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1 2	Acute interaction between oral glucose (75 g as Lucozade) and inorganic nitrate: decreased insulin clearance, but lack of blood pressure-lowering		
3			
4	Short running title: Acute glucose and nitrate interaction on insulin and blood pressure		
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8			
9 10	The authors confirm that the Principle Investigator for this paper is Dr AJ Webb and that he had direct clinical responsibility for patients.		
11			
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18			
19 20 21 22	Conflict of interest/Disclosures: AJW holds shares in HeartBeet Ltd, which receive a royalty from James White Drinks Ltd who manufacture beetroot juice (source of dietary nitrate). The other authors have stated explicitly that there are no conflicts of interest in connection with this article.		
23 24 25 26	Address for correspondence: Dr. Andrew J Webb: andrew.1.webb@kcl.ac.uk, +442071887188 ext 84700 (Senior Lecturer in Cardiovascular Clinical Pharmacology, King's College London, Department of Clinical Pharmacology, St. Thomas' Hospital London SE1 7EH)		
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1 ABSTRACT

- 2 Introduction: Dietary inorganic nitrate (NO₃⁻) lowers peripheral blood pressure (BP) in
- 3 healthy volunteers, but lacks such effect in individuals with, or at risk of, type two diabetes
- 4 mellitus. Whilst this is commonly assumed to be a consequence of chronic
- 5 hyperglycaemia/hyperinsulinaemia, we hypothesised that acute physiological elevations in
- 6 plasma [glucose]/[insulin] blunt the haemodynamic responses to NO₃; a pertinent question
- 7 for carbohydrate-rich Western diets.
- 8 Methods: We conducted an acute, randomised, placebo-controlled, double-blind, crossover
- 9 study on the haemodynamic and metabolic effects of potassium nitrate (8 or 24 mmol
- 10 KNO₃) versus potassium chloride (KCl; placebo) administered 1 h prior to an oral glucose
- 11 tolerance test in 33 healthy volunteers.
- 12 Results: Compared to placebo, there were no significant differences in systolic or diastolic
- 13 BP (*P*=0.27 and *P*=0.30 on ANOVA, respectively) with KNO₃, nor in pulse wave velocity or
- 14 central systolic BP (*P*=0.99 and *P*=0.54 on ANOVA, respectively). Whilst there were
- 15 significant elevations from baseline for plasma [glucose] and [C-peptide], no differences
- 16 between interventions were observed. A significant increase in plasma [insulin] was
- 17 observed with KNO_3 versus KCl (n=33; P=0.014 on ANOVA) with the effect driven by the
- high-dose cohort (24mmol, *n*=13; *P*<0.001 on ANOVA; at T=0.75 h mean difference 210.4
- 19 pmol/L (95% CI 28.5 to 392.3), *P*=0.012).
- 20
- 21 Conclusions: In healthy adults, acute physiological elevations of plasma [glucose] and
- 22 [insulin] result in a lack of BP-lowering with dietary nitrate. The increase in plasma [insulin]
- without a corresponding change in [C-peptide] or [glucose] suggests that high-dose NO₃
- 24 decreases insulin clearance; a likely mechanism is via NO-dependent inhibition of insulin-
- 25 degrading enzyme.

1 What is already known about this subject:

- Inorganic nitrate lowers blood pressure and pulse wave velocity in healthy
 individuals.
- These effects are absent in those with, or at risk of, type two diabetes mellitus.
- This is assumed to be a consequence of chronic hyperglycaemia/hyperinsulinaemia.
- 6

7 What this study adds:

- Acute physiological elevations of plasma [glucose] and [insulin] result in a lack of BPlowering with dietary nitrate.
- 10 High-dose inorganic nitrate reduced insulin clearance, probably via NO-dependent
- 11 inhibition of insulin-degrading enzyme.

