs were increased after INU+NO_3^- and INU, though the incremental area under the curve did not reach statistical significance, likely due to large inter-individual variability. No significant main effects were observed on BP; however, inverse correlations were identified between peak plasma nitrite and diastolic BP ($r_s = -0.61$, $p = 0.004$) and mean arterial pressure ($r_s = -0.59$, $p = 0.005$) following INU+NO_3^-. Peak nitrate concentrations were inversely correlated with diastolic BP following NO_3^- ($r_s = -0.47$, $p = 0.004$). Although co-supplementation with inulin and nitrate did not enhance plasma nitrate/nitrite or BP beyond nitrate alone, it modulated SCFAs profiles, suggesting potential interactions between fibre fermentation and nitrate metabolism for cardiovascular health.

Keywords: Fermentable fibre; Nitrate; Gut microbiome; Acetate; Vascular health

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1. Introduction

Hypertension is a significant modifiable risk factor for cardiovascular disease (CVD) and premature mortality, affecting 1.3 billion adults worldwide (Mishra et al., 2025). This highlights the crucial role of blood pressure (BP) management as a fundamental strategy for CVD prevention. Despite the availability of therapeutic interventions, approximately 80% of patients continue to have uncontrolled BP (Kario et al., 2024), underscoring the urgent need for dietary interventions (Jama et al., 2024; Norouzzadeh et al., 2025). This is particularly pertinent given the association between Western dietary patterns and the rising prevalence of hypertension (Clemente-Suárez et al., 2023). The consumption of foods rich in fermentable fibre, such as vegetables, fruits, cereal grains, and legumes, along with nitrate-rich foods such as leafy greens and beetroot, might benefit the gut microbiome and cardiovascular health (Azuma et al., 2023; Jama et al., 2024; Kaye et al., 2020; Wang et al., 2023), with subsequent health benefits likely due to the production of short-chain fatty acids (SCFAs) from fibre fractions (Boets et al., 2015) and the increased bioavailability of nitric oxide (NO) from inorganic nitrate (Norouzzadeh et al., 2025).

Inulin, a fermentable fibre mostly derived from chicory roots (Kaur et al., 2021), remains intact until it reaches the large intestine where it undergoes fermentation into SCFAs by anaerobic bacteria, thereby promoting bacterial growth (van der Beek et al., 2018). Although direct evidence linking inulin to BP modulation is lacking, studies have suggested that inulin selectively changes gut microbiota composition (Aldubayan et al., 2023). Its consumption results in the fermentation of SCFAs within 2-12 hours, with acetate being more prevalent than butyrate or propionate (Boets et al., 2015; Tarini & Wolever, 2010; van der Beek et al., 2018). SCFAs have been associated with enhanced cardiovascular health (Xu et al., 2022), including the reversal of hypertension due to a deficiency in fermentable fibre in mice diets (Kaye et al., 2020). These microbial metabolites are absorbed from the colon into the bloodstream via monocarboxylate transporters and passive diffusion (Xu et al., 2022). In the circulation, SCFAs may reduce BP by activating G protein-coupled receptors (GPR41 and GPR43) in vascular and renal tissues facilitating vasodilation (Xu et al., 2022). This effect has been demonstrated in preclinical studies (Kaye et al., 2020), with one human intervention study indicating that SCFA-enriched high-amylose maize starch can lower systolic blood pressure (SBP) in hypertensive patients (Jama et al., 2023). Additionally, a preclinical study demonstrated that inulin consumption ameliorated endothelial dysfunction in a hypertensive animal model by enhancing the nitric oxide synthase (NOS) pathway, improving endothelium-dependent relaxation, and increasing the phosphorylated endothelial nitric oxide synthase (eNOS) to total eNOS ratio at Ser-1177 (eNOS phosphorylation site) and NO-producing bacteria, including *E. coli* and *Bifidobacteriaceae* (Catry et al., 2018). In human umbilical vein endothelial cells (HUVECs), incubation with acetate (not derived from inulin) similarly increased NO bioavailability by stimulating eNOS phosphorylation at Ser-1177,

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2-4 hours post-incubation. Phosphorylation was dependent on AMP-activated protein kinase (AMPK) activation (Sakakibara et al., 2010).

Inorganic nitrate serves as a bioactive compound that functions as a precursor to NO, a signalling molecule essential for various physiological processes (Lundberg et al., 2008). Upon ingestion, nitrate is rapidly absorbed in the upper gastrointestinal tract, with approximately 25% actively sequestered by the salivary glands (Lundberg et al., 2018). Within the oral cavity, commensal bacteria located on the tongue facilitate the reduction of nitrate to nitrite, which is subsequently swallowed and metabolised in the stomach. Under acidic gastric conditions and in the presence of specific dietary components, nitrite is chemically reduced to NO. This process, referred to as the nitrate-nitrite-NO pathway, is particularly active under hypoxic conditions (Lundberg et al., 2018). Furthermore, NO is synthesised via the oxidation of L-arginine catalysed by NOS enzymes, with eNOS being primarily responsible for NO production within the vascular system (Lundberg & Weitzberg, 2005). NO acts as a vasodilator by diffusing into the vascular smooth muscle cells and activating the soluble guanylate cyclase (sGC)/cyclic guanosine monophosphate pathway (cGMP), leading to vasodilation and a reduction in BP (Carlström et al., 2018). Consumption of dietary nitrate, either as a salt, green leafy vegetables or through beetroot juice supplementation, has been consistently shown to lower BP within a timeframe ranging from a few hours to several weeks (Ashworth & Bescos, 2017; Bahadoran et al., 2017; Siervo et al., 2013). This strategy is proposed as a cost-effective means of preventing cardiovascular disease, particularly in adults with elevated baseline BP or chronic conditions such as hypertension (Ashworth & Bescos, 2017). However, this perspective has recently been questioned, with suggestions that other factors, such as oral health, might play a more crucial role in BP regulation, and that the only significant connection appears to be between salivary nitrate and BP (Bescos et al., 2025).

Although plasma nitrate and nitrite are well-established precursors for NO production and bioavailability (Kapil et al., 2010), the influence of inulin on NO bioavailability via eNOS, as evidenced in *in vitro* and animal studies (Catry et al., 2018; Sakakibara et al., 2010) or described hypothetically through modulation of the gut microbiota potentially enhancing NO bioavailability (González-Soltero et al., 2020), remains uncertain. Metabolites derived from inulin and nitrate have the potential to lower BP via distinct biological mechanisms, as previously described. However, whether their combined intake exhibits complementary or synergistic effects remains unknown, as evidence from human studies is currently lacking. Consequently, investigating the response of their respective metabolites to co-supplementation could inform future research aimed at enhancing vascular health in at-risk populations, including in adults with hypertension.

The primary aim of this study was to explore whether the combination of nitrate and inulin affects plasma nitrate and nitrite compared to the effects of consuming these supplements individually.

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The secondary aim was to assess the independent and combined effects of these supplements on the production of SCFAs. Furthermore, this study sought to examine the potential impact on BP when peak concentrations of nitrate, nitrite, and acetate were reached, following nitrate and inulin supplemented separately or together. We hypothesised that combining inulin and nitrate would lead to higher plasma nitrite than when each supplement was consumed alone and that peak plasma nitrite concentrations would be inversely correlated with BP, resulting in a significant reduction in BP.

