Berkeley Life
The Nitric Oxide Experts

Design Matters: A Comparison of Clinical Trial Outcomes and Nitrate Supplement Efficacy

A Clinical Research Review of: Houston et al. (2023) and Cherukuri et al. (2020)

This report was written by Dr. Chris Easton, Professor and head of the School of Health and Life Sciences at the University of Western Scotland.

About Dr. Chris Easton:

Professor Chris Easton has led research studies in the fields of applied exercise physiology, physical activity, and health for nearly twenty years. He has extensive experience conducting exercise tests, training studies, and measuring physical activity and physiological outcomes in a wide range of different populations including children, the elderly, clinical groups, sedentary adults, trained athletes and Olympic champions. His primary research focus is to establish the impact of modulating nitric oxide bioavailability, via the diet and exposure to sunlight, on parameters of cardiovascular health and exercise performance in different populations. His recent work has demonstrated the importance of the oral microbiome in the regulation of nitric oxide production and sheds further light on the interplay between oral health and cardiovascular homeostasis. Professor Easton has further interest in the evaluation of mobile methods of assessing physiological and health outcomes in free-living populations for application in health services.

Preface

This report comprises an independent critical review of the following two manuscripts:

- Houston et al. (2023): *Effects of S-Allylcysteine-Rich Garlic Extract and Dietary Inorganic Nitrate Formula on Blood Pressure and Salivary Nitric Oxide: An Open-Label Clinical Trial Among Hypertensive Subjects*
- Cherukuri et al. (2020): *Effect of a plant-based bioequivalent inorganic nitrate (NO3-) complex with vitamins, antioxidants and phytophenol rich food extracts in hypertensive individuals – A randomized, double-blind, placebo-controlled study*

Executive Summary

The study by Cherukuri et al. (2020) reported that 12 weeks of daily inorganic nitrate

supplementation (314 mg/day) with added vitamins and food extracts, increased the availability of nitric oxide – a molecule that is known to regulate key biological processes such as blood pressure and immune function. The authors further reported that nitrate decreased blood pressure and improved blood vessel function compared to a placebo in adults with hypertension (high blood pressure). While there are some concerns with the methods of statistical analyses, the study design (randomized, placebo-controlled and double blind), sample size (n=67), and measurement procedures were robust, and the conclusions are supported by the data. The more recent study by Houston et al. (2023) provided participants with a similar dose of inorganic nitrate (242 mg/day) with added extract of black garlic. The addition of the garlic extract was intended to increase the availability of hydrogen sulfide – a molecule which is thought to increase the body's natural production of nitric oxide. These authors also reported the supplementation regimen increased markers of nitric oxide availability and improved blood pressure in adults with hypertension. However, this study has substantial limitations, including the study design (no placebo or control group), small sample size (n=12), inadequate control methods (diet), and no direct measures of nitric oxide availability, which severely limit confidence in the findings. Furthermore, the study does not provide any indication that the addition of black garlic extract enhances the clinical effect of nitrate and so does not expand the findings of Cherukuri et al. (2020). The narrative on the following pages offers a more detailed review and comparison of key components of each study.

Study Design

Type of Trial

The study by Cherukuri et al. (2020) was a randomized, double-blind and placebo-controlled trial which is the "gold-standard" for research investigating the effects of novel treatment methods. In brief, the participants were randomly assigned to receive capsules which contained either the active intervention (nitrate supplementation) or an inert placebo. Neither the research team nor the participants knew which until after completion of the trial. Conversely, Houston et al. (2023) used an uncontrolled open-labelled design where study outcomes were simply compared before and after the intervention. While this type of study design may be useful in the early stages of pharmaceutical development to explore safety and efficacy, there is a considerable risk of bias. For example, participants may modify their behaviours (e.g. diet or physical activity) in line with the expected outcomes of the study. Although speculative, it is likely that this significant limitation in study design would result in rejection of the paper for publication in most reputable scientific journals. It is worthy of highlight that the timeline from submission of this paper to publication in this open access journal was only 11 days – a process that normally takes several months.

