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1 **Beetroot Juice versus Chard Gel: A Pharmacokinetic and Pharmacodynamic**
2 **Comparison of Nitrate Bioavailability**

3

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30 **Highlights**

- 31 • When matched for nitrate content both beetroot juice and chard gels, known to
32 be rich in nitrate, increased plasma nitrate and nitrite concentrations and
33 reduced blood pressure to a similar extent.
- 34 • Inter-individual variability to reach maximal plasma nitrite levels was
35 considerable and should be taken into account when utilizing acute dietary
36 nitrate supplementation.
- 37 • Plasma concentrations of total nitrosated products were higher with beetroot
38 juice than with chard gel despite comparable nitrate content.

39

40 **Abstract**

41 Dietary supplementation with inorganic nitrate (NO_3^-) has been shown to induce a
42 multitude of advantageous cardiovascular and metabolic responses during rest and
43 exercise. While there is some suggestion that pharmacokinetics may differ depending
44 on the NO_3^- source ingested, to the best of our knowledge this has yet to be
45 determined experimentally. Here, we compare the plasma pharmacokinetics of NO_3^- ,
46 nitrite (NO_2^-), and total nitroso species (RXNO) following oral ingestion of either
47 NO_3^- rich beetroot juice (BR) or chard gels (GEL) with the associated changes in
48 blood pressure (BP). Repeated samples of venous blood and measurements of BP
49 were collected from nine healthy human volunteers before and after ingestion of the
50 supplements using a cross-over design. Plasma concentrations of RXNO and NO_2^-
51 were quantified using reductive gas-phase chemiluminescence and NO_3^- using high
52 pressure liquid ion chromatography. We report that, $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ were increased
53 and systolic BP reduced to a similar extent in each experimental arm, with
54 considerable inter-individual variation. Intriguingly, there was a greater increase in

55 [RXNO] following ingestion of BR in comparison to GEL, which may be a
56 consequence of its higher polyphenol content. In conclusion, our data suggests that
57 while differences in circulating NO_2^- and NO_3^- concentrations after oral
58 administration of distinct NO_3^- -rich supplementation sources are moderate,
59 concentrations of metabolic by-products may show greater-than-expected variability;
60 the significance of the latter observation for the biological effects under study remains
61 to be investigated.

62 **Key Words:** nitrite, nitric oxide, dietary supplementation, blood pressure

63

64 **1. Introduction**

65 Dietary nitrate (NO_3^-) supplementation has been demonstrated to positively influence
66 parameters of exercise performance (2, 25, 36) and vascular health (26, 27, 50, 54).
67 These effects have been achieved utilizing a variety of different vehicles for NO_3^-
68 delivery, including simple sodium (28) or potassium salts (23), NO_3^- -rich foods (44),
69 concentrated beetroot juice (BR) (58), and chard gel (GEL) (37, 38). These studies
70 have consistently shown that circulating plasma $[\text{NO}_3^-]$ and nitrite ($[\text{NO}_2^-]$)
71 concentrations are increased following ingestion of NO_3^- supplements. Whilst the
72 biological consequences of dietary NO_3^- administration are not fully understood at
73 present, it is known that NO_3^- can be reduced to NO_2^- , which is believed to be
74 subsequently further converted to bioactive nitric oxide (NO) (1, 31). The entero-
75 salivary circulation plays a vital role in NO homeostasis with ~25% of all circulating
76 NO_3^- taken up by the salivary glands and concentrated in the saliva (51). The
77 reduction of NO_3^- to NO_2^- takes place in the oral cavity where commensal facultative
78 anaerobic bacteria on the surface of the tongue reduce NO_3^- to NO_2^- via NO_3^-

79 reductase enzymes (12, 29). Once swallowed, NO_2^- reaches the stomach where a
80 proportion is then converted to NO, with the remainder being absorbed into
81 circulation via the intestinal tract (3, 32, 33).

82 It is well-established that increases in plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ following dietary
83 NO_3^- supplementation occur in a dose-dependent manner (4, 19, 21, 23, 58, 59),
84 however the influence of the vehicle, if any, is less certain. Several studies have
85 reported that plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ reaches maximal quantities at $\sim 1\text{--}1.5$ h and
86 $2.5\text{--}3$ h, respectively, after ingestion of BR (23, 35, 54, 58). Recent work from our
87 laboratory has shown that consuming GEL results in similar plasma NO_3^-
88 pharmacokinetics but plasma $[\text{NO}_2^-]$ reaches maximal levels more quickly (~ 1.5 h)
89 after ingestion (37). It is currently unclear whether the variance in NO_2^-
90 pharmacokinetics between BR and GEL is simply due to the vehicle of administration
91 or profoundly influenced by inter-cohort differences in the response to NO_3^-
92 supplementation. Understanding if the vehicle of NO_3^- supplementation affects the
93 fate of NO-related metabolites may allow for the optimization of dosing strategies for
94 sports performance and other contexts. Therefore, the purpose of this study was to
95 compare the effects of ingesting BR and GEL on plasma NO metabolite
96 pharmacokinetics and blood pressure (BP) pharmacodynamics in healthy individuals.