1 INTRODUCTION

- 2 The role of dietary inorganic nitrate (NO_3) as an alternative source of <u>nitric oxide</u> (NO) via
- 3 the enterosalivary nitrate-nitrite-NO pathway is recognised as a physiological mediator of
- 4 blood pressure (BP), endothelial function and platelet aggregation (1-3). In both healthy
- 5 individuals and those with chronic cardiovascular conditions, NO₃ supplementation has
- 6 been shown to increase exercise capacity (4-8). This beneficial effect is thought to arise from
- 7 the action of NO on skeletal muscle where it modulates excitation-contraction coupling,
- 8 mitochondrial respiration, autoregulation of blood flow, and <u>glucose</u> homeostasis (9).
- 9 However, individuals with, or at risk of, type two diabetes mellitus (T2DM) fail to exhibit a
- 10 reduction in peripheral BP or pulse wave velocity (PWV) in response to NO₃⁻
- 11 supplementation (10-12). There are a number of mechanisms that might contribute to this
- 12 lack of effect including dysfunctional NO synthesis, increased NO scavenging and altered
- 13 redox balance (13). To what extent this is a consequence of acute or chronic
- 14 hyperglycaemia/hyperinsulinaemia is unknown.
- 15 Carbohydrate (CHO) ingestion also has established benefits on exercise performance (14).
- 16 However, the effects of concurrent NO₃⁻ and CHO intake on cardiovascular haemodynamics
- 17 and glucose homeostasis (both important determinants of exercise capacity) have not been
- 18 studied in detail.
- 19 Type two diabetes mellitus is a condition associated with excess CHO intake (15), although
- 20 the aetiology of the condition is more complex (16). It has been observed that in both
- 21 healthy individuals and those with T2DM, plasma [nitrate] and [nitrite] fall acutely in
- 22 response to an oral glucose tolerance test (OGTT) (17, 18), likely reflecting an increase in NO
- 23 consumption. However, there is a lack of agreement with regards to basal plasma [nitrate]
- and [nitrite], with conflicting results reported (17, 19). This lack of agreement regarding
- 25 basal concentrations may be the result of the use of the Griess reactions which measures
- 26 combined plasma [nitrate/nitrite] and is not sufficiently sensitive to measure physiological
- 27 plasma [nitrite].
- 28 Systemic inhibition of NO synthesis results in a deterioration in glucose tolerance in non-
- 29 diabetic individuals in response to an OGTT, accompanied by an elevation in BP (20, 21).
- 30 However, the effects of NO₃ supplementation on glucose homeostasis are less clear. In
- 31 healthy individuals, NO₃⁻ supplementation appears to result in lower plasma [glucose] post-
- 32 exercise (22, 23), but without changing homeostatic responses to glucose at rest (24-26). In
- 33 those with, or at risk of T2DM, studies are heterogeneous in their design and report either
- 34 an improvement or null effect of nitrate on insulin sensitivity following glucose
- 35 administration (26-29).
- 36 In studies investigating the haemodynamic effects of NO₃⁻ supplementation in individuals
- 37 with T2DM there is greater consistency, as neither peripheral BP nor exercise tolerance are
- 38 improved (10-12); although we have demonstrated a lowering of central SBP with 6 months'

- 1 dietary nitrate [24], with a decrease in left ventricular volumes (30). This lack of effect in
- 2 those with impaired glucose tolerance may be due to impaired insulin-mediated
- 3 vasodilation (31-33), but whether this is a consequence of acute or chronic
- 4 hyperglycaemia/hyperinsulinaemia has not been established.
- 5 The purpose of this study was to determine whether there is an interaction between NO₃⁻
- 6 and glucose on BP and glucose homeostasis in healthy individuals. We hypothesised that
- 7 acute physiological elevations in plasma [glucose] and [insulin] would blunt the
- 8 haemodynamic responses to NO_3^{-} . This study was therefore conducted to address two
- 9 complimentary questions; (i) is the BP response to NO₃ supplementation affected by
- 10 concurrent glucose ingestion? and (ii) is the metabolic response to an OGTT affected by NO₃⁻
- 11 supplementation?

1 METHODS

2 Participants

- 3 Participants were healthy, normotensive volunteers aged 18 to 45 years. All participants had
- 4 a body mass index (BMI) 18 to 35 kg/m², no current or recent illness and were not taking
- 5 systemic medication other than the oral contraceptive pill. A negative urine dipstick result
- 6 for nitrite was required on the morning of each visit.
- 7 The study was approved by the South East London Research Ethics Committee
- 8 (10/H0802/52). Written informed consent was obtained from all participants.
- 9

10 Study protocol

- 11 We conducted an acute, randomised, placebo-controlled, double-blind, crossover study of
- 12 potassium nitrate (KNO₃) versus potassium chloride (KCl; placebo) (both Martindale
- 13 Pharma) followed by an OGTT performed 1 h later. The study consisted of two independent
- 14 cohorts based on the dose of KNO₃/KCl ingested: (i) a 'high-dose' cohort received 24 mmol,
- 15 and (ii) a 'low-dose' cohort received 8 mmol. Each study visit lasted 4 h and was separated
- 16 by a minimum of 7 days. The order of allocation to KNO₃ or KCl for each participant was
- 17 performed using a random, computer-generated order produced by an independent
- 18 researcher.
- 19 Participants were asked to fast overnight (>12 hours) and to avoid nitrate-rich foods,
- 20 strenuous exercise, smoking and the use of mouthwash for 24 h before the study. To
- 21 minimise any dietary confounders, participants were asked to consume the same meals for
- 22 the day prior to each arm of the study.
- 23 On the day of the study and following an hour's equilibration period during which baseline
- 24 measurements were taken (see below), participants were randomised to receive KNO₃
- versus KCl at Time -1 h. Both were administered with low-nitrate water (300 ml; Buxton
- 26 Water) and an antacid (10-20 mL repeated if necessary; Gaviscon, GSK) to minimise
- 27 gastrointestinal discomfort from the potassium supplement. A standard OGTT (75g glucose
- as Lucozade, GSK) was performed at Time 0 h. A schematic of the events is presented in
- 29 Figure 1.