2. Methods

2.1. Study design

This study was a double-blind, randomised, crossover design. Participants were initially screened via a video call, and those who expressed interest and provided written informed consent underwent a laboratory-based screening procedure. This procedure included anthropometric measurements (height and body weight), a comprehensive medical history report, and office BP measurements. The primary inclusion criteria were adults aged between 18 and 45 years, with a body mass index (BMI) ranging from 18 to 25 kg/m^2 and BP measurements within the normotensive range, defined as a SBP of ≤ 120 mmHg and a diastolic blood pressure (DBP) of ≤ 80 mmHg (McEvoy et al., 2024). Participants were excluded if they had been taking antibiotics for three months prior to or during the study, were engaged in a weight loss intervention or adhered to any restrictive dietary practices (e.g., vegan, FODMAP, etc.), had a history of chronic gastrointestinal conditions, or pre-existing medical conditions, including hypertension, diabetes, cardiovascular or dental conditions requiring treatment. Additional exclusion criteria included regular use of antibacterial mouthwash or tongue scrapes, smoking, and consumption of prebiotics, probiotics, or nitrate supplements for at least one month prior to or during the study (see Supplementary Methods for a comprehensive list of exclusion criteria). The study protocol adhered to the core principles of the ICH-GCP and the Helsinki Declaration and was approved by the Public Health and Sport Sciences Ethics Committee (University of Exeter; approval number: 22-02-02 A-01).

2.2. Study procedures

All tests were performed at the University of Exeter, Faculty of Health and Life Sciences. Twenty normotensive participants attended three separate visits where they were randomly assigned to receive either 15 g Orafti inulin (Orafti Chicory Inulin powder, Hellenia, UK) (INU), 400 mg potassium nitrate (NO_3^-) (Vital Minerals, UK) (NO_3^- condition), or a combination of both (INU+NO_3^-) in a randomised order. The nutritional composition of the conditions is shown in Table S1. The doses of 15 g inulin and 400 mg nitrate were based on previous studies (Boets et al., 2015; Kapil et al., 2015). For inulin, we selected a dose previously demonstrated to be well-tolerated regarding reduced risk of gastrointestinal adverse effects, including abdominal distension, nausea, flatulence, constipation, and gastrointestinal cramping and rumbling (Bonnema et al., 2010). The 400 mg nitrate dose represents

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a practical amount consumable through vegetables (Li et al., 2024) and is within the range shown to be well tolerated and offer vascular benefits (Kapil et al., 2015). A seven-day washout period between visits ensured nitrate and nitrite levels returned to pre-supplementation levels (Capper et al., 2022) and eliminated potential carry-over effects from inulin supplementation (Depeint et al., 2008).

Visits occurred between 08:00 AM and 2:00 PM after a 12-hour overnight fast (water permitted). Participants received a list of nitrate-rich foods to avoid the day before and were instructed to avoid caffeine for 12 hours, vigorous exercise, and alcohol within 24 hours of the visit. Compliance was verified through a 24-hour dietary recall analysed using Nutritics software (Nutritics, 2019, Research Edition v6.04). At each visit, a cannula was inserted into an antecubital vein for baseline blood collection, followed by BP measurement. Participants consumed the allocated supplement(s) within 5 minutes. Blood samples were collected at 60, 120, and 180 minutes post-consumption, followed by a low-fibre, low-nitrate meal within 10 minutes (94 g white bread, 22 g lactose-free cheddar cheese, and 7 g spreadable plant-based butter). The nutritional composition of standard meal is presented in Table S2. Additional blood samples were obtained at 240, 300, and 360 minutes with BP measurements (Figure 1).

2.3 Blood pressure measurements and anthropometric measurements

Clinic BP measurements were taken according to previously described guidelines (Muntner et al., 2019). BP was measured four times with a one-minute rest between readings using an electronic sphygmomanometer (Dinamap Pro; GE Medical System, Tampa, FL) and appropriately sized upper-arm cuff after a 10-minute rest period. The readings were blinded to the participant and an average of the last three measurements at each timepoint were taken for analysis. Participants' body mass and height were measured using a digital balance scale (precision of 0.1 kg) and a wall-mounted stadiometer (accurate to 0.1 cm). During these measurements, the participants were asked to remove their shoes and wear minimal clothing.

2.4 Blood sampling

Whole blood samples were obtained through a cannula and collected in 10 mL lithium heparin vacutainers (BD). Following each blood sample collection, 5 mL of non-coagulant saline solution was injected through the cannula to prevent clotting and line blockage. The samples were centrifuged immediately at 3,300 x g for 10 min at 4°C, and the plasma was divided into aliquots and frozen at -80°C. Plasma samples were used to measure concentrations of nitrate, nitrite, and SCFAs.

2.5 Plasma nitrate and nitrite analysis

Plasma nitrate and nitrite samples were deproteinised by cold ethanol precipitation prior to analysis. Briefly, plasma samples were immediately frozen at -80°C for the subsequent determination of nitrate and nitrite. Each sample was mixed with cold ethanol at a ratio of 1:2 (sample: ethanol) and

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centrifuged at 13,000 rpm (4°C) for 15 minutes to precipitate proteins. The supernatant was then analysed for nitrate and nitrite concentrations using a Sievers gas-phase chemiluminescence nitric oxide analyser (NOA 280i, Analytix), in accordance with a previously outlined methodology (Piknova et al., 2016), as previously described (Wylie et al., 2013).

2.6 Plasma short-chain fatty acids analysis

Plasma SCFAs levels were measured using LC-MS/MS, as previously described (Dei Cas et al., 2020). Briefly, 40 µL of plasma was diluted with 500 µL of ice-cold methanol, followed by incubation on dry ice and centrifugation at 14,800 rpm for 5 minutes. The supernatants were filtered, and the extracts were evaporated using Savant™ SpeedVac™, followed by reconstitution with 40 µL of methanol. To each 20 µL of the reconstituted sample, an internal standard mix (acetic acid d3, propionic acid d2, and isobutoxyacetic acid) was added. For derivatisation, 10 µL of 3-NPH and 10 µL of EDC were added, and the mixture was incubated at 37°C for 30 minutes, followed by quenching with 20 µL of 0.1% formic acid. The derivatised samples were then transferred to autosampler vials and subjected to LC-MS/MS analysis. Stock solutions of the metabolites in methanol were prepared and stored at -80°C. Calibration standards, including acetic acid, propionic acid, and other SCFAs, were run at the beginning, middle, and end of each analytical queue to construct calibration curves based on the analyte–internal standard response ratios, facilitating the accurate quantification of SCFAs. Detailed information on the procedure for analysing SCFA in plasma samples can be found in the Supplementary Methods section.

2.7 Sample size

This pilot study sought to offer initial insights into the feasibility of these interventions and their overall impact on both the primary and secondary outcomes. The study sample size was determined on the basis of two key considerations. Previous studies have demonstrated positive effects of various types and doses of inorganic nitrate supplementation on plasma nitrite levels in healthy adults (Jakubcik et al., 2021; McDonagh et al., 2018). Second, we used the predicted effect size estimates proposed by Whitehead et al. (2016) to calculate the sample size for a pilot trial. According to these recommendations, a sample size of 20 participants per group would be sufficient to detect a small effect size (δ = 0.10-0.30) with a power of 0.80 and a p-value of less than 0.05.

