Effect of acute nitrate-containing beverage consumption on oxygen consumption efficiency in adults

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Submitted: April 5, 2024

Abstract

Background: Gas exchange in the lungs facilitates the uptake of oxygen and removal of carbon dioxide into tissues, crucial for cellular respiration. Nitrate-rich beverages, like beetroot juice (BRJ), have been demonstrated to affect oxygen consumption efficiency (OCE) during exercise, with multiple potential mechanisms being proposed. The process involves the nitrate-nitrite-nitric oxide pathway, where the different intermediates have varying roles in physiology and respiration.
Materials and Methods: Eight healthy young adults participated, all providing informed consent and meeting the exclusion criteria. Experimental and control trials were conducted following the ingestion of BRJ or a control drink, respectively. Moderate-intensity cycling sessions were conducted after 3 hours to measure OCE. Data analysis utilized statistical tests to compare oxygen consumption and respiratory exchange ratio between both conditions.
Results: A statistical difference was not observed for both VO_2 ($p = 0.888$) and RER ($p = 0.093$). The lack of statistical difference for RER was expected in the literature, and the VO_2 data eludes that BRJ has no significant effect on OCE.
Conclusion: The null hypothesis of the study can not be rejected, leading to the conclusion acute nitrate-containing beverages have no effect on OCE.

Keywords Oxygen Consumption · Beetroot Juice · Nitrate · Nitric Oxide · VO_2

Abbreviations

BRJ	Beetroot Juice
HR	Heart Rate
V_E	Minute Volume
OCE	Oxygen Consumption Efficiency
VO_2	Oxygen Consumption
RER	Respiratory Exchange Ratio

Introduction

Gas Exchange

Oxygen is utilized through pulmonary gas exchange at the alveoli in the lungs. Gas exchange is a process where inspired oxygen diffuses through a blood-gas barrier into the blood and is transported throughout the body where it can be a part of metabolic processes (Powell & Hopkins 2004). Once the required oxygen is used and the blood is deoxygenated, it travels back to the lungs. The carbon dioxide produced in respiration reactions diffuses through the blood gas barrier and is exhaled (Powell & Hopkins 2004).

Oxygen Consumption

Oxygen consumption refers to the rate at which an organism utilizes oxygen to produce energy through cellular respiration, relative to its metabolic demands or activity level. OCE reflects how effectively an organism converts oxygen into usable energy, typically measured as the ratio of oxygen consumed to the amount of energy generated, such as ATP produced, during metabolic processes (Betteridge et al. 2016). It is also known as the P:O ratio, or oxidative phosphorylation efficiency, as the amount of phosphorylation to the unit of oxygen consumed (Hinkle, 2005). Higher oxygen consumption efficiency indicates more effective energy production per unit of oxygen consumed, while lower efficiency may suggest inefficiencies in energy metabolism or increased oxygen demands (Pool and Richarson 1997).

There is uncertainty around the impact of ingestion of nitrate-containing beetroot juice (BRJ) supplements concerning oxygen consumption efficiency (OCE) while exercising. It has been previously shown that, in healthy young adults, a decrease in VO_2 at moderate intensity is induced by BRJ (Bailey et al. 2009). Healthy young adults have also shown no significant effect on their VO_2 after ingesting BRJ in other studies (Betteridge et al. 2016). Bailey et al. (2010) found that consuming BRJ does not affect the respiratory exchange ratio in human exercise studies. Contradictions in the literature lead to a knowledge gap in this area of research.

Nitrate Pathway

The nitrate-nitrite-nitric oxide (NO) pathway is responsible for converting dietary nitrate into nitrite and subsequently NO within the human body (Jones et al. 2018). When nitrate is taken orally through ingestion of BRJ, it is concentrated in the oral cavity by saliva (Govoni et al. 2008). The conversion of nitrate (NO_3^-) into nitrite (NO_2^-), known as bioactivation, is done by anaerobic oral bacteria (Pignatelli et al. 2020). Humans are unable to perform this conversion, but these bacteria possess the enzymes necessary to catalyze the reduction of nitrate to nitrite as part of their metabolic processes, making them an essential component of the pathway. Nitrite, once swallowed and absorbed in the gastrointestinal tract, undergoes further reduction to nitric oxide, facilitated by various enzymatic and chemical reactions in tissues, particularly in conditions of low oxygen and/or high acidity (Jones et al. 2018). This process has been found to take around 2-3 hours in adults (Stanaway et al. 2019).