97

98 **2. Methods**

99 **2.1 Participants**

100 Nine healthy adult males (age 28 ± 4 years, stature: 181 ± 8 cm, body mass: $83.4 \pm$
101 10.4 kg) volunteered to take part in the study, which was approved by the School of
102 Science and Sport Ethics Committee of the University of the West of Scotland. All

103 participants provided written informed consent and a medical questionnaire before the
104 study began. Healthy males between the ages of 18 and 45 who were physically active
105 (taking part in recreational activity a minimum of 3 times per week) were eligible to
106 participate in the study. Participants were excluded if they were currently taking
107 dietary supplements or any medication, regularly used mouthwash, were smokers, had
108 a current illness or virus within the previous month, had a known disorder or history
109 of disorders of the hematopoietic system, were hypertensive ($\geq 140/90$ mmHg) or had
110 a family history of premature cardiovascular disease. All procedures were conducted
111 in accordance with the Declaration of Helsinki.

112

113 **2.2 Experimental Design**

114 Our study had a simple randomized cross-over design. Participants visited the
115 laboratory on two separate occasions with a minimum 7-day washout period and a
116 maximum of 14 days between visits. Participants consumed either concentrated BR
117 (Beet It Organic Shot, James White Drinks, Ipswich, UK) or GEL (Science in Sport,
118 GO+ Nitrates, Lancashire, UK) during each trial.

119

120 Participants were asked to refrain from the consumption of alcohol, caffeine, NO_3^-
121 rich foods as outlined by Hord and colleagues (22), and to avoid any strenuous
122 exercise for 24 h before each trial. Participants were also asked to refrain from the use
123 of anti-bacterial mouthwash and chewing gum for the duration of the study as they
124 have been shown to disturb the oral bacterial flora required for the conversion of NO_3^-
125 to NO_2^- in the saliva (17, 41). Compliance to these factors was determined at the start
126 of each visit.

127

128 Following a 12 h overnight fast, participants reported to the lab in the morning where
129 they were asked to void the contents of their bladder and lie supine on a medical bed.
130 After 15 min, BP was determined using an automated sphygmomanometer (Omron
131 M10, Kyoto, Japan) three times, at 1 min intervals. A cannula was then inserted into
132 the antecubital vein of the arm or a superficial vein on the dorsal surface of the hand
133 and the line was kept patent by regular flushing with intravenous 0.9% saline solution.
134 A sample of venous blood was then collected in a vacutainer containing EDTA and
135 immediately centrifuged at 4000 rpm at 4°C for 10 min (Harrier 18/80, MSE, UK).
136 The plasma was extracted carefully ensuring the cell layer was not disturbed and
137 immediately frozen at -80°C for later analysis of plasma [NO₃⁻], [NO₂⁻], and total
138 nitrosospecies [RXNO]. Participants then ingested either the BR or GEL supplements
139 within 1 min of pre supplementation blood sampling. The GEL supplement
140 comprised 120 ml of peach flavored sports gel containing 500 mg of NO₃⁻ from
141 natural chard and rhubarb sources. In the BR trial, participants ingested 117 ml of
142 concentrated BR that also contained 500 mg of NO₃⁻. The NO₃⁻ content of the
143 supplements was later verified using high-pressure liquid ion chromatography
144 (section 2.3).

145

146 As outlined in Fig. 1 venous blood samples were collected simultaneously with
147 measurements of BP pre-supplementation then at 1, 1.5, 2, 2.5, 3, 3.5 and 6 h post-
148 ingestion of each supplement. The measurement of BP was carried out in triplicate,
149 with the measurement being performed as close as possible to blood draw. The BP
150 Cuff was placed on the opposite arm to the cannula. Participants remained supine

151 from the first blood sample until the 3.5 h sample, after which they were allowed to
152 sit at a desk, returning 30 min before the final sample. During the experimental trials,
153 participants were provided with standardized meals, which had a low NO_3^- content.
154 Specifically, participants consumed a cereal bar after 1.5 h and a cheese sandwich 3.5
155 h after ingestion of BR or GEL. Participants were provided with *ad libitum* access to
156 tap water. The volume consumed in trial 1 was recorded and kept consistent for trial
157 2.

158

159 **2.3 Additional Experimental Arm**

160 The aforementioned procedures were conducted to address the primary objective of
161 this experiment whereby doses of GEL and BR matched for NO_3^- content were
162 compared. Whereas the dose of GEL used in this experiment comprised two full gels
163 as provided by the manufacturer (2 x 60g), 23 ml of BR was removed from one 70 ml
164 bottle to ensure a matched NO_3^- content. Given that both researchers and end-users are
165 more likely to utilize the full 140 ml (e.g. (21, 58) the dose of BR used in this
166 experiment was considered to be lacking in ecological validity. To this end, eight of
167 the participants completed an additional experimental trial where they received 140
168 ml of BR (600 mg of NO_3^- , H-BR) with the procedures repeated as previously
169 described.