2.8 Statistical analysis

Data were analysed using SPSS (IBM SPSS Statistics, Version 29) and visualised using GraphPad Prism (GraphPad Software V 10.1.1; San Diego, CA, USA). Two-way repeated measures ANOVA assessed time x treatment effects on plasma nitrite, nitrate and SCFAs, with Bonferroni correction for timepoint comparisons. For non-significant interactions, main effects of time and condition were analysed separately. Total and incremental areas under the curve (tAUC and iAUC)

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were calculated using the trapezium rule via R Statistical Software, with differences analysed using one-way repeated-measures ANOVA and Bonferroni post-hoc test. For non-normal data with violated sphericity, the Friedman test was used (Blanca et al., 2023). Spearman's and Pearson's correlations examined relationships between nitrite, nitrate, acetate, and BP variables, with strengths categorised as weak (0.2), moderate (0.5), and strong (0.8) (Mukaka, 2012). Data are expressed as mean ± SD, with significance at $p \leq 0.05$. For full statistical methods, see online supplementary methods.

3. Results

3.1 Participants Characteristics

Figure 2 presents a flowchart of participants recruitment and Table 1 presents their baseline characteristics. Twenty normotensive participants with an average age of 27.4 ± 6.3 years (mean ± SD), a BMI of 24.6 ± 3.1 kg/m^2, and a waist circumference of 81.5 ± 8.5 cm were included in the study. Three participant discontinued the intervention due to time constraints, which prevented the completion of the remaining two visits. None of the participants reported any adverse reactions or discomfort after consuming supplements during the study visits. All participants adhered to a low-nitrate diet according to their completed food diaries. No significant differences were observed in micronutrient, dietary fibre, or macronutrient intake across the three laboratory visits, except for the percentage of fat intake for the total daily energy consumption (Table 2).

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256 **Table 1.** Baseline values of the participants (*n* = 20).

Characteristics (*n* = 20)	
Age (y)	27.4 ± 6.3
Male:Female	11:9
Height (cm)	172.4 ± 9.8
Body weight (kg)	73.5 ± 12.5
BMI (kg/m^2)	24.6 ± 3.1
Waist (cm)	81.5 ± 8.5
Waist-to-hip ratio	0.8 ± 0.1
Resting SBP (mmHg)	114.4 ± 8.8
Resting DBP (mmHg)	62.7 ± 6.2
Resting MAP (mmHg)	82.3 ± 6.5
Plasma NO_2^- (nM)	72.3 ± 29.3
Plasma NO_3^- (μM)	24.4 ± 9.3
Plasma acetate (μM)	79.3 ± 19.3
Plasma butyrate (μM)	0.6 ± 0.3
Plasma propionate (μM)	2.2 ± 0.4
Ethnic group	18 Caucasian, 2 South Asian

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258 Data are expressed as means ± standard deviation. BMI, body mass index; SBP, systolic blood pressure, DBP,
259 diastolic blood pressure; MAP, mean arterial pressure; ethnic group, self-declared.

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Table 2. Macronutrient, micronutrient and fibre intake fata from the 24-h dietary recalls from 20 normotensive young adults participating in the study before each laboratory visit.

Nutrient	INU + NO_3^-	NO_3^-	INU	*p*-value
Energy (kcal/day)	1841.3 ± 473.4	1784.7 ± 471.5	1878.0 ± 665.0	0.545
Carbohydrates (%E kcal)	46.0 ± 9.8	43.5 ± 9.6	43.6 ± 9.8	0.274
Carbohydrate (g/day)	210.2 ± 63.2	193.1 ± 63.8	199.2 ± 67.7	0.300
Protein (%E kcal)	17.3 (8.8)	17.1 (8.7)	16.8 (6.8)	0.253
Protein (g/day)	89.6 ± 29.2	83.6 ± 31.1	82.8 ± 35.3	0.360
Fat (%E kcal)	34.0 ± 7.6	37.2 ± 8.1	38.5 ± 8.6	**0.021***
Fat (g/day)	71.4 ± 29.9	75.3 ± 28.2	83.3 ± 47.3	0.194
Saturated Fat (g/day)	22.9 (12.9)	27.1 (16.3)	24.8 (23.3)	0.287
Cholesterol (mg/day)	313. 1 ± 262.4	326.5 ± 353.1	237.7 ± 319.4	0.468
Dietary Fibre (g/day)	18.2 ± 6.9	17.3 ± 6.7	18.2 ± 8.9	0.756
Potassium (mg/day)	1414.7 ± 964.6	1397.0 ± 631.7	1572.9 ± 919.2	0.522
Magnesium (mg/day)	200.6 ± 103.9	191.9 ± 65.9	195.9 ± 100.7	0.896
Vitamin E (mg/day)	4.8 ± 4.8	4.0 ± 2.5	5.1 ± 4.1	0.286
Vitamin C (mg/day)	10.5 ± 15.6	12.7 ± 15.5	17.3 ± 26.1	0.493

Data are presented as mean ± SD or median (IQR) for non-normally distributed variables. Energy adjusted values (%E kcal) are expressed as the percentage of total energy contributed by the nutrient. * *P* value < 0.05 indicate a significant difference between the INU+NO_3^- and the INU conditions.

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297 *3.2 Plasma nitrate and nitrite*

298 Plasma nitrite and nitrate did not differ between the baseline conditions ($p > 0.05$).
299 The rise in plasma nitrite above baseline following both INU+NO_3^- and NO_3^- reached peak
300 concentrations at 120 minutes (233 ± 177 nM, 95% CI, 150-315 nM and 235 ± 169 nM, 95%
301 CI, 156-313 nM, respectively), in contrast to INU (69 ± 38 nM, 95% CI, 50-87 nM) ($p = 0.001$
302 and $p < 0.001$, respectively) (Figure 3A). The iAUC for plasma nitrite was 35883 ± 35914
303 nM*min (95% CI, 19074-52691 nM*min) and 33990 ± 27030 nM*min (95% CI, 21339-46640
304 nM*min) following INU+NO_3^- and NO_3^-, respectively, both significantly exceeding the nitrate
305 iAUC following INU (2520 ± 3685 nM*min, 95% CI, 795-4244 nM*min, $p < 0.001$) (Figure 3B).
306 A similar trend was observed for nitrite tAUC across all three conditions (Figure S1, A). The
307 increase in plasma nitrate above baseline following both INU+NO_3^- and NO_3^- reached peak
308 concentrations at 60 minutes (132 ± 45 μM, 95% CI, 110-153 μM and 137 ± 69 μM, 95% CI,
309 105-170 μM, respectively), compared to INU (28 ± 19 μM, 95% CI, 19-37 μM) ($p < 0.001$ for
310 both) (Figure 3C). Nitrate iAUC after INU+ NO_3^- (25709 ± 10849 μM*min, 95% CI, 20631-
311 30787 μM*min) was comparable to that of NO_3^- (26584 ± 13843 μM*min, 95% CI, 20105-
312 33063 μM*min), both significantly greater than the nitrate iAUC after INU (719 ± 829 μM*min,
313 95% CI, 331–1107 μM*min, $p < 0.001$) (Figure 3D). A similar pattern was observed for nitrate
314 tAUC across all three conditions (Figure S1, B).