Study Duration

While most studies in the nitrate field tend to be limited to the short-term effects of

supplementation (1 hour – 7 days after ingestion), the study by Cherukuri et al. (2020) lasted for 12 weeks and the study by Houston et al. (2023) for 4 weeks. Cherukuri et al. (2020) measured study outcomes in both the active and placebo groups at baseline, 2 hours, 2 weeks and 12 weeks after commencing supplementation. Houston et al. (2023) collected data at baseline and then 2, 6, and 24 hours after the first dose. Subsequently, they collected data prior to, and 2 hours following, ingestion of the supplement on weeks 2 and 4. The additional measurement points in the study by Houston et al. (2023) provide some useful insight on nitric oxide kinetics (i.e. how long the supplement elevates nitric oxide availability) but this was limited by the measurement method (only saliva test strips) and the absence of blood markers (collected by Cherukuri et al. (2020)). The extended duration of the study by Cherukuri et al. (2020) provides a greater degree of confidence that the supplementation regimen is tolerable, safe, and effective in the longer term.

Recruitment Methods

Both manuscripts have limited detail on recruitment methods but seemed to employ a similar approach. Briefly, patients presenting with high blood pressure (see patient population details below) were recruited by each research team to participate in the trial. Each trial was pre-registered, had the relevant IRB approval, and obtained written informed consent from participants.

Patient Population

The patient populations in both studies were predominantly female and of a similar age. Participants in the study by Cherukuri et al. (2020) had a higher mean systolic blood pressure (Table 1). The inclusion and exclusion criteria were also similar, although Cherukuri and colleagues (2020) reported more detail on the number of participants excluded. The reporting of participant exclusions is deemed good practice in the National Institute for Health and Care Excellence Research Guidelines[1]. Participants in the study by Cherukuri et al. (2020) were split into a placebo group and an active (nitrate supplementation) group. There were no differences in demographics, blood pressure, or nitric oxide availability between the groups at baseline.

[1] NICE Research Guidelines

Table 1: Participant Characteristics

STUDY	HOUSTON ET AL. (2023)	CHERUKURI ET AL. (2020)
INCLUSION CRITERIA	• Elevated blood pressure (Systolic > 120 mmHg or Diastolic > 80 mmHg) • Ability to provide informed consent • Absence of any significant cardiac or other medical history • No medication changes in the preceding six months	• 40–75 years of age • Blood pressure >120/80 mmHg • On a stable hypertensive treatment regimen
EXCLUSION CRITERIA	Failure to meet all of the inclusion criteria	• History of coronary artery disease (n = 2) • Myocardial infarction (n=1) • Stroke or life-threatening arrhythmia within the prior 6 months (n=1) • New York Heart Association Functional Classification II-IV heart failure (n=2) • Renal impairment (serum creatinine > 1.4 mg/dL) (n=3) • Current tobacco use (n=2) • History of bleeding disorders or use of anticoagulants (n=1) • Hypertensive encephalopathy or cerebrovascular accident (n=1) • Currently enrolled in another placebo-controlled trial (n=0)

		PLACEBO GROUP	NITRATE GROUP
PARTICIPANTS	N=12 (8 females)	N=32 (23 females)	N=30 (18 females)
AGE	52 – 73 years	61 ± 9 years	58 ± 9 years
BASELINE SYSTOLIC BLOOD PRESSURE	134 ± 4 mmHg	143 ± 11 mmHg	143 ± 11 mmHg
BASELINE DIASTOLIC BLOOD PRESSURE	62 – 94 mmHg [mean and standard deviation not reported for the full cohort]	81 ± 11 mmHg	81 ± 11 mmHg

Interventions

The patient populations in both studies were predominantly female and of a similar age. Participants in the study by Cherukuri et al. (2020) had a higher mean systolic blood pressure (Table 1). The inclusion and exclusion criteria were also similar, although Cherukuri and colleagues (2020) reported more detail on the number of participants excluded. The reporting of participant exclusions is deemed good practice in the National Institute for Health and Care Excellence Research Guidelines1. Participants in the study by Cherukuri et al. (2020) were split into a placebo group and an active (nitrate supplementation) group. There were no differences in demographics, blood pressure, or nitric oxide availability between the groups at baseline.

Table 2: Intervention and Control Treatments

STUDY	HOUSTON ET AL. (2023)	CHERUKURI ET AL. (2020)
INTERVENTION	Vascanox®: A proprietary formulation that combines dietary nitrates in the form of beetroot extract with a source of hydrogen sulfide (black garlic extract), vitamin C, various berry extracts, and other vitamins and essential metals	Berkely Life Nitric Oxide Capsules: 20 mg nitrate rich beetroot extract, 90 mg thiamine mononitrate, 480 mg potassium nitrate, 150 mg ascorbic acid, 200 mcg folic acid, 200 mcg methyl cobalamin, 115 mg calcium, 5mg pomegranate fruit extract, and 115 mg of green coffee bean extract (Coffea canephora),
DOSAGE	2 capsules per day	2 capsules per day
NITRATE CONTENT	242 mg	314 mg
CONTROL	N/A: Pre-post measurements with the intervention only	2 capsules of similar shape, size, and color with no active ingredients
DURATION	4 weeks	12 weeks