170

171 **2.4 Analysis of Plasma NO Metabolites**

172 High-pressure liquid ion chromatography was used to determine plasma $[\text{NO}_3^-]$ and
173 $[\text{NO}_2^-]$. Due to high variability in the NO_2^- measurements, which may relate to lack of
174 specific sample processing without addition of N-ethylmaleimide prior to

175 centrifugation, the NO_2^- data were re-analyzed using chemiluminescence and the latter
176 was used in all calculations. Gas-phase chemiluminescence was used to determine
177 plasma [RXNO]. Samples were thawed at room temperature in the presence of 5 mM
178 N-ethylmaleimide and subsequently analyzed using an automated NO_x detection
179 system (Eicom, ENO-20, Kyoto, Japan, combined with a Gilson auto-sampler for
180 [NO_3^-])(46) and a NO analyzer (Sievers NOA 280i, Analytix, UK for [NO_2^-] and CLD
181 77AM sp, ECOphysics, Durnten, Switzerland for [RXNO]) in conjunction with a
182 custom-designed reaction chamber. NO_2^- levels were determined using 1% potassium
183 iodide in 5ml glacial acetic acid at room temperature for reduction of NO_2^- to NO
184 (42); RXNO levels were determined using the triiodide method (13). All samples
185 were analyzed within 3 months of sample collection in order to minimize degradation
186 of NO metabolites.

187

188 **2.5 Data Analysis**

189 All analyses were carried out using the Statistical Package for the Social Sciences,
190 Version 22 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism version 6 (GraphPad
191 Software Inc., San Diego, USA) for kinetic analyses. For brevity, data from the
192 additional H-BR trial are not displayed in figures. The sample size was determined *a*
193 *priori* using a power calculation which revealed that a minimum of eight participants
194 was required to detect differences in the time taken for NO_2^- to peak between GEL and
195 BR conditions. To establish the time to reach maximal [NO_2^-] and [NO_3^-] a log
196 (Gaussian) non-linear regression model was applied to the data using the following
197 equation:

$$198 \quad Y = \text{Amplitude} * \exp(-0.5 * (\ln(X/\text{Center})/\text{Width})^2).$$

199 Data are expressed as the change in the mean (Δ) \pm standard error of the mean
200 (S.E.M) as compared to baseline or the mean and 95% confidence interval (CI) for
201 time to reach maximal values. The distribution of the data was tested using the
202 Shapiro-Wilk test. A two-way repeated-measures ANOVA was used to examine the
203 differences between condition and over time for plasma NO_3^- , NO_2^- , RXNO, and BP.
204 *Post-hoc* analysis to determine the difference from the baseline was conducted using a
205 paired samples t-tests with Bonferroni correction. Statistical significance was declared
206 when $P < 0.05$.

207

208 **3. Results and Discussion**

209 Plasma [NO_3^-] and [NO_2^-] at baseline amounted to $26 \pm 5.7 \mu\text{M NO}_3^-$, $95 \pm 31.9 \text{ nM}$
210 NO_2^- for BR and $33 \pm 3.4 \mu\text{M NO}_3^-$ and $25 \pm 6.7 \text{ nM NO}_2^-$ for GEL. As expected, oral
211 NO_3^- supplementation significantly increased plasma [NO_3^-] and [NO_2^-] in each
212 experimental arm ($P < 0.001$) (Δ [NO_3^-] with BR: $319.4 \pm 32.1 \mu\text{M}$, with GEL: 383.9
213 $\pm 35.7 \mu\text{M}$, Fig. 2; Δ [NO_2^-] with BR: $205.4 \pm 51.9 \text{ nM}$, with GEL: $207.4 \pm 58.1 \text{ nM}$,
214 Fig. 3). The magnitude of the increase, however, was not different between BR and
215 GEL ($P > 0.10$). In the H-BR arm, [NO_2^-] and [NO_3^-] increased to a greater extent
216 than BR and GEL (Δ [NO_2^-] $277 \pm 161 \text{ nM}$, Δ [NO_3^-] $457 \pm 22 \mu\text{M}$, both $P < 0.01$).
217 Following ingestion of BR, [NO_2^-] reached maximal values at 3 h (95% CI 2.1 – 3.9
218 h), which was not different to GEL (2.8 h, 95% CI 2.3 – 3.2 h, $P = 0.739$). Likewise,
219 the time taken for plasma [NO_3^-] to reach maximal concentrations was not different
220 between BR and GEL (BR: 1.4 h 95% CI 0.8 – 1.9 h, GEL: 1.4 h 95% CI 0.7 – 2.1 h, P
221 $= 0.737$). In the H-BR arm, [NO_2^-] and [NO_3^-] reached maximal concentration in the
222 plasma after 3.2 h (95% CI 2.1 – 4.2 h) and 1.5 h (95% CI 0.9 – 2.1 h), respectively.