Nitrate Effect on Mitochondria

In humans, the mitochondria in cells produce ATP by using the ATP synthase complex in the electron transport chain. This complex converts ADP to ATP using the proton gradient across the inner mitochondrial membrane (Ahmad et al. 2023). There are a few proposed mechanisms of how NO can reduce oxygen consumption. Firstly, when the proton gradient is dissipated without ATP synthesis, the energy is lost to heat, known as uncoupling (Ahmad et al. 2023). The inefficient energy loss is caused by uncoupling reactions of transport proteins such as adenine nucleotide translocase (ANT) and uncoupling protein 3 (UCP-3) that dissipate the proton gradient (Ricquier & Bouillaud 2013). NO

has been theorized to improve mitochondrial efficiency by downregulating the expression of these proteins (Brand et al. 2005 & Larsen et al. 2011). The inhibition of ANT and UCP-3 allows for less dissipation of protons during energy production, allowing for less energy waste (Larsen et al. 2011). After nitrate ingestion occurs, it is improbable that there is sufficient time for alterations in protein expression to appear, suggesting the acute effects on oxygen consumption would have to be a more post-transcriptional mechanism.

Additionally, Basu et al. (2008) claim that an increased presence of nitrite and NO can inhibit mitochondrial electron carrier cytochrome C. The inhibition of cytochrome C could potentially slow down the electron carrier, reducing the amount of oxygen used at complex IV. Furthermore, NO is also able to bind to complex IV competitively with oxygen, forming a partial inhibition of mitochondrial respiration (Brown & Cooper 1994).

All of the mechanisms listed above would decrease the overall usage of oxygen in the electron transport chain. However, some of the proposed mechanisms may not be useful in increasing the OCE since they would not increase the amount of ATP produced per molecule of oxygen consumed. Whilst the amount of oxygen used would decrease, backed by the literature above. The amount of oxidative phosphorylation occurring was not demonstrated to remain either constant or increase. Due to the lack of support, it is inconclusive in the literature if the acute physiological effects of nitrate, nitrite, and NO can increase OCE through the downregulation of ANT and binding to complex IV.

Nitrate Effect in Physiology

An increase in NO can also cause further vasodilation through the activation of signaling cascades in blood vessels affecting the smooth muscle (Palmer et al. 1987). Literature also states that when NO is present in the blood or corresponding blood vessels the molecule increases the production of cGMP by activating guanylyl cyclase leading to vasodilation (Zhao et al. 1999). By vasodilating, blood vessels will widen and decrease the peripheral resistance causing additional blood flow in these tissues. Although this study focuses on OCE, the increase in oxygen delivery by the blood may further increase the available oxygen to be utilized by myocytes. The use of oxygen within skeletal myocytes mirrors the measured pulmonary VO_2 (Bailey et al. 2009). For this reason, the measured VO_2 in this study was collected via expired gas.

Steady State

When cycling on a stationary bike, with each watt of power increase, an individual's oxygen use increases by about 10mL per minute (Bailey et al. 2009). When reaching a steady state, ATP turnover at the cross bridges increases immediately but there is a time delay in mitochondrial oxygen consumption in the muscle (Jones & Poole 2005). The delay is correlated to a Gas Exchange Threshold, where an individual's body will use oxygen less efficiently (Bailey et al. 2009). When working at a constant moderate intensity, participants maintain homeostasis by shifting between anaerobic and aerobic glycolysis as well as fatty acid oxidation (Loon et al. 2001). A steady state is referred to as a state of constant exercise where the heart rate and other metabolic processes stop increasing or fluctuating. When at a steady state, it gives information on the metabolic energetic cost of the required power to perform processes at a cellular level for exercise (Selinger & Donelan 2014). It is also found that when exercising at a constant work rate, it takes participants 3 minutes to reach their steady state when measured in terms of VO_2 (Koike et al., 1995).