315 *3.3 Plasma Short-Chain Fatty Acids*

316 Plasma SCFAs concentrations did not differ between the baseline conditions ($p >$
317 0.05). The mean plasma acetate were 79.56 ± 13.06 μM (95% CI: 73.45-85.67 μM) for
318 INU+NO_3^-, 79.23 ± 17.48 μM (95% CI: 71.05-87.41 μM) for INU, and 66.97 ± 21.42 μM (95%
319 CI: 56.94-77.00 μM) for NO_3^-. Plasma acetate was significantly higher in the INU+NO_3^- (mean
320 difference: 12.59 ± 18.60 μM, 95% CI: 1.66-23.52 μM, $p = 0.021$) and INU (mean difference:
321 12.26 ± 20.48 μM, 95% CI: 0.23-24.29 μM, $p = 0.045$) compared to the NO_3^- (Figure 4A).
322 Plasma acetate iAUC was higher following INU+NO_3^- (53.47 ± 64.57 μM, 95% CI, 23.25-83.69
323 μM) and INU (47.86 ± 61.66 μM, 95% CI, 19.00-76.72 μM) compared to NO_3^- (24.38 ± 37.58
324 μM, 95% CI, 6.80-41.96 μM); however these differences did not reach statistical significance
325 ($p = 0.241$) (Figure 4B). In contrast, plasma tAUC, was significantly higher following INU+NO_3^-
326 and INU compared to NO_3^- ($p = 0.020$ and $p = 0.037$, respectively) (Figure S2, A). The mean
327 plasma propionate was 2.73 ± 0.63 μM (95% CI: 2.45-3.01 μM) for INU+NO_3^-, 2.26 ± 0.45 μM
328 (95% CI: 2.04-2.47 μM) for INU, and 2.07 ± 0.63 μM (95% CI: 1.79-2.36 μM) for NO_3^-. Plasma
329 propionate concentrations were significantly higher following INU+NO_3^- compared to NO_3^-
330 (mean difference: 0.66 ± 0.89 μM, 95% CI: 0.15-0.17 μM, $p = 0.010$), while INU and NO_3^-
331 showed no significant difference (mean difference: 0.18 ± 0.54 μM, 95% CI, -0.13-0.50 μM, *p*

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= 0.416) (Figure 4C). Plasma propionate iAUC was higher following INU+NO_3^- (2.16 ± 2.59 μM, 95% CI, 0.95-3.38 μM) and INU (2.08 ± 1.74 μM, 95% CI, 1.26-2.89 μM) compared to NO_3^- (1.53 ± 1.72 μM, 95% CI, 0.72-2.34 μM), although this difference was not statistically significant (p = 0.591) (Figure 4D). Plasma propionate tAUC was significantly higher following INU+NO_3^-, but not INU, compared to NO_3^- (p = 0.010 and p = 0.379, respectively) (Figure S2, B). The mean plasma butyrate concentrations were 0.81 ± 0.31 μM (95% CI: 0.66-0.96 μM) for INU+NO_3^-, 0.76 ± 0.49 μM (95% CI: 0.52-0.99 μM) for INU, and 0.50 ± 0.22 μM (95% CI: 0.39-0.60 μM) for NO_3^-. Plasma butyrate concentrations were significantly higher following INU+NO_3^- (mean difference: 0.32 ± 0.22 μM, 95% CI: 0.18-0.46 μM, p < 0.001) and tended higher following INU (mean difference: 0.26 ± 0.45 μM, 95% CI: -0.01-0.53 μM, p = 0.058) compared to the NO_3^- condition (Figure 4E). Plasma butyrate iAUC was higher following INU+NO_3^- (1.18 ± 1.19 μM, 95% CI, 0.62-1.74 μM) and INU (1.79 ± 2.61 μM, 95% CI, 0.56-3.01 μM), compared to NO_3^- (0.76 ± 0.65 μM, 95% CI, 0.45-1.06 μM), although this difference was not statistically significant (p = 0.166) (Figure 4F). Plasma butyrate tAUC was significantly higher following INU+NO_3^- and INU compared to NO_3^- (p = 0.008) (Figure S2, C).

3.4 Resting blood pressure

There were no significant baseline differences in SBP, DBP, and MAP across the various conditions (p > 0.05) (Table 3). SBP remained stable over time and did not differ between conditions. However, DBP and MAP exhibited significant changes over time (p < 0.001 and p = 0.022, respectively), without any effects from the conditions or interactions. No associations were identified between acetate and BP variables after INU or INU+NO_3^-. When NO_3^- was consumed alone, peak plasma nitrite did not correlate with BP. In contrast, following INU+NO_3^-, peak plasma nitrite showed a moderate negative correlation with DBP (r_s = -0.61, p = 0.004) and MAP (r_s = -0.59, p = 0.005). Additionally, a moderate negative correlation was found between DBP and plasma nitrate following NO_3^- (r_s = -0.47, p = 0.04) (Table 4).

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357 **Table 3.** Mean ± SD of clinic blood pressure measurements following consumption of the three supplements in normotensive adults (*n* = 20).

Timepoint	SBP INU+NO_3^-	SBP NO_3^-	SBP INU	DBP INU+NO_3^-	DBP NO_3^-	DBP INU	MAP INU+NO_3^-	MAP NO_3^-	MAP INU
Baseline (mmHg)	113 ± 11	116 ± 9	114 ± 10	62 ± 6	63 ± 6	64 ± 7	81 ± 8	83 ± 6	82 ± 8
60 min (mmHg)	115 ± 11	116 ± 7	115 ± 11	64 ± 6	66 ± 7	63 ± 7	83 ± 7	84 ± 6	82 ± 8
120 min (mmHg)	115 ± 10	114 ± 8	114 ± 10	63 ± 6	64 ± 6	62 ± 6	82 ± 7	82 ± 6	82 ± 7
180 min (mmHg)	117 ± 9	116 ± 10	117 ± 9	64 ± 7	64 ± 6	64 ± 7	84 ± 6	83 ± 7	83 ± 7
240 min (mmHg)	115 ± 9	114 ± 7	116 ± 8	61 ± 5	61 ± 5	61 ± 5	81 ± 5	81 ± 5	82 ± 5
300 min (mmHg)	113 ± 8	113 ± 7	116 ± 9	62 ± 5	61 ± 5	61 ± 5	81 ± 5	80 ± 5	82 ± 6
360 min (mmHg)	113 ± 8	116 ± 8	114 ± 8	62 ± 4	62 ± 5	62 ± 6	81 ± 5	82 ± 5	81 ± 6
p-value (time)	0.374			< 0.001***		0.022*			
p-value (condition)	0.794			0.903		0.795			
p-value (interaction)	0.394			0.401		0.568			

358

359 *$p < 0.05$, ** $p < 0.01$. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; INU, inulin, NO_3^-, nitrate; min,
360 minutes.