223 These data collectively suggest that the vehicle of delivery, be it liquid or gel, does
224 not impact the kinetics of the reduction of NO_3^- to NO_2^- or the maximal plasma
225 concentrations of these metabolites. Nevertheless, it remains to be established
226 whether NO_3^- supplementation in solid forms, such as whole vegetables or
227 concentrated BR flapjacks, results in different NO_x pharmacokinetics.

228

229 In the present study, plasma $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ reached maximal quantities within a
230 similar timeframe to previous research with BR (19, 29, 40, 43). However, on this
231 occasion $[\text{NO}_2^-]$ took substantially longer after GEL (2.8 h) compared with our own
232 previous work (1.5 h) (37). Given that descriptive and anthropometric variables were
233 similar between the two study cohorts, it seems likely that physiological variations
234 between individuals may account for these differences in time. Although plasma
235 $[\text{NO}_2^-]$ is likely to be substantially elevated in most individuals 2.5 h after ingestion of
236 either BR or GEL, the peak may reasonably occur anywhere between 2.1 and 3.9 h.
237 To further highlight this Figure 4 displays the individual variability in the plasma
238 NO_2^- response to both vehicles of supplementation. Another important factor to
239 acknowledge when comparing different studies is the methods of analysis for NO
240 metabolites. The sensitivity of chemiluminescence and HPLC has been highlighted
241 with factors such as sample preparation, type of analyzer used, and duration of sample
242 storage, all potentially influencing the result acquired (8, 42). Whilst the precise
243 mechanisms explaining the disparity in plasma $[\text{NO}_2^-]$ pharmacokinetics between
244 these studies are unclear, we speculate that this may at least be partially explained by
245 variances in the gut microbiota (14), pH of oral cavity and stomach (18, 43), and
246 differences in the composition of the oral bacterial flora required for NO_3^- reduction
247 (11, 18). The importance of the oral microbiome for NO_3^- reduction has been clearly

248 established, with the oral reductase capacity substantially interrupted when using anti-
249 bacterial mouthwash (5, 41, 55) or spitting of saliva following NO_3^- supplementation
250 (30, 54). Equally, physical fitness has been suggested to affect the individual response
251 to NO_3^- supplementation (18). In contrast to the direct association between endothelial
252 NO production (as measured by plasma NO_2^-) and exercise performance (47, 53).
253 Porcelli and colleagues (45) demonstrated that there was a negative association
254 between aerobic capacity ($\text{VO}_{2\text{peak}}$) and the increase in plasma $[\text{NO}_2^-]$ following
255 ingestion of a NO_3^- supplement. Although not measured in either the present study or
256 our previous work on NO_3^- pharmacokinetics (37), it is conceivable that individual
257 differences in physical fitness, diet, or other lifestyle habits may contribute to the
258 between-group variation reported here and elsewhere within the literature (18).
259 Although it has not been thoroughly investigated, it is also conceivable that oral (and
260 gut) microbial flora changes as a result of frequent NO_3^- supplementation. It has been
261 recently demonstrated following 2 weeks of NO_3^- supplementation via BR there is an
262 increase in salivary pH suggesting a role of NO_3^- supplementation in altering
263 composition of the oral microbiome (20).

264

265 Whilst the NO_3^- and NO_2^- responses were similar between experimental arms, an
266 unexpected finding was that ingestion of BR tended to increase plasma $[\text{RXNO}]$ to a
267 greater extent in comparison to GEL (Δ in BR: 408.1 ± 127.9 nM vs. Δ in GEL: 148.1
268 ± 35.1 nM, $P = 0.08$, Fig. 5.). Plasma $[\text{RXNO}]$ at baseline amounted to 79.5 ± 13.1
269 nM for BR and 71.9 ± 10.9 nM for GEL. There was, however, a high degree of
270 variability in the change in $[\text{RXNO}]$ between individuals and the small sample size
271 likely explains why this finding was not statistically significant. The increase in
272 $[\text{RXNO}]$ was even greater in the H-BR trial ($\Delta 563.8 \pm 116.7$ nM) at 2 h post ingestion

273 than in GEL ($P = 0.004$) and BR ($P=0.03$). Although plasma [RXNO] is not
274 measured routinely in NO_3^- supplementation studies, the magnitude by which
275 [RXNO] increased following BR in the present study is greater than what has been
276 previously reported [6]. Equally surprising was that the rise in [RXNO] exceeded that
277 of $[\text{NO}_2^-]$ following ingestion of BR. The explanation for this is presently uncertain
278 and while differences in supplementation regimen, NO_3^- dose, and study participants
279 may explain the disparity with previous research, further work is required to explore
280 the changes in [RXNO] and $[\text{NO}_2^-]$ following ingestion of BR.