Outcomes and Endpoints

Cherukuri et al. (2020) defined systolic blood pressure as their primary endpoint and the study was appropriately powered (i.e. had an adequate sample size) to detect a clinically significant difference in systolic blood pressure between the active and placebo groups. The primary outcome of the study by Houston et al. (2023) was not defined and the authors did not report a power calculation. Cherukuri et al. (2000) collected blood pressure using best practice guidelines (i.e. three times in both arms) and standardized the posture of the participant during measurement (Table 3). This is important as blood pressure will vary significantly depending on whether the participant is supine, sitting, or standing. Blood pressure was measured in triplicate by Houston et al. (2023) but it was not reported whether this was on the left, right, or dominant arm. Nor was the posture of the participants during measurement. Cherukuri et al. (2020)

included an additional measure of vascular (endothelial) function – flow mediated dilation of the brachial artery. In this method, the artery diameter is measured using an ultrasound probe while blood flow restriction by a tourniquet. The tourniquet is then released and the change in artery diameter (% change) is recorded. When the tourniquet is released, a healthy artery should dilate due to the biological action of nitric oxide and other vasodilators. If dilation changes by less than 7% then this is suggested to indicate endothelial dysfunction.

The data from Houston et al. (2023) provides limited insight regarding the change in nitric oxide availability following the intervention as saliva nitrite was only estimated using saliva test strips and plasma samples were not collected. Conversely, Cherukuri and colleagues (2020) measured the concentration of nitrite and nitrate in plasma and saliva using gold-standard techniques. This is important given plasma nitrite provides the best approximation of whole-body nitric oxide availability.

Table 3: Measurement of outcomes in both studies

STUDY	HOUSTON ET AL. (2023)	CHERUKURI ET AL. (2020)
BLOOD PRESSURE	Measured after resting for 5 min with back support, feet flat and arm bared at heart level. Three readings were recorded on both right and left arms.	Measured in the brachial artery three times, five minutes apart.
ENDOTHELIAL FUNCTION	Not measured	Measured using gold-standard method (brachial flow-mediated dilation).
PLASMA NITRATE AND NITRITE	Not measured	Measured using gold-standard method (gas phase chemiluminescence)
SALIVA NITRATE AND NITRITE	Saliva nitrite was measured at defined measurement points using a nitric oxide test strip (MyFitStrip® LLC). The authors stated that saliva nitrate was measured but did not state the method or report the data.	Measured using gold-standard method (gas phase chemiluminescence) at defined measurement points. Saliva nitrite was also self-measured daily using a nitric oxide test strip (Berkeley Life).
OTHER PLASMA MARKERS	Not measured	c-reactive protein (CRP), creatinine, serum glucose, hemoglobin A1c (HbA1c) and a lipid profile, including serum LDL cholesterol, HDL cholesterol, and triglycerides, by automated diagnostic equipment

Results

As expected, both studies reported a significant increase in saliva nitrite concentration following the intervention (Table 4). This suggests that the pharmacological interventions increased nitrate concentration in the saliva and that oral bacteria were able to convert some of this to nitrite. While a meaningful comparison between the studies is limited by differences in the measurement method (Table 3), the Cherukuri et al. (2020) intervention resulted in a greater saliva nitrite concentration, perhaps due to the higher dose of nitrate provided (Table 2). Nevertheless, the increase in saliva and plasma nitric oxide markers was lower than that typically observed following the ingestion of nitrate-rich beetroot juice. For example, Burleigh et al. (2018)[2] reported saliva nitrite increased to ~1200 µM and plasma nitrite to 330 µM following ingestion of this dietary supplement.

Both studies reported similar and clinically meaningful reductions in blood pressure following the intervention (Table 4). Nevertheless, the aforementioned lack of control group in the Houston et al. (2023) study limits confidence in their findings. To emphasize this point, the placebo group in the study by Cherukuri et al. (2020) also experienced a significant reduction in systolic blood pressure over the course of the study, perhaps due to deliberate behavioral adjustments or simply due to biological variation. Despite this, the authors found the reduction in systolic blood pressure was significantly greater in the nitrate group and thus can safely conclude that the pharmacological intervention reduces blood pressure in hypertensive adults and can estimate the likely magnitude of the effect (Table 4). The improvevment in endothelial function reported by Cherukuri et al. (2020) provides further corroboration that the intervention can improved vascular health outcomes in this population.