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Table 4. Correlation coefficients of peak changes in plasma nitrite, nitrate and acetate with their corresponding blood pressure variables following acute ingestion of the three supplements.

Metabolites: Treatment	**ΔSBP**	**ΔDBP**	**ΔMAP**
Δ Peak Plasma Nitrite : INU + NO_3^-	r_s 0.02, p = 0.955	**r_s -0.61, p = 0.004****	**r_s -0.59, p = 0.005****
Δ Peak Plasma Nitrite : NO_3^-	r_s -0.41, p = 0.06	-0.13, p = 0.56	-0.29, p = 0.20
Δ Peak Plasma Nitrate: INU + NO_3^-	r_s 0.29, p = 0.22	r_s 0.13, p = 0.58	r_s -0.02, p = 0.94
Δ Peak Plasma Nitrate: NO_3^-	r_s 0.24, p = 0.32	**r_s -0.47, p = 0.04***	r_s -0.06, p = 0.79
Δ Peak Plasma Acetate : INU + NO_3^-	r 0.20, p = 0.39	r 0.13, p = 0.57	r 0.20, p = 0.41
Δ Peak Plasma Acetate : INU	r -0.21, p = 0.37	r -0.33, p = 0.15	r -0.30, p = 0.20

Δ changes in systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP), and mean arterial pressure (ΔMAP). Δ changes in peak nitrite, peak nitrate, and peak acetate concentrations in plasma. Abbreviations: INU, inulin; NO_3^-, nitrate. 'rs' indicates Spearman's rank correlation coefficient, 'r' indicates Pearson's correlation coefficient. *p < 0.05, ** p < 0.01.

4. Discussion

This study represents the first double-blind crossover investigation examining the direct impacts of acute dietary intervention with inorganic nitrate and inulin, focusing on their potential synergistic effects on their derived metabolites. Based on prior preclinical research (Catry et al., 2018), an *in vitro* study (Sakakibara et al., 2010), and the understanding that nitrate supplementation enhances NO bioavailability via the nitrate-nitrite-NO pathway (Lundberg et al., 2018), we hypothesised that combining inulin and nitrate might increase NO bioavailability, as indicated by elevated plasma nitrate and nitrite concentrations. However, combined inulin and nitrate did not elevate plasma nitrate or nitrite concentrations beyond those observed with nitrate alone. Plasma nitrate and nitrite increased following nitrate but remained unchanged with inulin ingestion, suggesting that neither inulin nor its metabolite acetate significantly influenced NO bioavailability in humans. Inulin has been proposed as a dietary approach to increase the production of SCFAs, including acetate (Boets et al., 2015),

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potentially exceeding the levels observed with other indigestible carbohydrates (van der Beek et al., 2018). Nevertheless, in this study, we observed only modest increases in plasma acetate, as well as butyrate and propionate, within a 360-minute timeframe. This lack of a distinct peak is likely attributable to the limited duration of supplementation. Additionally, while reductions in DBP and MAP from baseline were observed, no significant differences were found across the different conditions. Nonetheless, significant inverse relationships were identified between DBP, MAP and plasma nitrite following combined inulin and nitrate supplementation suggesting potential interactive effects on BP. A similar inverse association was also noted between nitrate intake and DBP, corroborating findings from previous research (Wei et al., 2024; Wei et al., 2023).

4.1 Plasma nitrate and nitrite

After nitrate ingestion, plasma nitrate and nitrite concentrations increase via the nitrate-nitrite-NO pathway, peaking at 1-2 hours and 2-3 hours, respectively, before gradually declining and returning to baseline levels within 24 hours (Webb et al., 2008). Various enzymes and proteins, including deoxyhemoglobin, can catalyse the conversion of nitrite to NO within the blood and other tissues (Cosby et al., 2003). Consistent with previous research on adults with normal BP, hypertension, and obesity (Kapil et al., 2015; Webb et al., 2008; Wylie et al., 2013), this study observed increases in plasma nitrate and nitrite after nitrate supplementation, both with and without inulin. After supplementation, nitrate peaked at 60 minutes and nitrite at 120 minutes, with elevated concentrations persisting for up to 360 minutes. No differences in peak timing were observed between combined inulin and nitrate and nitrate alone, indicating that their combination does not alter the pharmacokinetics of nitrate or nitrite, as seen with other prebiotics (Rodriguez-Mateos et al., 2015).

Nitrate and inulin have been suggested to enhance NO bioavailability via distinct mechanisms. However, unlike nitrate, the effects of which have been documented in clinical studies (Wei et al., 2024), the effects of inulin have only been demonstrated in preclinical (Catry et al., 2018) and *in vitro* (Sakakibara et al., 2010) studies, with no clinical trials conducted in humans to date. Previous *in vivo* studies have shown that the dietary context can modulate the effects of inorganic nitrate on NO metabolism, particularly when co-ingested with prebiotics compounds that influence eNOS phosphorylation (Álvarez-Cilleros et al., 2018; Lovegrove et al., 2017). However, the combination of apples (a source of flavonoids) with spinach (a source of nitrate) did not enhance NO bioavailability, but instead attenuated SBP responses (Bondonno et al., 2012). Similarly, cocoa flavanols, another prebiotic-rich food, markedly reduced plasma nitrite concentrations when consumed with dietary nitrate (Rodriguez-Mateos et al., 2015), an effect we did not observe with inulin and nitrate co-supplementation. The relationship between plasma nitrite levels and eNOS phosphorylation

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is complex. While elevated plasma nitrite concentrations suggest enhanced NO availability and eNOS activity under normal physiological conditions (Kleinbongard et al., 2003; Lauer et al., 2001), others have proposed that dietary nitrate-derived plasma nitrite increases, while eNOS-derived nitrite might decrease, resulting in unchanged plasma nitrite concentrations. This supports the hypothesis that activation of the nitrate-nitrite-NO pathway downregulates eNOS activity (Carlström et al., 2015). Although we did not observe changes in plasma nitrite levels, we cannot exclude the possibility that inulin and nitrate supplementation influenced eNOS phosphorylation, as this was not directly evaluated.