281

282 What is also unclear is why ingestion of BR increases [RXNO] to a greater extent (at
283 least in the H-BR trial) compared to GEL. Although care was taken to match the
284 supplements for total NO_3^- content, differences in the polyphenol content between
285 beetroot and chard may account for this outcome (24, 57). Furthermore, alongside the
286 primary sources of NO_3^- the BR supplement contained additional ingredients
287 including lemon juice and the GEL contained rhubarb juice, gelling agents,
288 preservatives, and flavorings. While the total antioxidant and polyphenol content of
289 BR has been defined (56, 57) there is no comparable data on GEL. The total
290 polyphenol content of each supplement may be important for overall NO
291 bioavailability. Ingestion of flavonoid rich apples, for example, has been shown to
292 increase [RXNO] in healthy adults (6), and nitrated polyphenols are formed from
293 acidified NO_2^- under simulated stomach conditions (40). Moreover, it has been shown
294 that polyphenols augment the reduction of NO_2^- to NO in the gut (48, 49). Given that
295 S-nitrosothiols (RSNO), a component of RXNO, act as a carrier and store of NO in
296 the blood, a polyphenol-induced increase in the bioavailability of NO may reasonably
297 be exhibited by an increase in total nitroso products following BR. The importance of

298 the polyphenol content of NO_3^- supplements and the role of RXNO in the translation
299 to consequent physiological outcomes has yet to be established. However, the high
300 polyphenol content of BR (56, 57), may explain the greater reduction in oxygen
301 consumption following BR compared to sodium NO_3^- (15). RXNOs are protected
302 from direct NO scavenging by reactive oxygen species allowing NO to be transported
303 by e.g. serum albumin and red blood cells (7, 52). This establishes an NO reservoir
304 for the sustained release of NO from these biological storage forms (9, 16, 34).
305 Potentially allowing for the targeted delivery of NO to where it is required such as
306 sites of ischemia during exercise.

307

308 Systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) at baseline were as
309 follows SBP: 123 ± 2 mmHg, DBP: 70 ± 1 mmHg, MAP: 88 ± 1 mmHg for BR and
310 SBP: 124 ± 2 mmHg, DBP: 73 ± 2 mmHg, MAP: 90 ± 2 mmHg for GEL. In the
311 present study, both BR and GEL reduced SBP and MAP (Δ SBP with BR: -10 ± 2
312 mmHg, $P < 0.001$, vs. Baseline; with GEL: -12 ± 2 mmHg, $P < 0.001$; Δ MAP with
313 BR: -5 ± 2 mmHg, $P = 0.012$ vs Baseline; with GEL: -7 ± 2 mmHg, $P = 0.010$, Fig.
314 6). The magnitude of the reductions in SBP and MAP were not different between BR
315 and GEL ($P \geq 0.12$). Neither GEL nor BR significantly altered DBP ($P = 0.18$) nor
316 was there any difference between experimental arms ($P = 0.197$). Likewise, SBP (Δ -
317 11 ± 2 mmHg, $P < 0.001$) and MAP (Δ -8 ± 3 mmHg, $P < 0.001$) were reduced and
318 DBP remained unchanged from baseline in the H-BR arm. It must be acknowledged
319 that maintenance of the supine position for a prolonged period of time also likely
320 contributed to a reduction in BP. Without a control condition, however, it is
321 impossible to determine the extent of this effect. Nevertheless, these findings are
322 consistent with previous literature demonstrating that ingestion of either BR or GEL

323 reduces SBP and MAP among healthy individuals (23, 37, 54, 58). The response in
324 DBP appears to be more variable, however, although several previous studies have
325 reported comparable data (2, 10, 23). Given the data presented here, it appears that the
326 plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ mirrors acute hemodynamic response to dietary NO_3^-
327 closely. Of notable interest, however, is that the changes in $[\text{RXNO}]$ did not appear to
328 be associated with the magnitude of the reduction in BP. This is in contrast to work by
329 Oplander and colleagues (39) who demonstrated that reductions in BP were
330 associated with an increased plasma availability of RXNO but not NO_2^- following
331 exposure of the skin to ultraviolet radiation. It is conceivable, therefore, that the
332 method by which NO bioavailability is augmented will alter the mechanisms by
333 which BP is reduced.

334

335 **4. Conclusion**

336 Our data suggests that dietary NO_3^- supplementation via BR and GEL elicits similar
337 plasma $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ pharmacokinetics when examined within the same
338 participant cohort. Likewise, both BR and GEL are capable of reducing SBP and
339 MAP with little difference in the magnitude of these effects. Nevertheless, we here
340 present data demonstrating that the time course of ingesting the NO_3^- supplements to
341 maximal $[\text{NO}_2^-]$ in blood plasma is profoundly variable between individuals. This is
342 of major relevance for researchers wishing to determine the same. We also report, for
343 the first time, that ingesting BR leads to a greater availability of RXNO compared to
344 GEL, which we speculate may be attributed to the higher polyphenol content of the
345 BR supplement.

