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SUMMARY

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*

Overview

Cherukuri et al. (2020) conducted a well-designed, randomized, double-blind, placebo-controlled study, demonstrating that 12 weeks of inorganic nitrate supplementation significantly increased nitric oxide availability, leading to improvements in blood pressure and endothelial function among hypertensive individuals. Despite concerns about statistical methods, the study's robust design and larger sample size contribute to the credibility of the reported outcomes.

In contrast, Houston et al. (2023) explored a similar nitrate supplementation approach but with the addition of black garlic extract. While reporting positive effects on nitric oxide markers and blood pressure, the study's limitations, such as a small sample size and lack of a control group, raise doubts about the reliability of the findings. Moreover, the study did not provide evidence that the inclusion of black garlic extract enhanced the clinical benefits observed by Cherukuri et al. These discrepancies emphasize the importance of rigorous study design and larger sample sizes in drawing reliable conclusions regarding the efficacy of nitric oxide-supporting supplements.

Key Takeaways

- **Nitric Oxide Increase:** Both studies, by Cherukuri et al. (2020) and Houston et al. (2023), demonstrated that inorganic nitrate supplementation led to an increase in nitric oxide availability. Cherukuri et al. reported a notable improvement in flow-mediated dilation, a measure of endothelial function, while Houston et al. observed an increase in saliva nitrite levels.
- **Blood Pressure Reduction:** Both studies indicated a positive impact on blood pressure, with a reduction in systolic blood pressure observed. Cherukuri et al. reported a significant decrease of 12.5 ± 13.3 mmHg in the nitrate group, while Houston et al. showed a reduction of 11 mmHg after 4 weeks. Diastolic blood pressure also showed improvements in both studies.
- **Study Design Discrepancies:** Cherukuri et al. (2020) employed a robust randomized, double-blind, and placebo-controlled trial design with a longer duration (12 weeks), while Houston et al. (2023) utilized an uncontrolled open-label design with a shorter duration (4 weeks). The differences in study design raise concerns about the reliability of Houston et al.'s findings.
- **Sample Size Variation:** Cherukuri et al. had a larger sample size (n=67) compared to Houston et al. (n=12). Larger sample sizes generally contribute to the statistical robustness and generalizability of findings, enhancing the reliability of Cherukuri et al.'s results.
- **Garlic Extract Impact:** Houston et al. introduced an additional component, black garlic extract, to the nitrate supplementation, aiming to increase hydrogen sulfide production. However, this addition did not seem to enhance the clinical effects, and the study's limitations, including the lack of a control group, raise questions about the validity of this approach.

Which was the stronger study?

Based on the information provided, the study by Cherukuri et al. (2020) appears to be more robust, better designed, and with more favorable clinical outcomes compared to the study by Houston et al. (2023). Here are the key factors supporting this assessment:

- **Study Design:** Cherukuri et al. employed a randomized, double-blind, and placebo-controlled trial design, which is considered the gold standard in clinical research. This design helps minimize bias and ensures rigorous scientific investigation. On the other hand, Houston et al. used an uncontrolled open-label design, which is generally less robust in terms of controlling for confounding variables and placebo effects.
- **Sample Size:** Cherukuri et al. had a larger sample size (n=67) compared to Houston et al. (n=12). A larger sample size enhances the statistical power of a study, increasing the confidence in the reliability and generalizability of the results.
- **Study Duration:** Cherukuri et al. conducted a 12-week study, allowing for a more extended evaluation of the intervention's effects. In contrast, Houston et al.'s study had a shorter duration of 4 weeks, which might limit the ability to capture sustained or long-term effects.
- **Control Group:** Cherukuri et al. included a placebo-controlled group, providing a proper baseline for comparison. Houston et al. lacked a control group, making it challenging to attribute observed effects solely to the intervention, as confounding factors could influence the outcomes.
- **Clinical Outcomes:** Cherukuri et al. reported significant improvements in nitric oxide availability, blood pressure reduction, and endothelial function. The study by Houston et al. also showed positive outcomes but with substantial limitations, particularly the absence of a control group and a small sample size, which raises concerns about the validity and generalizability of the findings.

STUDY	HOUSTON ET AL. (2023)	CHERUKURI ET AL. (2020)
TRIAL TYPE	Uncontrolled open-labelled design (i.e. comparison of outcomes before and after the intervention)	Randomized, double-blind and placebo-controlled trial
STUDY DURATION	4 weeks	12 weeks
MEASUREMENT POINTS	Baseline followed by 2, 6, and 24 hours after the first dose. Then prior to, and 2 hours following, ingestion of the supplement on weeks 2 and 4.	Baseline followed by 2 hours, 2 weeks and 12 weeks after commencing supplementation.
RECRUITMENT METHODS	Direct recruitment from hypertension clinic following trial pre-registration and IRB approval.	Direct recruitment from hypertension clinic following trial pre-registration and IRB approval.

Table 2: 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

Table 3: 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	Berkeley 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

Table 4: 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

Table 5: 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.

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.