Importance

As a population, there is a need for better OCE for health and competitive reasons tied to performance. Exploring the potential benefits of nitrate-containing juices on OCE holds significance.

Understanding the effect of BRJ on performance is valuable for society's well-being and contributes to ongoing physiological research.

Materials and Methods

Ethical Approval

This study was approved by the Thompson Rivers University Research Ethics Committee on Human Subjects and Faculty Supervisor, Mark Rakobowchuk. All participants provided their written consent after they received a verbal and written description of the methods and procedures involved in the study. Each participant understood their right to withdraw. Verbal consent was also received before each task within the experiment.

Participants

Eight healthy young adults (4 males and 4 females, 20 ± 2 years) were recruited and volunteered 2 hours for this study. Participants were recruited from Thompson Rivers University. Before data collection started all participants were briefed on the safety and study procedures.

All participants provided informed consent and completed a health history questionnaire to ensure eligibility. A health history questionnaire was used to ensure each participant's safety and avoid factors that could affect the results, such as respiratory issues or known inability to complete trials. Participants excluded from the study were those who checked 'yes' to any of the following: having health concerns disabling elevated HR, inadequate shape to ride a stationary bike for 20 minutes at a moderate-high pace, risk of causing damage to health by conducting exercise, a current muscle injury, respiratory issues or heart conditions, or known allergy to BRJ. Participants were continuously monitored throughout their time in the lab regarding HR, comfort, and overall health, and were allowed to stop at any time during the trials.

Equipment

The following equipment was used: stationary bike (NEO Tacx Bike Smart), BRJ shot (Beet It Sport Nitrate 400 Concentrated Beet Juice), chest-strap heart rate monitor (Polar H10), spirometer pod, respiratory flow heads and tubes, gas analyzer, Douglas bags, stopwatch, and Power Lab.

The Tacx stationary bike enabled power output to be controlled while monitoring rotations per minute (rpm) and HR. Controlling the power output is key in the study as it maintains all participants at their personalized work rate to maintain a steady state of aerobic respiration.

Baseline testing

The study consisted of a preliminary submaximal power output test, followed by two trial days separated by at least 48 hours. Participants' resting HR was recorded and their maximum HR was calculated using the standard equation of 220 minus age (Equation 1). All participants underwent a baseline ramp trial, where participants underwent a predictive submaximal test session on a stationary bike. Resting HR was recorded, and participants cycled through a ramping protocol, consisting of a warm-up at a low power output of 30W, followed by 4W increases every 15 seconds until an HR of 160 bpm was reached. This value approaches the vigorous exercise intensity, while being well below the maximum HR, to prevent negative side effects. HR and the corresponding power output were recorded continuously throughout the ramping procedure. The obtained data was used to calculate each participant's maximum aerobic power output using a linear regression line and calculating 15% and 30% of their maximum power output.

Experimental Design

Participants refrained from consuming nitrate-rich foods or BRJ for at least 24 hours before the experiment to minimize confounding effects. During the control session, Exercise Protocol Trial 1 (Control) was conducted after participants drank a

calorically equivalent juice to the beetroot shot. Exercise Protocol Trial 2 (Experiment) was conducted after participants consumed a BRJ shot containing 400mg of nitrate. Jones et al. (2018) state that the minimum recommended dose of nitrate is 5mmol, equating to 310mg, validating the amount used in this study. A time gap of 2.5 hours between BRJ consumption and the experimental trial was used.

Experiment Trial

Participants consumed a BRJ shot 2.5 hours before the experimental trial. Participants warmed up at 15% of their maximum power output for 2 minutes before cycling at 30% for 15 minutes. The first 5 minutes at 30% involved no gas collection, as individuals are predicted to reach a steady state within this time frame, once VO_2 has become constant (Koike et al. 1995). After a steady state was reached, gas collection began. Gas exchange was measured using Douglas bags, with 3 samples of 1-minute collection intervals, spaced 3 minutes apart. The time gap allowed time to analyze gas samples, and to account for any minor fluctuations in VO_2 during the steady state. Each participant cycled for a total of 17 minutes. Once the gas collection was finished, participants were advised to do a cool down, typically 2 minutes of 30W power, then rest sitting down, until their HR lowered back to resting.