346

347 **References**

- 348 1. Bailey JC, Feelisch M, Horowitz JD, Frenneaux MP, Madhani M.
349 Pharmacology and therapeutic role of inorganic nitrite and nitrate in
350 vasodilatation. *Pharmacol Ther* 2014;144(3):303–20.
- 351 2. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary nitrate supplementation
352 reduces the O₂ cost of low-intensity exercise and enhances tolerance to
353 high-intensity exercise in humans. *J Appl Physiol* 2009;107(4):1144–55.
- 354 3. Benjamin N, O’Driscoll F, Dougall H, et al. Stomach NO synthesis. *Nature*
355 1994;368(6471):502.
- 356 4. Bondonno CP, Croft KD, Puddey IB, et al. Nitrate causes a dose-dependent
357 augmentation of nitric oxide status in healthy women. *Food Funct*
358 2012;3(5):522.
- 359 5. Bondonno CP, Liu AH, Croft KD, et al. Antibacterial mouthwash blunts oral
360 nitrate reduction and increases blood pressure in treated hypertensive
361 men and women. *Am J Hypertens* 2015;28(5):572–5.
- 362 6. Bondonno CP, Yang X, Croft KD, et al. Flavonoid-rich apples and nitrate-
363 rich spinach augment nitric oxide status and improve endothelial function
364 in healthy men and women: A randomized controlled trial. *Free Radic Biol*
365 *Med* 2012;52(1):95–102.
- 366 7. Bryan NS, Fernandez BO, Bauer SM, et al. Nitrite is a signaling molecule
367 and regulator of gene expression in mammalian tissues. *Nat Chem Biol*
368 2005;1(5):290–7.
- 369 8. Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites
370 in biological samples. *Free Radic Biol Med* 2007;43(5):645–57.
- 371 9. Bryan NS, Rassaf T, Maloney RE, et al. Cellular targets and mechanisms of
372 nitros(yl)ation: an insight into their nature and kinetics in vivo. *Proc Natl*
373 *Acad Sci U S A* 2004;101(12):4308–13.
- 374 10. Coles LT, Clifton PM. Effect of beetroot juice on lowering blood pressure in
375 free-living, disease-free adults: a randomized, placebo-controlled trial.
376 *Nutr J* 2012;11(1):106.
- 377 11. Doel JJ, Benjamin N, Hector MP, Rogers M, Allaker RP. Evaluation of
378 bacterial nitrate reduction in the human oral cavity. *Eur J Oral Sci*
379 2005;113(1):14–9.
- 380 12. Duncan C, Dougall H, Johnston P, et al. Chemical generation of nitric oxide
381 in the mouth from the enterosalivary circulation of dietary nitrate. *Nat*
382 *Med* 1995;1(6):546–51.
- 383 13. Feelisch M, Rassaf T, Mnaimneh S, et al. Concomitant S-, N-, and heme-
384 nitros(yl)ation in biological tissues and fluids: implications for the fate of
385 NO in vivo. *FASEB J* 2002;16(13):1775–85.
- 386 14. Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in

- 387 nutrition and health. *Nat Rev Gastroenterol Hepatol* 2012;9(10):577–89.
- 388 15. Flueck JL, Bogdanova A, Mettler S, Perret C. Is beetroot juice more effective
389 than sodium nitrate? The effects of equimolar nitrate dosages of nitrate-
390 rich beetroot juice and sodium nitrate on oxygen consumption during
391 exercise. *Appl Physiol Nutr Metab* 2016;41(4):421–9.
- 392 16. Ford PC, Wink DA, Stanbury DM. Autoxidation kinetics of aqueous nitric
393 oxide. *FEBS Lett* 1993;326(1–3):1–3.
- 394 17. Govoni M, Jansson EA, Weitzberg E, Lundberg JO. The increase in plasma
395 nitrite after a dietary nitrate load is markedly attenuated by an
396 antibacterial mouthwash [Internet]. *Nitric Oxide* 2008;19(4):333–7.
- 397 18. Hezel MP, Weitzberg E. The oral microbiome and nitric oxide
398 homeostasis. *Oral Dis* 2015;21(1):7–16.
- 399 19. Hobbs DA, Kaffa N, George TW, Methven L, Lovegrove JA. Blood pressure-
400 lowering effects of beetroot juice and novel beetroot-enriched breads in
401 normotensive male subjects. *Br J Nutr* 2012;108(11):2066–74.
- 402 20. Hohensinn B, Haselgrübler R, Müller U, et al. Sustaining elevated levels of
403 nitrite in the oral cavity through consumption of nitrate-rich beetroot juice
404 in young healthy adults reduces salivary pH [Internet]. *Nitric Oxide*
405 2016;Ahead of Print
- 406 21. Hoon MW, Jones AM, Johnson NA, et al. The effect of variable doses of
407 inorganic nitrate-rich beetroot juice on simulated 2000-m rowing
408 performance in trained athletes. *Int J Sports Physiol Perform*
409 2014;9(4):615–20.
- 410 22. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the
411 physiologic contact for potential health benefits. *Am J Clin Nutr*
412 2009;90(6):1–10.
- 413 23. Kapil V, Milsom AB, Okorie M, et al. Inorganic Nitrate Supplementation
414 Lowers Blood Pressure in Humans: Role for Nitrite-Derived NO.
415 *Hypertension* 2010;56(2):274–81.
- 416 24. Kazimierczak R, Hallmann E, Lipowski J, et al. Beetroot (*Beta vulgaris* L.)
417 and naturally fermented beetroot juices from organic and conventional
418 production: Metabolomics, antioxidant levels and anticancer activity. *J Sci*
419 *Food Agric* 2014;94(13):2618–29.
- 420 25. Lansley KE, Winyard PG, Bailey SJ, et al. Acute dietary nitrate
421 supplementation improves cycling time trial performance. *Med Sci Sports*
422 *Exerc* 2011;43(6):1125–31.
- 423 26. Lara J, Ashor AW, Oggioni C, Ahluwalia A, Mathers JC, Siervo M. Effects of
424 inorganic nitrate and beetroot supplementation on endothelial function: a
425 systematic review and meta-analysis. *Eur J Nutr* 2016;55(2):451–9.
- 426 27. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of Dietary
427 Nitrate on Blood Pressure in Healthy Volunteers To the Editor : Nitric
428 oxide , generated by nitric. *N Engl J Med* 2006;355(26):2792–3.