30

31 Measurements

- 32 Blood pressure and heart rate (HR) readings were taken in triplicate every 15 min using an
- 33 oscillometric BP monitor (Omron 705CP, UK) according to guidelines. The average of the
- 34 second and third readings were used for analysis to diminish the impact of any alerting
- 35 response. Central systolic blood pressure (cSBP), pulse wave velocity (PWV) and

- 1 augmentation index (Alx) were measured (Time -1 h and Time 2 h) using Finometer
- 2 (Finopress Medical Systems, Netherlands) and Vicorder (SMT Medical, Germany) devices
- 3 according to manufacturers' instructions.
- 4
- 5 Blood samples were taken from a cannula in the antecubital vein at time intervals shown in
- 6 Figure 1. An initial 2 mL of blood was discarded, before 6 mL of blood was collected and
- 7 transferred into chilled lithium heparin blood collection tubes. Blood samples were
- 8 immediately centrifuged at 4500 rpm for 5 min at 4°C (Hettich Mikro 220R, Germany).
- 9 Plasma was stored in duplicate in 1 mL aliquots at -80°C prior to analysis.
- 10 Plasma concentrations of glucose, insulin and C-peptide were measured using standardised
- 11 clinical assays (Viapath, St Thomas' Hospital). Nitrate and nitrite concentrations in urine and
- 12 plasma were measured by ozone-based chemiluminescence as previously described (1, 34).
- 13 The coefficient of variation was <10% for both nitrite and nitrate quantification. Exhaled NO
- 14 (eNO) was measured using a NObreath monitor (Bedfont Scientific, UK), according to the
- 15 manufacturer's instructions.
- 16 Insulin sensitivity during each study arm was calculated via the Matsuda index, where a
- 17 higher value represents greater insulin sensitivity (35).
- 18

19 Data and Statistical Analyses

- 20 All data were analysed with GraphPad Prism software (v7.03), and are expressed as
- 21 mean±SEM unless otherwise stated. Repeated-measures two-way ANOVA with Sidak's post-
- 22 test was used for comparison of the data between the two interventions. Repeated-
- 23 measures one-way ANOVA with Dunn's post-test was used for comparison with baseline.
- 24 Correlation was assessed using Pearson's correlation. Where data were non-parametric,
- 25 appropriate equivalent statistical tests were used. *P*<0.05 was considered statistically
- 26 significant.
- 27

28 Nomenclature of Targets and Ligands

- 29 Key protein targets and ligands in this article are hyperlinked to corresponding entries in
- 30 http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS
- 31 Guide to PHARMACOLOGY (36), and are permanently archived in the Concise Guide to
- 32 PHARMACOLOGY 2017/18 (37, 38).

1 **RESULTS**

- 2 Thirty-three participants completed both visits of the study, of which 13 received high-dose
- 3 (24 mmol) and 20 received low-dose (8 mmol) KNO₃/KCl. Mild gastrointestinal discomfort
- 4 lasting <15 min was reported by 42.4% (14/33) of participants following dosing, with no
- 5 significant difference between dose or intervention. Demographic data for participants are
- 6 summarised in **Table 1**.
- 7

8 Nitrate metabolism

- 9 The metabolism of ingested NO₃ was confirmed by a significant time-dependent increase in
- 10 plasma and urinary [nitrate] and [nitrite] and eNO following KNO₃ compared to KCI (Figure
- **2**). In the high-dose cohort, plasma [nitrite] was significantly increased for KNO₃ versus KCl
- 12 at both the time of the OGTT (Time 0 h, 399±104 versus 81±16 nmol/L; P<0.01) and at peak
- 13 plasma [glucose] (Time 1 h, 721±95 versus 60±13 nmol/L; P<0.001); see Figure 2.
- 14

15 Haemodynamic response

- 16 Haemodynamic parameters pre-intervention (Time -2 h to -1 h) were similar for KNO₃ versus
- 17 KCl interventions (Table 2). There were no significant differences in BP or HR for KNO_3 versus
- 18 KCl throughout the study (Time -2 h to +2 h; SBP *P*=0.27; DBP *P*=0.30; PP *P*=0.74; HR *P*=0.12)
- 19 (Figure 3). Similarly, there were no significant differences in PWV, cSBP or Alx pre- and post-
- 20 OGTT (Time -1 h versus +2 h; all *P*>0.05; **Figure 4**).
- 21 Subgroup analyses of high-dose (24 mmol) and low-dose (8 mmol) cohorts also revealed
- 22 similar haemodynamic parameters at baseline (*data not shown*). However, in contrast to the
- 23 main analysis, significant differences in HR were observed between interventions within
- 24 each cohort. In the high-dose cohort, HR was reduced with KNO₃ versus KCl (mean
- 25 67.29±0.55 versus 68.36±0.56 mmHg; *P*=0.01) (**Figure 5**).
- For the low-dose cohort, the opposite effect was observed with a significantly higher HR
 with KNO₃ versus KCl (mean 65.89±0.62 versus 64.40±0.40 mmHg; *P*<0.01) (Figure 6). There
- 28 were no significance differences in ΔPWV , $\Delta cSBP$ or ΔAIx for the interventions within either
- 29 cohort (all P>0.05; data not shown).