4.2 Plasma short-chain fatty acids

Inulin is not absorbed until it reaches the colon, where it is fermented into SCFAs by the gut bacteria. Studies have shown that inulin consumption can elevate plasma SCFAs concentrations within hours (Boets et al., 2015; Tarini & Wolever, 2010; van der Beek et al., 2018). We observed that inulin intake resulted in higher plasma acetate, butyrate, and propionate concentrations, with and without nitrate, than with nitrate alone. However, these increases were not statistically significant when baseline values were excluded from the iAUC analysis, possibly because of individual variations in postprandial SCFAs responses. Our findings partly align with those of Fernandes et al. (2011), who observed a significant increase in breath hydrogen and methane at 120 minutes following inulin consumption (24 g) and only observed a non-significant trend in serum SCFAs up to 240 minutes post-consumption. However, our findings contrast with those of other studies that reported a significant increase in SCFAs after acute inulin consumption. (van der Beek et al., 2018) found a significant rise in plasma acetate 240-420 minutes post-ingestion compared to maltodextrin. Tarini and Wolever (2010) found that 24 g of inulin significantly increased all three serum SCFAs within a 6-hour period, while Rahat-Rozenbloom et al. (2017) showed an increase in SCFAs 240-360 minutes after 24 g of inulin intake. The discrepancies between our findings and those of previous studies may stem from differences in the types of inulin used. van der Beek et al. (2018) used short-chain inulin, leading to rapid fermentation and elevated SCFAs at 240 minutes post-ingestion. In contrast, we used long-chain inulin, which results in slower fermentation (Stewart et al., 2008). Our inulin dose was 9 g lower than the amounts used in most studies to limit common side effects with higher doses of this supplement (i.e., bloating, flatulence) (Bonnema et al., 2010), which may have affected timing and quantities of SCFAs production, as inulin effects are dose-dependent (Vinelli et al., 2022). Studies using 15 g inulin found increases in all SCFAs at 12 hours post-consumption (Boets et al., 2015). By measuring only up to 6 hours (360 minutes) post-consumption, we might have missed interindividual variability on transit time, meaning a further increase in SCFAs after 6 hours in those participants with a slower digestion.

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The impact of dietary nitrate on the gut microbiome and SCFAs is not yet well understood. For instance, Wang et al. (2023) discovered that while nitrate supplementation through red beetroot juice does not seem to affect alpha and beta diversity, it does lead to significant changes in the abundance of certain taxa, such as *Romboutsia*, *Bacteroidales*, and *Akkermansia muciniphila*, after a 14-day supplementation period. In contrast, Messiha et al. (2025) reported mixed outcomes with nitrate supplementation in the form of sodium nitrate, which modified the gut microbiome and elevated proatherogenic metabolites such as trimethylamine N-oxide (TMAO). This study also observed an increase in *Akkermansia muciniphila* compared to placebo, suggesting a compensatory response to elevated TMAO levels, as this bacterium can reduce TMAO. Additionally, the authors noted an increase in *Clostridiales*, which contribute to TMAO production (Messiha et al., 2025). However, much like our findings, where treatment differences were likely due to inulin fermentation into SCFAs, Messiha et al. (2025) showed no significant reduction in plasma SCFAs (acetate, propionate, butyrate, and caproate) following nitrate supplementation.

4.3 Blood pressure

Nitrate acts as a reservoir for NO and reduces BP through vasodilation via the sGC-cGMP pathway, which decreases reactive oxygen species, inhibits oxidative stress enzymes, and enhances eNOS function (Carlström et al., 2018). Despite an increase in plasma nitrate and nitrite concentrations, we observed no significant differences in BP between conditions. This finding contrasts with those of some studies (Kapil et al., 2010; Vanhatalo et al., 2010) but aligns with others (Miller et al., 2012; Wei et al., 2023). Studies have demonstrated BP reductions with nitrate supplementation in adults with normal BP (Bahra et al., 2012; Kapil et al., 2010; Wei et al., 2023) and hypertension (Ghosh et al., 2013; Kapil et al., 2015). However, nitrate does not consistently lower BP, even when plasma nitrate and nitrite concentrations are elevated (Bescos et al., 2025). Baseline BP seems to influence the BP reduction achieved with nitrate supplementation (Kapil et al., 2010), with supplementation potentially being more effective in older adults with BMI > 30 kg/m² or prehypertension (He et al., 2021). Despite we showed no reduction in BP, our findings revealed correlations between changes in peak nitrite and DBP and MAP following combined inulin and nitrate supplementation, and peak nitrate and DBP following nitrate supplementation alone, potentially suggesting individual variations in BP responses linked to NO bioavailability. These interindividual differences support the concept of higher and lower responses to nitrate supplementation (Hayes et al., 2025).

4.4 Strengths and limitations

The strengths of this study include its crossover design, which effectively controlled for baseline differences, and the assessment of plasma SCFAs rather than faecal SCFAs,

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491 which provides a representative measure of systemic circulation (den Besten et al., 2013). In
492 addition, this study controlled for dietary nitrate intake. However, this study has several
493 limitations that warrant consideration. The young, healthy study population may not be
494 representative of the general population and may have a limited potential for BP improvement.
495 The analysis did not account for habitual fibre intake, which could have influenced the results.
496 Future studies should consider participants' regular fibre intake and dietary history to
497 contextualise their responses to these interventions, offering a more comprehensive
498 understanding of the potential benefits (Whelan et al., 2024). Moreover, the 360-minute post-
499 intervention blood collection may have failed to capture peak SCFAs concentrations in some
500 participants due to variability in gut microbiota fibre fermentation rates, attributable to diverse
501 bacterial metabolite profiles and gut physiology, among other factors (Thomson et al., 2021).
502 In this study, BP was assessed using office-based measurements, which are not regarded as
503 the gold standard in comparison to ambulatory BP monitoring (Asayama et al., 2024). Lastly,
504 the absence of a placebo control and the small sample size limit the ability to distinguish
505 supplement effects from natural variations, reducing the statistical power to identify significant
506 differences, which could affect the generalisability of the findings.

507 *4.5 Future perspectives*

508 Investigating the chronic consumption of inulin and nitrate is essential to understanding
509 their combined health effects in real-world dietary contexts, as individuals consume foods
510 rather than isolated compounds. This approach provides a realistic assessment of potential
511 health benefits. Long-term studies on the synergistic effects of inulin and nitrate may elucidate
512 their impact on vascular function, gut microbiome composition, and cardiovascular health,
513 particularly in populations at an elevated risk of cardiovascular diseases. Such research
514 should prioritise assessing the effectiveness of these supplements in reducing BP in adults
515 with hypertension, rather than those with normal BP. For example, elevation in plasma nitrite
516 concentration has been demonstrated to be significantly greater in older individuals than in
517 their younger counterparts (Stanaway et al., 2019), indicating that enhancement of the
518 enterosalivary pathway may potentially result in unexpectedly more favourable outcomes with
519 respect to cardiovascular parameters in the older population. Additionally, it is crucial to
520 examine the impact of inulin consumption in older adults, who may respond differently to age-
521 related changes in the gut microbiota (Kiewiet et al., 2021). For instance, older individuals with
522 reduced caloric needs may require foods enriched with fibre or the use of fibre supplements
523 (McKeown et al., 2022).

524 **5. Conclusion**

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In the short term, acute supplementation with inulin and nitrate did not demonstrate any additional effect on plasma nitrate and nitrite levels compared to nitrate supplementation alone. Similarly, nitrate supplementation did not appear to adversely affect plasma SCFAs; however, a longer duration may be required to observe inulin-derived plasma SCFAs. BP was not significantly influenced by the supplements; nonetheless, significant negative correlations were identified between peak plasma nitrite and both DBP and MAP for the combined supplementation, as well as between peak plasma nitrate and DBP following nitrate supplementation alone. Consequently, further research is necessary to investigate the effects of chronic inulin and nitrate supplementation in adults with hypertension, with the aim of evaluating the impact on BP, vascular function, and gut microbiota composition, while considering variations in dietary history and fibre intake.

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Declaration of interests

The authors declare none.

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842 **Figure 1.** Study visit overview, including timings of blood sampling and blood pressure
843 measurements. Created with BioRender.com.