Control Procedure

The control procedure involved participants ingesting a calorically equivalent control juice 2.5 hours before cycling. The caloric equivalence was to ensure that any observed effects were not solely due to differences in caloric intake between the experimental and control groups. The procedure followed the same protocol as the experimental, with participants warming up at 15% of their maximum power output for 2 minutes before cycling at 30% for 15 minutes. Gas collection parameters remained constant, as well as the resting protocol.

Sample Collection

Douglas bags were utilized to collect expired gas samples during exercise trials. The percentages of oxygen and carbon dioxide in the gas samples and room air were determined using a gas analyzer and recorded. The airflow of expelled air was measured by a spirometer pod connected to the PowerLab device. HR was continuously monitored throughout each trial for the participant's safety. The HR data was recorded using Garmin Connect. The flow volume and gas analyzer data were recorded using Lt Labstation (v1.3.7) with a provided lab lesson file of Energy Expenditure and Exercise.

Data Analysis

The integral of the flow over each Douglas bag's collection period, typically 1 minute, was computed, producing the volume of air being expelled into each Douglas bag. To calculate the minute volume (V_E), the total volume in the bag was divided by the time of collection. For all the BRJ samples, the minute volume for the control session was used due to an error with the spirometer pod. With the V_E and the average percentage of oxygen in the air and the sample for each session, VO_2 was calculated for every sample using Equation 2. VCO_2 was calculated similarly to VO_2 using Equation 3. The RER was calculated by dividing the VCO_2 by the VO_2 shown in Equation 4.

Statistical Analysis

All data was expressed as a mean ± SD with a 95% confidence interval. All descriptive statistics, figures, and tables were produced using JASP (v0.18.3). Statistical analysis included comparing the means and standard deviations of VO_2 and RER between the control and experimental trials. To determine the statistical significance of the mean differences, a Paired Sample T-test was conducted in JASP. The threshold value for p to declare statistical significance used was p=0.05.

Results

Oxygen Consumption (VO_2)

The measured VO_2 was not significantly different after the ingestion of BRJ ($p=0.888$, Figure 1A). The VO_2 decreased by 0.5% after the acute ingestion of BRJ. The mean VO_2 after ingestion of BRJ was measured at 245.6 ± 56.9 and the control at 246.8 ± 62.1. The null hypothesis is unable to be rejected.

Respiratory Exchange Ratio (RER)

The measured RER was not significantly different after the ingestion of BRJ ($p=0.093$, Figure 1B). The RER increased by 2.8% after the acute ingestion of BRJ. The mean RER after ingestion of BRJ was measured at 1.059 ± 0.039 and the control was measured at 1.030 ± 0.051. The null hypothesis is unable to be rejected.

Discussion

Findings

Contrary to the hypothesis, nitrate-containing BRJ shows no significant effect on OCE when working at a moderate intensity, aerobically. No significant results of nitrate influencing the RER in the participants were found. An increase in VO_2 after the ingestion of BRJ containing a recommended dose (400mg) of nitrate was expected, as found by Bailey et al. (2009). However, studies supporting nitrate having no acute effect on VO_2 during moderate-intensity exercise, such as Betteridge et al. (2016) exist, supporting the current findings.

The RER results were insignificant because of the increased RER rate after ingestion of BRJ. The increase of RER with BRJ ingestion suggests that participants were not exercising aerobically. An RER higher than 1 indicates anaerobic exercise, increased carbohydrate oxidation, and decreased fat oxidation (O'Neill et al. 2004). The participant's RER should not have increased to above one if they were working aerobically. If participants were at an anaerobic threshold, they would be partaking in anaerobic glycolysis instead of aerobic glycolysis (Loon et al. 2001). Participants may have been using glucose as a substrate for ATP generation instead of free fatty acids (Loon et al. 2001). This leads to the belief that participants were not engaging in beta-oxidation, which would have been the ideal measure for this study because it is more stable.