30

31 Glucose Homeostasis

- 32 There were no significant differences between interventions for plasma [glucose] (*P*=0.58)
- 33 or [C-peptide] (*P*=0.84), but significantly higher plasma [insulin] was observed for KNO₃
- 34 versus KCl (*P*=0.01) (**Figure 7**). Insulin sensitivity, as represented by the Matsuda index, was
- not significantly different for KNO_3 versus KCl (mean 4.26±0.48 versus 4.37±0.48; P=0.59).

- 1
- 2 Subgroup analyses of high-dose (24 mmol) and low-dose (8 mmol) cohorts revealed that the
- 3 significant difference in plasma [insulin] was driven by the high-dose cohort (P<0.001 on
- 4 ANOVA; at t=0.75 h mean difference 210.4 pmol/L (95% CI 28.5 to 392.3), P=0.012) (Figure
- 5 8). There was no significant difference in the Matsuda index for KNO₃ versus KCl with either
- 6 24 mmol (mean 5.42±0.86 versus 5.60±0.75; *P*=0.77) or 8 mmol (mean 3.50±0.51 versus
- 7 3.57±0.57; *P*=0.31).
- 8 high- or low-dose. No significant correlation was observed between ΔSBP/DBP and
- 9 Δ [insulin]/[glucose] at timepoint 1 h and 2 h (*data not shown*).

1 **DISCUSSION**

- 2 This acute, crossover study investigated the effects of concurrent inorganic nitrate and
- 3 glucose ingestion on blood pressure and glucose homeostasis in healthy individuals. The
- 4 principal findings of this study were as follows: (i) physiological elevation of plasma [glucose]
- 5 and [insulin] resulted in a lack of BP-lowering with inorganic nitrate, despite elevated
- 6 plasma [nitrite], and (ii) the increase in plasma [insulin] without a corresponding change in
- 7 [C-peptide] or [glucose] suggests that high-dose NO₃⁻ decreases insulin clearance.
- 8 A dose-response relationship has previously been demonstrated between NO₃⁻ ingestion (as
- 9 beetroot juice or nitrate capsules) and peripheral BP reduction (1, 39, 40). Doses as low as
- 10 5.1 mmol have been shown to cause significant SBP reductions (39, 40), with higher doses
- 11 (up to 22 mmol as beetroot juice, and 24 mmol as potassium nitrate, as used here) resulting
- 12 in SBP/DBP reductions of 10.4/8.0 mmHg, and 9.4/6.0 mmHg, respectively (1, 39).
- 13 Reductions in arterial stiffness have also occurred with both acute and chronic dosing (41,
- 14 42).Whilst several studies in healthy individuals failed to show a peripheral BP decrease with
- 15 NO_3 supplementation, this is the first study with a neutral effect for $\ge 12 \text{ mmol/d } NO_3$.
- 16 There is a strong correlation between PWV and PP, and so the lack of change in PWV is
- 17 consistent with the peripheral measurements (43). Based on our previous work in those
- 18 with, or at risk of, T2DM we would have expected to observe a reduction in cSBP following
- 19 NO_3^{-1} ingestion through a selective dilatory effect on medium-sized conduit vessels (12, 44).
- 20 However, nitrate had no effect on cSBP with an acute glucose load.
- 21 The lack of effect on both peripheral and central haemodynamics suggest that normal,
- 22 physiological responses to glucose are sufficient to prevent the BP-lowering effects of NO₃⁻
- 23 supplementation. The observed differences in HR between interventions were small and, as
- 24 the magnitude of change was opposite to that expected for the two doses, their biological
- validity is uncertain. The lack of BP-lowering is consistent with other studies that have
- 26 demonstrated inhibition of NO-dependent flow mediated dilatation of conduit and small
- 27 resistance arteries following acute physiological elevations in plasma [glucose] and [insulin]
- 28 (45-47). Furthermore, in a study of overweight men Joris *et al* reported that co-ingestion of
- 29 beetroot juice (approximately 8 mmol NO₃⁻) counteracted the decrease in FMD associated
- 30 with the intake of a mixed meal, without differences in PWV or peripheral BP between
- 31 groups (48). Whilst our study was not designed to disentangle the relative contributions
- 32 from glucose and insulin, we hypothesise that lack of effect was modulated by elevated
- 33 plasma glucose given that insulin-mediated vasodilatation within skeletal muscle is NO-
- 34 dependent (31). The elevated exhaled NO demonstrated an increase in systemic NO
- 35 availability following nitrate supplementation, and that the lack of BP-lowering was
- 36 therefore unlikely due to interruption of the nitrate-nitrite-NO pathway.
- 37 In agreement with previous studies, NO₃⁻ supplementation did not lower resting plasma
- 38 [glucose] or improve insulin sensitivity as assessed by the Matsuda index (24-26). However,
- 39 in the high-dose cohort we did observe an increase in plasma [insulin] without a