844 **Figure 2.** CONSORT diagram flowchart for the recruitment and retention of the study
845 participants.

846 **Figure 3.** Plasma nitrite and nitrate following INU+NO_3^- (pink), NO_3^- (green), and INU (orange)
847 conditions. A) plasma nitrite (nM) over 360 minutes; B) plasma nitrite iAUC from baseline to
848 360 minutes; C) plasma nitrate (μM) over 360 minutes; D) plasma nitrate iAUC from baseline
849 to 360 minutes. All results are expressed as means ± SD (n = 20). ^{f}Significant differences
850 between INU+NO_3^- and INU. *Significant differences between NO_3^- and INU. ns $p > 0.05$, **p
851 = 0.001, ***$p < 0.001$. Abbreviations: iAUC, incremental area under the curve; INU, inulin; NO_3^-
852 , nitrate; min, minutes.

853 **Figure 4.** Plasma acetate, propionate and butyrate following INU+NO_3^- (pink), NO_3^- (green),
854 and INU (orange) supplements. A) plasma acetate (μM) over 360 minutes; B) plasma acetate
855 iAUC from baseline to 360 minutes; C) plasma propionate (μM) over 360 minutes; D) plasma
856 propionate iAUC from baseline to 360 minutes; E) plasma butyrate (μM) over 360 minutes; F)
857 plasma butyrate iAUC from baseline to 360 minutes. All results are expressed as means ± SD
858 (n = 20). ns $p > 0.05$. Abbreviations: iAUC, incremental area under the curve; INU, inulin; NO_3^-
859 , nitrate; min, minutes.

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861 Figure 1

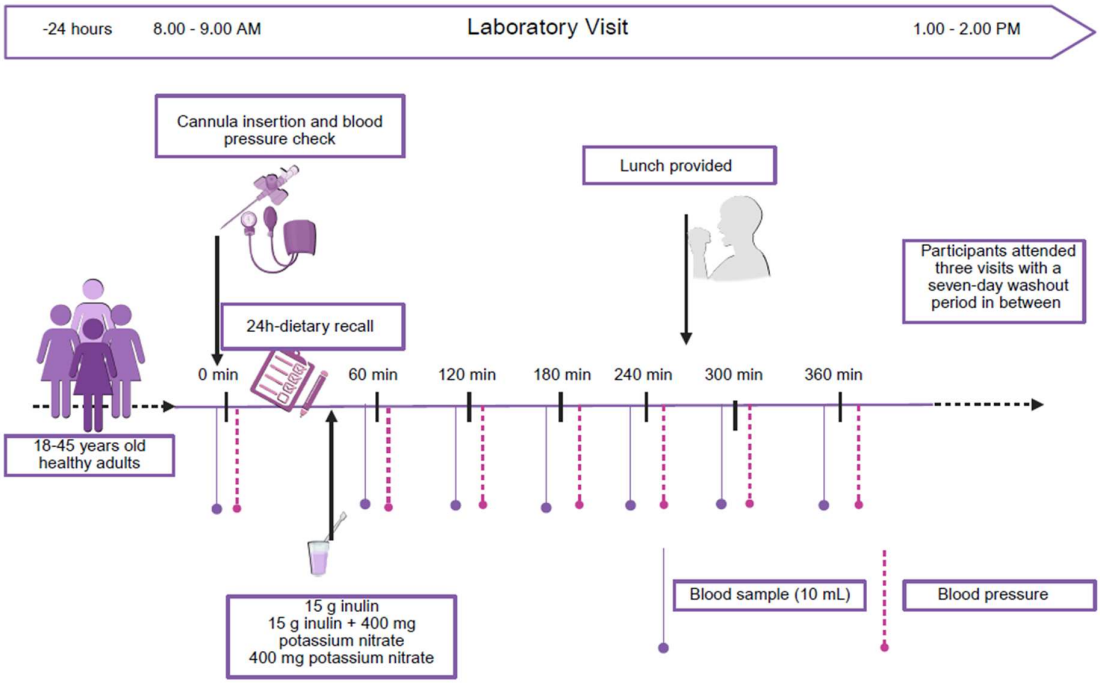

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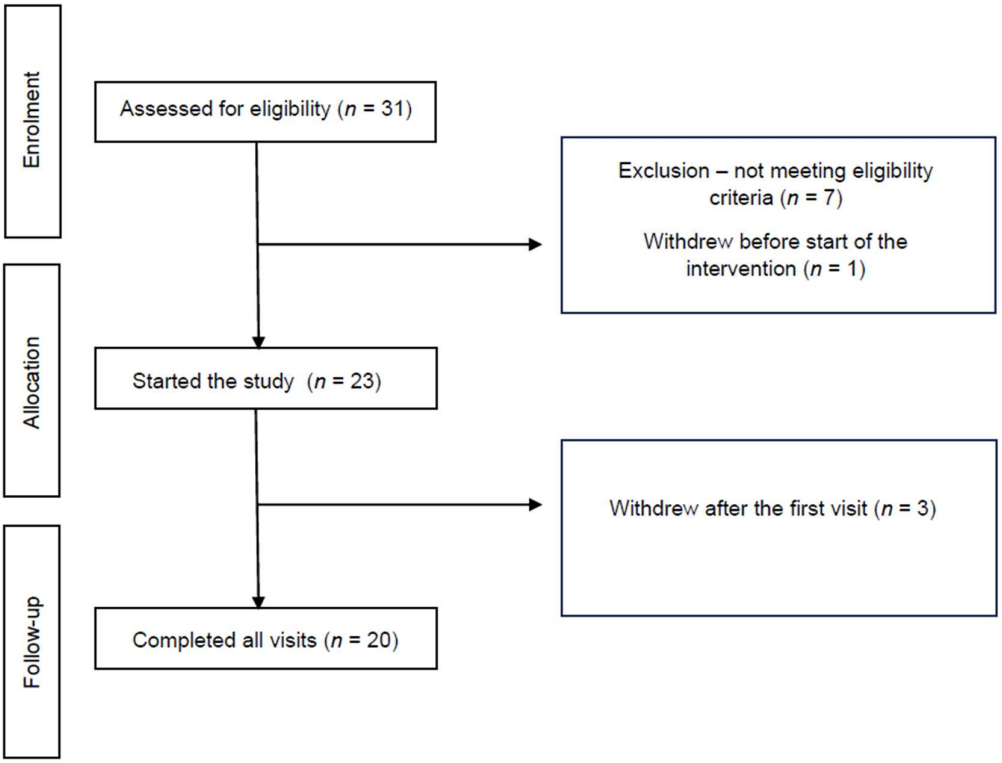
Figure 2