- 429 28. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate
430 on oxygen cost during exercise. *Acta Physiol* 2007;191(1):59–66.
- 431 29. Li H, Duncan C, Townend J, et al. Nitrate-reducing bacteria on rat tongues.
432 *Appl Environ Microbiol* 1997;63(3):924–30.
- 433 30. Lundberg JO, Govoni M. Inorganic nitrate is a possible source for systemic
434 generation of nitric oxide. *Free Radic Biol Med* 2004;37(3):395–400.
- 435 31. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide
436 pathway in physiology and therapeutics. *Nat Rev Drug Discov*
437 2008;7(2):156–67.
- 438 32. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intra-gastric nitric oxide
439 production in humans: measurements in expelled air. *Gut*
440 1994;35(11):1543–6.
- 441 33. McKnight GM, Smith LM, Drummond RS, Duncan CW, Golden M, Benjamin
442 N. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in
443 humans. *Gut* 1997;40(2):211–4.
- 444 34. Miersch S, Mutus B. Protein S-nitrosation: Biochemistry and
445 characterization of protein thiol-NO interactions as cellular signals. *Clin*
446 *Biochem* 2005;38(9):777–91.
- 447 35. Miller GD, Marsh AP, Dove RW, et al. Plasma nitrate and nitrite are
448 increased by a high-nitrate supplement but not by high-nitrate foods in
449 older adults. *Nutr Res* 2012;32(3):160–8.
- 450 36. Muggeridge DJ, Howe CCF, Spendiff O, Pedlar C, James PE, Easton C. A
451 single dose of beetroot juice enhances cycling performance in simulated
452 altitude. *Med Sci Sports Exerc* 2014;46(1):143–50.
- 453 37. Muggeridge DJ, Sculthorpe N, Grace FM, et al. Acute whole body UVA
454 irradiation combined with nitrate ingestion enhances time trial
455 performance in trained cyclists. *Nitric Oxide - Biol Chem* 2015;48:3–9.
- 456 38. Muggeridge DJ, Sculthorpe N, James PE, Easton C. The effects of dietary
457 nitrate supplementation on the adaptations to sprint interval training in
458 previously untrained males. *J Sci Med Sport* 2016; Ahead of Print
- 459 39. Oplander C, Volkmar CM, Paunel-go A, et al. Whole Body UVA Irradiation
460 Lowers Systemic Blood Pressure by Release of Nitric Oxide From
461 Intracutaneous Photolabile Nitric Oxide Derivates. *Circ Res*
462 2009;105(10):1031–40.
- 463 40. Peri L, Pietraforte D, Scorza G, Napolitano A, Fogliano V, Minetti M. Apples
464 increase nitric oxide production by human saliva at the acidic pH of the
465 stomach: A new biological function for polyphenols with a catechol group?
466 *Free Radic Biol Med* 2005;39(5):668–81.
- 467 41. Petersson J, Carlström M, Schreiber O, et al. Gastroprotective and blood
468 pressure lowering effects of dietary nitrate are abolished by an antiseptic
469 mouthwash. *Free Radic Biol Med* 2009;46(8):1068–75.