- 1 corresponding increase in [C-peptide], thus suggesting decreased insulin clearance. A
- 2 change in plasma [insulin] without a corresponding change in [glucose] is consistent with
- 3 the multifaceted mechanisms responsible for glucose homeostasis (49, 50). Dietary nitrate
- 4 has been demonstrated to enhance glucose uptake in skeletal muscle independent of insulin
- 5 via translocation of <u>glucose transporter 4</u> (GLUT4) (51). It is therefore possible that high-
- 6 dose dietary nitrate facilitated glucose uptake via insulin-independent mechanisms, thus
- 7 reducing insulin clearance at the same site. Our finding is also consistent with a previous
- 8 study which showed that systemic inhibition of <u>nitric oxide synthase</u> (NOS) with N^{G} -
- 9 monomethyl-L-arginine (L-NMMA) in healthy volunteers increased insulin clearance without
- 10 an effect on peripheral insulin sensitivity (21). The mechanism of increased insulin clearance
- 11 following NOS inhibition was attributed to activation of the specific protease hepatic insulin-
- 12 degrading enzyme (IDE), which is largely responsible for whole-body insulin clearance (52).
- 13 IDE is dose-dependently inhibited by NO *in vitro* and provides a plausible mechanism for our
- 14 observation of decreased insulin clearance. Furthermore, as NO mediates glucose uptake by
- 15 skeletal muscle *in vitro* through insulin-independent mechanisms, decreased insulin
- 16 clearance may also occur peripherally following NO₃⁻ (53, 54).
- 17 This study differs from those previously conducted with regards to the nitrate dose, glucose
- 18 load and relative timing of ingestion. Our use of high-dose nitrate, a full OGTT and
- 19 coordination of peak plasma [glucose] with elevated [nitrite], optimised any interaction and
- 20 may explain why other studies did not observe changes in plasma [insulin]. Furthermore, we
- 21 opted to deliver NO₃ via capsules rather than beetroot juice, to avoid additional
- 22 uncontrolled CHO ingestion (37.5 g sugar per 500 mL; James White Drinks Ltd). It is a
- 23 limitation of this study that although Lucozade is routinely used to administer OGTTs in
- 24 clinical practice, we cannot exclude confounders mediated by other ingredients. However,
- 25 the ingredients of Lucozade are similar to those in many other sports drinks and so the
- 26 potential impact on exercise may represent a 'class effect'. Thus, the lack of an effect of
- 27 concomitant administration of glucose with nitrate on BP suggests the possibility that
- 28 glucose might also negate the beneficial effects of nitrate on exercise performance.
- 29 In summary, our findings describe decreased insulin clearance as a previously unidentified
- 30 consequence of NO_3^{-} supplementation and provide further information regarding how diet
- 31 can acutely modulate blood pressure. Further investigation is required into the potentially
- 32 antagonistic interaction between glucose and NO₃.

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- 3 submitted work. We acknowledge internal infrastructure financial support from King's
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- 6 Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation
- 7 Trust and King's College London. The views expressed are those of the authors and not
- 8 necessarily those of the NHS, the NIHR or the Department of Health.

1 Tables with Legends

- 2 **Table 1.** Demographic data for participants. Data expressed as mean±SD. [BMI: body mass
- 3 index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate]

	All participants	Subgroups	
		24 mmol	8 mmol
Number of participants (n)	33	13	20
Gender (<i>n</i> male)	15	6	9
Age (years)	27.1±6.5	27.8±7.2	26.5±6.0
Weight (kg)	70.1±13.9	69.4±9.9	70.5±16.1
Height (m)	1.7±0.1	1.7±0.1	1.7±0.1
BMI (kg/m ²)	23.3±2.9	23.7±3.2	23.1±2.8
SBP (mmHg)	113.4±10.1	115.0±11.1	112.4±9.6
DBP (mmHg)	71.2±5.8	72.0±5.3	70.7±6.2
HR (bpm)	67.9±9.4	69.4±10.3	67.1±8.0
Fasting glucose (mmol/L)	4.7±0.6	4.7±0.4	4.7±0.7
Fasting insulin (pmol/L)	44.1±22.2	39.9±16.2	46.9±25.3

4

- 5 **Table 2.** Baseline haemodynamic parameters. Time -2 h to -1 h. Data expressed as
- 6 mean±SD.

7 [SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; HR: heart

8 rate]

	KNO ₃	КСІ
SBP (mmHg)	113.1±10.0	113.2±10.9
DBP (mmHg)	70.8±6.7	71.1±6.4
PP (mmHg)	42.3±7.3	41.2±7.6
HR (bpm)	66.0±7.2	65.7±6.1

1 Figure Legends

- 2
- 3



4

5 Figure 1: Schematic of events. After acclimatisation (-2 h to -1 h), participants received

6 KNO₃ or KCl tablets (Time -1 h) followed by an oral glucose tolerance test (OGTT; 75 mg

7 glucose) at Time 0 h. Blood pressure (BP) measurement, blood tests and urine collection

8 occurred as indicated.