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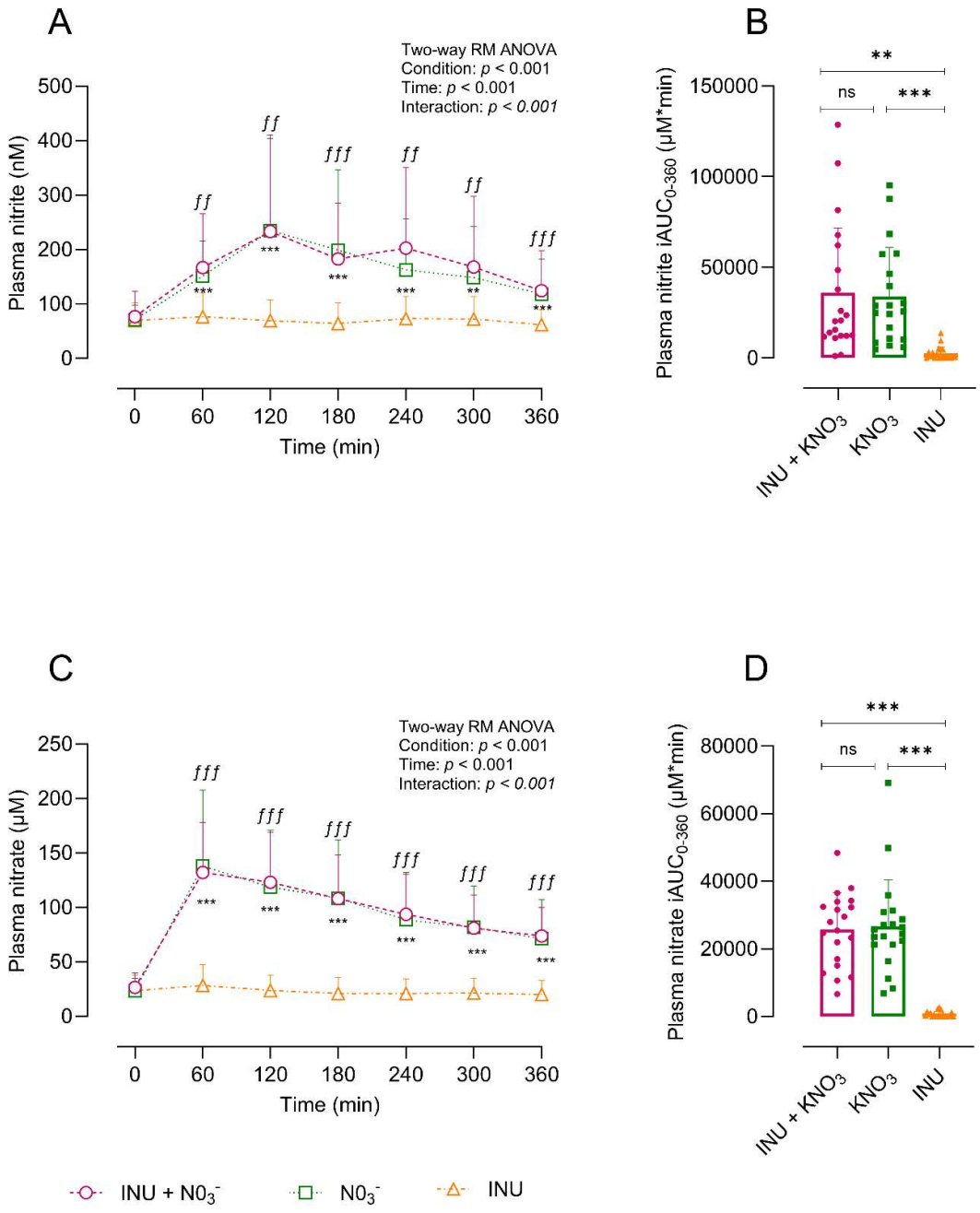
868 Figure 3



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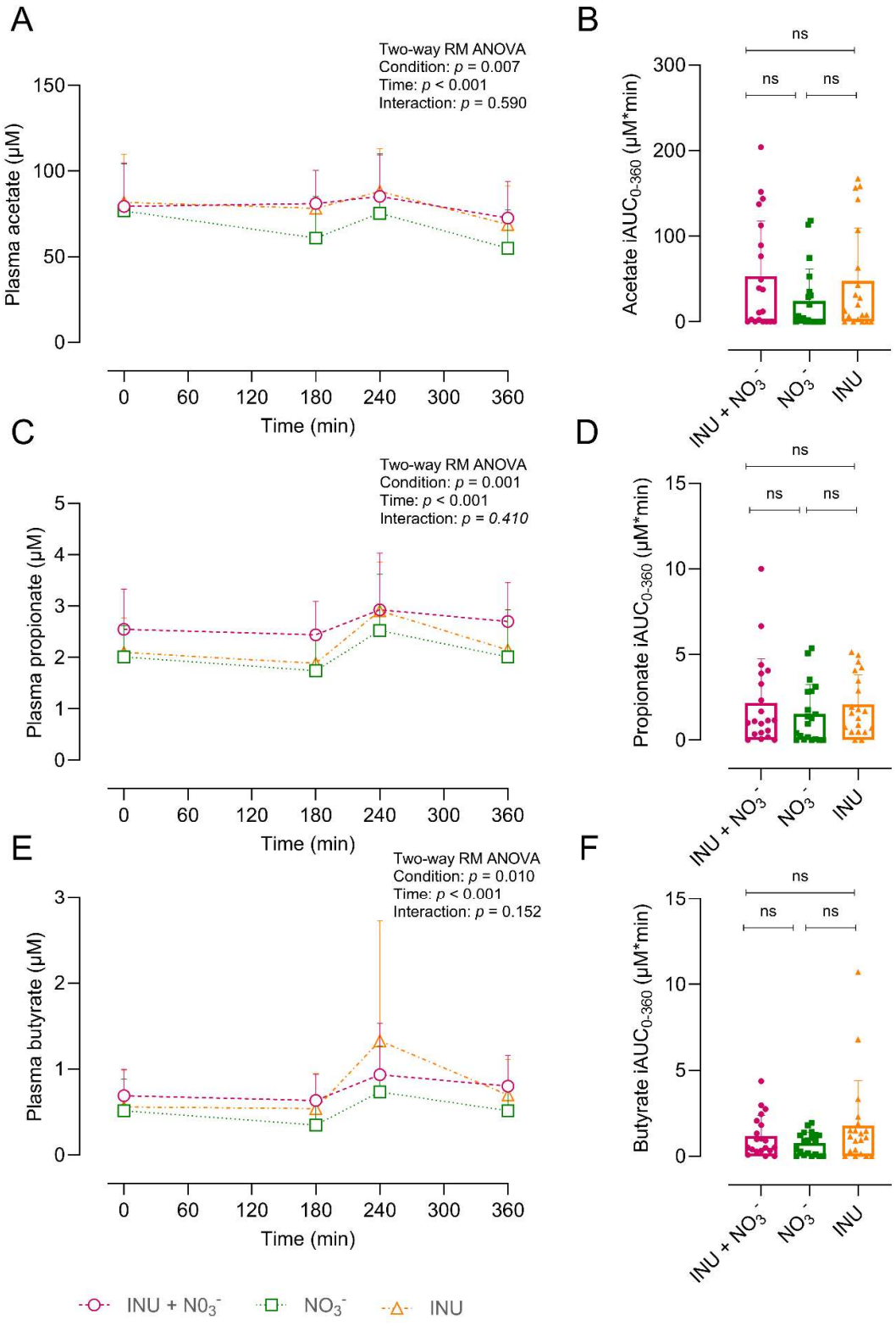
871 Figure 4



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