[2] Burleigh et al. (2018)

Table 4: Key results in both studies

STUDY	HOUSTON ET AL. (2023)	CHERUKURI ET AL. (2020)
BLOOD PRESSURE	Systolic blood pressure was reduced by 10 mmHg (P<0.01) after 2 weeks of the intervention and remained lower (11 mmHg, P<0.001) after 4 weeks. Overall, diastolic blood did not change from pre- to post-intervention. However, participants with an elevated diastolic blood pressure at baseline (n=6) experienced a reduction of 10 mmHg following the intervention.	Systolic blood pressure was reduced by 12.5 ± 13.3 mmHg from baseline in the nitrate group (P<0.001). The nitrate group had a greater reduction in systolic blood pressure than the placebo group (6.3 mmHg, P=0.04). Diastolic blood pressure was reduced by 4.7 ± 10.3 mmHg from baseline in the nitrate group (P=0.01). The nitrate group had a greater reduction in diastolic blood pressure than the placebo group (2.7 mmHg), but this did not reach statistical significance.
ENDOTHELIAL FUNCTION	Not measured	Nitrate significantly improved flow mediated dilation from 3.1% at baseline to 3.7% after 12 weeks (P=0.03). There was no change in the placebo group
PLASMA NITRATE AND NITRITE	Not measured	Plasma nitrite and nitrate significantly increased following nitrate supplementation compared to the placebo group at all time points. Plasma nitrite peaked at 0.2 ± 0.4 µM, 2 hours after ingestion on day 0.
SALIVA NITRATE AND NITRITE	Saliva nitrite was higher than baseline two hours after administration on day 0, 14, and 28 of the study. The highest concentration of saliva nitrite was measured on day 14, two hours after administration of the dose (734 ± 258 µM).	Saliva nitrite and nitrate significantly increased following nitrate supplementation compared to the placebo group at all time points. Saliva nitrite peaked at 1316 ± 1801 µM, 2 hours after ingestion on day 0.
OTHER PLASMA MARKERS	Not measured	No differences between nitrate and placebo groups.

Both studies have some issues with the methods of statistical analyses that were used to analyze the results of the study. In the first instance, Houston et al. (2023) used an analysis of variance (ANOVA) to determine whether the outcomes (e.g. blood pressure) significantly changed over the repeated measures. If they found a significant change, they explored this further by using paired t-tests to compare values between specific time points (e.g. baseline

vs. 2 hours after ingestion of the supplement). An issue with this approach is that each individual statistical test (e.g. paired t-test) has a degree of error associated it. By undertaking repeated paired t-tests on the same data set, it compounds this error. While there are statistical methods to correct for this, the authors do not report whether these were used in their analyses. In the first instance, Cherukuri et al. (2020) used a two-factor repeated measures ANOVA to interrogate their data. This statistical method simultaneously compares the differences in outcomes across measurement points between the placebo and active intervention groups. This is a robust statistical method that enables the researchers to determine whether the measured outcome was different between nitrate and active groups and also whether the pattern of change differed. However, the authors found no differences between active and placebo groups using this technique but did not report the statistical data in their manuscript. They then proceeded to use a less robust (and less conservative) method of analyzing their data. In short, they used a similar method to Houston et al. (2023) (i.e. repeated t-tests) with no mention of correction for the error associated with repeated measures analysis.

Discussion and Conclusions

The discussion section of the study by Cherukuri et al. (2020) provides a fairly balanced synthesis of the data and the conclusions are generally supported by the data. Key limitations of the study are detailed, albeit concerns with the methods of statistical analyses should be acknowledged. The conclusion section of the study by Houston et al. (2023) starts by suggesting the purpose of the study was to determine the safety and efficacy of the supplement. While the authors reported no side effects or other safety concerns, a longer duration trial would be needed to provide further assurances. Further, the conclusion that the supplement is effective (i.e. reduces blood pressure) is compromised by the lack of placebo/control group. Lastly, the authors suggestion that the addition of black garlic (which was intended to increase hydrogen sulfide availability and production of nitric oxide via nitric oxide synthases) offers further benefit to the individual than nitrate alone, is not supported by the study. Further research to compare nitrate (with and without added garlic) to a placebo would be needed, with the inclusion of further physiological measurements.

In summary, Cherukuri et al.'s study, done on Berkeley Life's Nitric Oxide capsule, is more reliable due to its rigorous design, larger sample size, longer duration, inclusion of a control group, and positive clinical outcomes.