9

10



- 1 2
- 2 **Figure 2:** Effect of 24 mmol KNO₃ versus KCl (*n*=13) on: (A) plasma [nitrate], (B) plasma
- 3 [nitrite], (C) urine [nitrate], (D) urine [nitrite], and (E) exhaled nitric oxide (NO). Effect of 8
- 4 mmol KNO₃ versus KCl (*n*=20) on (F) exhaled NO. Data expressed as mean±SEM. Significance
- 5 shown as: +*P*<0.05, ++*P*<0.01, +++*P*<0.001 on ANOVA, followed by +*P*<0.05, ++*P*<0.01,
- 6 ***P<0.001, Sidak's post-test of KNO₃ versus KCl.
- 7 [OGTT: oral glucose tolerance test]
- 8
- 9



Figure 3: Effect of KNO₃ versus KCl (*n*=33) on (A) systolic blood pressure (SBP), (B) diastolic
blood pressure (DBP), (C) pulse pressure (PP), and (D) heart rate (HR). Data expressed as

5 mean±SEM. [OGTT: oral glucose tolerance test]



Figure 4. Effect of KNO₃ versus KCl (n=29) on (A) pulse wave velocity (PWV), (B) central
systolic blood pressure (cSBP), and (C) augmentation index (AIx). Plots show range, median
and 25 to 75th percentiles.



Figure 5: Effect of 24 mmol KNO₃ versus KCl (*n*=13) on (A) systolic blood pressure (SBP), (B)

3 diastolic blood pressure (DBP), (C) pulse pressure (PP), and (D) heart rate (HR). Data

4 expressed as mean±SEM. Significance shown as: †*P*<0.05 on ANOVA.

- 5 [OGTT: oral glucose tolerance test]



- 1
- 2 Figure 6: Effect of 8 mmol KNO₃ versus KCl (*n*=20) on (A) systolic blood pressure (SBP), (B)
- 3 diastolic blood pressure (DBP), (C) pulse pressure (PP), and (D) heart rate (HR). Data
- 4 expressed as mean±SEM. Significance shown as: +++P<0.001 on ANOVA.
- 5 [OGTT: oral glucose tolerance test]
- 6
- 7
- 8



- 1
- 2
- Figure 7: Effect of KNO₃ versus KCl (*n*=33) on (A) plasma [glucose], (B) plasma [insulin], and
 (C) plasma [C-peptide]. Data expressed as mean±SEM. Significance shown as: +*P*<0.05 on
- 5 ANOVA, followed by *P<0.05, Sidak's post-test of KNO₃ versus KCl. ¥ P<0.01 on ANOVA for
- 6 KNO₃ versus baseline (-1 h), with Dunn's post-test. $\ddagger P < 0.01$ on ANOVA for KCl versus
- 7 baseline, with Dunn's post-test.
- 8 [OGTT: oral glucose tolerance test]
- 9



1



5 and (B) plasma [glucose], (C) and (D) plasma [insulin], and (E) and (F) plasma [C-peptide].

6 Data expressed as mean±SEM. Significance shown as: +++P<0.001 on ANOVA, followed by

- 7 *P<0.05, Sidak's post-test of KNO₃ versus KCl.
- 8 [OGTT: oral glucose tolerance test]
- 9

1 References

Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering,
 vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite.
 Hypertension. 2008;51(3):784-90.

5 2. Khatri J, Mills CE, Maskell P, et al. It is rocket science - why dietary nitrate is hard to 6 'beet'! Part I: twists and turns in the realization of the nitrate-nitrite-NO pathway. Br J Clin 7 Pharmacol. 2017;83(1):129-39.

8 3. Mills CE, Khatri J, Maskell P, et al. It is rocket science - why dietary nitrate is hard to
9 'beet'! Part II: further mechanisms and therapeutic potential of the nitrate-nitrite-NO pathway.
10 Br J Clin Pharmacol. 2017;83(1):140-51.