- 470 42. Pinder AG, Rogers SC, Khalatbari A, Ingram TE, James PE. The
471 Measurement of Nitric Oxide and Its Metabolites in Biological Samples by
472 Ozone-Based Chemiluminescence. In: *Redox-Mediated Signal Transduction:
473 Methods and Protocols*. NJ: Humana Press; 2008 p. 11–28.
- 474 43. Pinheiro LC, Amaral JH, Ferreira GC, et al. Gastric S-nitrosothiol formation
475 drives the antihypertensive effects of oral sodium nitrite and nitrate in a
476 rat model of renovascular hypertension. *Free Radic Biol Med* 2015;87:252–
477 62.
- 478 44. Porcelli S, Pugliese L, Rejc E, et al. Effects of a Short-Term High-Nitrate Diet
479 on Exercise Performance. *Nutrients* 2016;8(9):534. 5
- 480 45. Porcelli S, Ramaglia M, Bellistri G, et al. Aerobic Fitness Affects the Exercise
481 Performance Responses to Nitrate Supplementation. *Med Sci Sports Exerc*
482 2014;47(8); 1643-1651.
- 483 46. Rassaf T, Bryan NS, Kelm M, Feelisch M. Concomitant presence of N-
484 nitroso and S-nitroso proteins in human plasma. *Free Radic Biol Med*
485 2002;33(11):1590–6.
- 486 47. Rassaf T, Lauer T, Heiss C, et al. Nitric oxide synthase-derived plasma
487 nitrite predicts exercise capacity. *Br J Sport Med* 2007;41(2):669–73;
488 discussion 673.
- 489 48. Rocha BS, Gago B, Barbosa RM, Laranjinha J. Dietary polyphenols generate
490 nitric oxide from nitrite in the stomach and induce smooth muscle
491 relaxation. *Toxicology* 2009;265(1–2):41–8.
- 492 49. Rocha BS, Nunes C, Pereira C, Barbosa RM, Laranjinha J. A shortcut to
493 wide-ranging biological actions of dietary polyphenols: modulation of the
494 nitrate-nitrite-nitric oxide pathway in the gut. *Food Funct*
495 2014;5(8):1646–52.
- 496 50. Siervo M, Lara J. Inorganic nitrate and beetroot juice supplementation
497 reduces blood pressure in adults: a systematic review and meta-analysis.
498 *The Journal of Nutrition* 2013;143(6):818–26.
- 499 51. Spiegelhalter B, Eisenbrand G, Preussmann R. Influence of dietary nitrate
500 on nitrite content of human saliva: Possible relevance to in vivo formation
501 of N-nitroso compounds. *Food Cosmet Toxicol* 1976;14(6):545–8.
- 502 52. Stamler JS, Jaraki O, Osborne J, et al. Nitric oxide circulates in mammalian
503 plasma primarily as an S-nitroso adduct of serum albumin. *Proc Natl Acad
504 Sci U S A* 1992;89(16):7674–7.
- 505 53. Totzeck M, Hendgen-Cotta UB, Rammos C, et al. Higher endogenous nitrite
506 levels are associated with superior exercise capacity in highly trained
507 athletes. *Nitric Oxide - Biol Chem* 2012;27(2):75–81.
- 508 54. Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering,
509 vasoprotective, and antiplatelet properties of dietary nitrate via
510 bioconversion to nitrite. *Hypertension* 2008;51(3):784–90.
- 511 55. Woessner M, Smoliga JM, Tarzia B, Stabler T, Van Bruggen M, Allen JD. A

- 512 stepwise reduction in plasma and salivary nitrite with increasing strengths
513 of mouthwash following a dietary nitrate load. *Nitric Oxide* 2016;54(16):1-
514 7.
- 515 56. Wootton-Beard PC, Moran A, Ryan L. Stability of the total antioxidant
516 capacity and total polyphenol content of 23 commercially available
517 vegetable juices before and after in vitro digestion measured by FRAP,
518 DPPH, ABTS and Folin-Ciocalteu methods. *Food Res Int* 2011;44(1):217-
519 24.
- 520 57. Wootton-Beard PC, Ryan L. A beetroot juice shot is a significant and
521 convenient source of bioaccessible antioxidants. *J Funct Foods*
522 2011;3(4):329-34.
- 523 58. Wylie LJ, Kelly J, Bailey SJ, et al. Beetroot juice and exercise:
524 pharmacodynamic and dose-response relationships. *J Appl Physiol*
525 2013;115(3):325-36.
- 526 59. Wylie LJ, Ortiz de Zevallos J, Isidore T, et al. Dose-dependent effects of
527 dietary nitrate on the oxygen cost of moderate-intensity exercise: Acute vs.
528 chronic supplementation. *Nitric Oxide* 2016;
- 529
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533 **Figure Captions**

534

535 **Figure 1:** Study overview: time-points for beetroot juice/chard gel administration,
536 venous blood sampling, blood pressure measurements and food intake.

537 **Figure 2:** Changes in plasma nitrate concentrations following supplementation with
538 BR and GEL (Δ Mean \pm S.E.M). * Significant difference from baseline (pre-
539 supplementation) ($P < 0.001$).

540 **Figure 3:** Changes in plasma nitrite concentrations following supplementation with
541 BR and GEL (Δ Mean \pm S.E.M). * Significant difference from baseline (pre-
542 supplementation)

543 **Figure 4:** Individual plasma nitrite pharmacokinetics and Systolic BP for BR and
544 GEL. Each participant is represented by the same different colour in each figure.

545 **Figure 5:** Changes in total nitroso species concentrations following supplementation
546 with BR and GEL (Δ Mean \pm S.E.M). * Significant difference from baseline (pre-
547 supplementation)

548 **Figure 6:** Systolic (A), diastolic (B) and mean arterial pressure (C) changes following
549 supplementation with BR and GEL (Δ Mean \pm S.E.M). * Significant difference from
550 baseline (pre-supplementation)