- Larsen FJ, Weitzberg E, Lundberg JO, et al. Effects of dietary nitrate on oxygen cost
 during exercise. Acta Physiol (Oxf). 2007;191(1):59-66.
- 5. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary nitrate supplementation reduces the
 O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in
 humans. J Appl Physiol (1985). 2009;107(4):1144-55.
- 16 6. Zamani P, Rawat D, Shiva-Kumar P, et al. Effect of inorganic nitrate on exercise
 17 capacity in heart failure with preserved ejection fraction. Circulation. 2015;131(4):371-80;
 18 discussion 80.
- Figgebeen J, Kim-Shapiro DB, Haykowsky M, et al. One Week of Daily Dosing With
 Beetroot Juice Improves Submaximal Endurance and Blood Pressure in Older Patients With
 Heart Failure and Preserved Ejection Fraction. JACC Heart Fail. 2016;4(6):428-37.
- Coggan AR, Broadstreet SR, Mahmood K, et al. Dietary Nitrate Increases VO2peak
 and Performance but Does Not Alter Ventilation or Efficiency in Patients With Heart Failure
 With Reduced Ejection Fraction. Journal of cardiac failure. 2017.
- 9. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. Physiol Rev.
 26 2001;81(1):209-37.
- 27 10. Gilchrist M, Winyard PG, Aizawa K, et al. Effect of dietary nitrate on blood pressure,
 28 endothelial function, and insulin sensitivity in type 2 diabetes. Free radical biology &
 29 medicine. 2013;60:89-97.
- 30 11. Shepherd AI, Gilchrist M, Winyard PG, et al. Effects of dietary nitrate
- supplementation on the oxygen cost of exercise and walking performance in individuals with
 type 2 diabetes: a randomized, double-blind, placebo-controlled crossover trial. Free radical
 biology & medicine. 2015;86:200-8.
- Mills CE, Govoni V, Faconti L, et al. Reducing Arterial Stiffness Independently of
 Blood Pressure: The VaSera Trial. J Am Coll Cardiol. 2017;70(13):1683-4.
- 36 13. Omar SA, Webb AJ, Lundberg JO, et al. Therapeutic effects of inorganic nitrate and
 37 nitrite in cardiovascular and metabolic diseases. J Intern Med. 2016;279(4):315-36.
- 38 14. Jeukendrup AE. Carbohydrate intake during exercise and performance. Nutrition.
 39 2004;20(7-8):669-77.
- Alhazmi A, Stojanovski E, McEvoy M, et al. Macronutrient intakes and development
 of type 2 diabetes: a systematic review and meta-analysis of cohort studies. J Am Coll Nutr.
 2012;31(4):243-58.
- 43 16. Taylor R. Pathogenesis of type 2 diabetes: tracing the reverse route from cure to44 cause. Diabetologia. 2008;51(10):1781-9.
- 45 17. Derosa G, D'Angelo A, Salvadeo SA, et al. Modification of vascular and inflammation
 46 biomarkers after OGTT in overweight healthy and diabetic subjects. Microvasc Res.
 47 2010;79(2):144-9.
- 48 18. Weiss EP, Park JJ, McKenzie JA, et al. Plasma nitrate/nitrite response to an oral 49 glucose load and the effect of endurance training. Metabolism. 2004;53(5):673-9.
- 50 19. Ghasemi A, Zahediasl S, Azizi F. Nitric oxide and clustering of metabolic syndrome 51 components in pediatrics. European journal of epidemiology. 2010;25(1):45-53.
- 52 20. Gentilcore D, Visvanathan R, Russo A, et al. Role of nitric oxide mechanisms in
- 53 gastric emptying of, and the blood pressure and glycemic responses to, oral glucose in

healthy older subjects. American journal of physiology Gastrointestinal and liver physiology.
 2005;288(6):G1227-32.

Natali A, Ribeiro R, Baldi S, et al. Systemic inhibition of nitric oxide synthesis in non diabetic individuals produces a significant deterioration in glucose tolerance by increasing
 insulin clearance and inhibiting insulin secretion. Diabetologia. 2013;56(5):1183-91.

6 22. Wylie LJ, Mohr M, Krustrup P, et al. Dietary nitrate supplementation improves team 7 sport-specific intense intermittent exercise performance. Eur J Appl Physiol.

8 2013;113(7):1673-84.

9 23. Vasconcellos J, Henrique Silvestre D, Dos Santos Baiao D, et al. A Single Dose of
10 Beetroot Gel Rich in Nitrate Does Not Improve Performance but Lowers Blood Glucose in
11 Physically Active Individuals. J Nutr Metab. 2017;2017:7853034.

12 24. Larsen FJ, Schiffer TA, Ekblom B, et al. Dietary nitrate reduces resting metabolic 13 rate: a randomized, crossover study in humans. Am J Clin Nutr. 2014;99(4):843-50.

Shepherd AI, Wilkerson DP, Fulford J, et al. Effect of nitrate supplementation on
hepatic blood flow and glucose homeostasis: a double-blind, placebo-controlled, randomized
control trial. American journal of physiology Gastrointestinal and liver physiology.

17 2016;311(3):G356-64.

Beals JW, Binns SE, Davis JL, et al. Concurrent Beet Juice and Carbohydrate
Ingestion: Influence on Glucose Tolerance in Obese and Nonobese Adults. J Nutr Metab.
2017;2017:6436783.

- 27. Cermak NM, Hansen D, Kouw IW, et al. A single dose of sodium nitrate does not
 improve oral glucose tolerance in patients with type 2 diabetes mellitus. Nutr Res.
 2015:35(8):674-80.
- Fuchs D, Nyakayiru J, Draijer R, et al. Impact of flavonoid-rich black tea and beetroot
 juice on postprandial peripheral vascular resistance and glucose homeostasis in obese,

insulin-resistant men: a randomized controlled trial. Nutrition & metabolism. 2016;13:34.
Ashor AW, Chowdhury S, Oggioni C, et al. Inorganic Nitrate Supplementation in

Young and Old Obese Adults Does Not Affect Acute Glucose and Insulin Responses but
 Lowers Oxidative Stress. J Nutr. 2016;146(11):2224-32.

30. Faconti L, Mills CE, Govoni V, et al. Cardiac effects of 6 months' dietary nitrate and
spironolactone in patients with hypertension and with/at risk of type 2 diabetes, in the
factorial design, double-blind, randomized controlled VaSera trial. Br J Clin Pharmacol.
2018.

34 31. Barrett EJ, Eggleston EM, Inyard AC, et al. The vascular actions of insulin control its
35 delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action.
36 Diabetologia. 2009;52(5):752-64.

37 32. Laakso M, Edelman SV, Brechtel G, et al. Decreased effect of insulin to stimulate
38 skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J Clin
39 Invest. 1990;85(6):1844-52.

40 33. Laakso M, Edelman SV, Brechtel G, et al. Impaired insulin-mediated skeletal muscle 41 blood flow in patients with NIDDM. Diabetes. 1992;41(9):1076-83.

42 34. Nair A, Khan S, Omar S, et al. Remote ischaemic preconditioning suppresses
43 endogenous plasma nitrite during ischaemia-reperfusion: a randomized controlled crossover
44 pilot study. British journal of clinical pharmacology. 2017;83(7):1416-23.

- 45 35. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose
 46 tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care.
- 47 1999;22(9):1462-70.
- 48 36. Southan C, Sharman JL, Benson HE, et al. The IUPHAR/BPS Guide to

PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein
 targets and 6000 ligands. Nucleic Acids Res. 2016;44(D1):D1054-68.

- 51 37. Alexander SP, Benson HE, Faccenda E, et al. The Concise Guide to
- 52 PHARMACOLOGY 2013/14: enzymes. Br J Pharmacol. 2013;170(8):1797-867.
- 53 38. Alexander SP, Benson HE, Faccenda E, et al. The Concise Guide to
- 54 PHARMACOLOGY 2013/14: transporters. Br J Pharmacol. 2013;170(8):1706-96.

- 1 39. Kapil V, Milsom AB, Okorie M, et al. Inorganic nitrate supplementation lowers blood 2 pressure in humans: role for nitrite-derived NO. Hypertension. 2010;56(2):274-81.
- 40. Bailey SJ, Fulford J, Vanhatalo A, et al. Dietary nitrate supplementation enhances
 muscle contractile efficiency during knee-extensor exercise in humans. J Appl Physiol
 (1985). 2010;109(1):135-48.
- Hughes WE, Ueda K, Treichler DP, et al. Effects of acute dietary nitrate
 supplementation on aortic blood pressure and aortic augmentation index in young and older
 adults. Nitric Oxide. 2016;59:21-7.
- 42. Kapil V, Khambata RS, Robertson A, et al. Dietary nitrate provides sustained blood
 pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebocontrolled study. Hypertension. 2015;65(2):320-7.
- 43. Kim EJ, Park CG, Park JS, et al. Relationship between blood pressure parameters
 and pulse wave velocity in normotensive and hypertensive subjects: invasive study. Journal
 of human hypertension. 2007;21(2):141-8.
- 44. Omar SA, Fok H, Tilgner KD, et al. Paradoxical normoxia-dependent selective
 actions of inorganic nitrite in human muscular conduit arteries and related selective actions
 on central blood pressures. Circulation. 2015;131(4):381-9; discussion 9.
- 45. Akbari CM, Saouaf R, Barnhill DF, et al. Endothelium-dependent vasodilatation is
 impaired in both microcirculation and macrocirculation during acute hyperglycemia. J Vasc
 Surg. 1998;28(4):687-94.
- 46. Title LM, Cummings PM, Giddens K, et al. Oral glucose loading acutely attenuates
 endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented
 by vitamins C and E. J Am Coll Cardiol. 2000;36(7):2185-91.
- 47. Watanabe K, Oba K, Suzuki T, et al. Oral glucose loading attenuates endothelial
 function in normal individual. European journal of clinical investigation. 2011;41(5):465-73.
 48. Joris PJ, Mensink RP. Beetroot juice improves in overweight and slightly obese men
- postprandial endothelial function after consumption of a mixed meal. Atherosclerosis.
 2013;231(1):78-83.
 49 Kabn SE Prigeon RL McCulloch DK et al. The contribution of insulin-depend
- 49. Kahn SE, Prigeon RL, McCulloch DK, et al. The contribution of insulin-dependent
 and insulin-independent glucose uptake to intravenous glucose tolerance in healthy human
 subjects. Diabetes. 1994;43(4):587-92.
- 32 50. Roder PV, Wu B, Liu Y, et al. Pancreatic regulation of glucose homeostasis. Exp Mol 33 Med. 2016;48:e219.
- Jiang H, Torregrossa AC, Potts A, et al. Dietary nitrite improves insulin signaling
 through GLUT4 translocation. Free radical biology & medicine. 2014;67:51-7.
- 52. Cordes CM, Bennett RG, Siford GL, et al. Redox regulation of insulin degradation by insulin-degrading enzyme. PLoS One. 2011;6(3):e18138.
- 53. Etgen GJ, Jr., Fryburg DA, Gibbs EM. Nitric oxide stimulates skeletal muscle glucose
 transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent
 pathway. Diabetes. 1997;46(11):1915-9.
- 41 54. Deshmukh AS, Long YC, de Castro Barbosa T, et al. Nitric oxide increases cyclic
- 42 GMP levels, AMP-activated protein kinase (AMPK)alpha1-specific activity and glucose
- 43 transport in human skeletal muscle. Diabetologia. 2010;53(6):1142-50.