The VO_2 showed a decrease after the consumption of nitrate-containing BRJ when graphing the results, but it was not significant. Since the decline was insignificant, there may not have been enough nitrite available to the mitochondria. Larson et al. (2011) isolated mitochondria from skeletal muscle and tested whether the rate of higher ATP resynthesis to oxygen consumption was due to

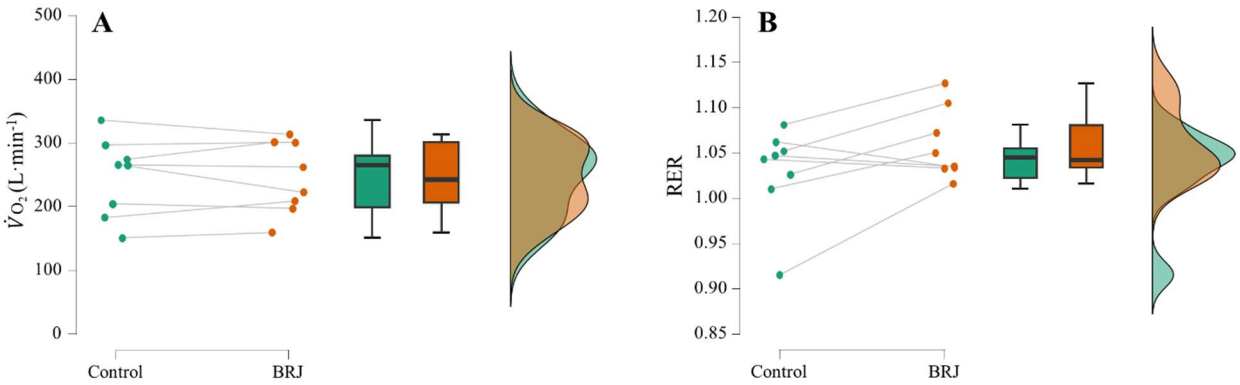

Figure 1. Raincloud plots demonstrating the relationship between acute ingestion of BRJ and control samples in VO_2 (A) and RER (B) measured at steady state during each bout of exercise.

supplement $NaNO_3$. They found that mitochondria with this supplementation increased the rate of ATP resynthesized compared to oxygen consumption, overall decreasing oxygen consumption (Larson et al. 2011). If isolated mitochondria are directly supplemented and show a decrease in oxygen consumption, it is possible that orally ingested nitrate may not have been an adequate approach to nitrate supplementation.

Previous research has shown that antiseptic mouthwash, which eliminates oral bacteria, effectively prevents the increase in plasma levels of nitrite following nitrate consumption (Govoni et al. 2008). Considering this, participants who use mouthwash as part of their daily routines may have limited the amount of nitrite entering the blood, diminishing any potential increase in OCE caused by nitrite.

The study also had limitations because it was done in three months, allowing time for a small sample size of n=8. After analyzing the data, it was unclear if there were enough participants to make a statistical difference. The ideal sample size to show statistical significance using G*Power (v3.1.9.7 Kiel, Germany) was determined. With a α level of 0.05 and a β level of 0.8, G*Power analysis determined the experiment needed n=33 to observe statistical significance in RER. Furthermore, with a α level of 0.05 and a β level of 0.8, G*Power analysis determined that n=1056 was required to observe statistical significance in VO_2. A sample size of n=1056 is quite unreasonable for the timeline of a project like this, therefore it would not be attainable to demonstrate significance in this timeframe.

Sources of Error

When analyzing the data, the experimental sessions showed irrationally high minute volume values; they ranged close to 300 L/min. Considering this, it was concurred there had to be an error as these values were not logical. Maximum minute volume values, although variable, should remain below 100 L/min (Blackie et al 1991). Following further investigation, it was found that there was a small slit in the flowmeter tubing. This caused the airflow volumes to be skewed and not realistic. To work around this, it was assumed minute volume was equivalent between both the control and experimental sessions.

In regard to the high RER values, another potential source could be due to the heart rate monitor during the baseline ramp test. Two of the participants were having difficulty raising their heart rate to 160 bpm during the ramping protocol. Originally, it was assumed it was because of their fitness levels, but now it could be that the heart rate monitor was reading their heart rate incorrectly, leading to the participants having a higher maximum power output. If the participants' target power output was too high it could have led to them exercising more vigorously, using glucose as a substrate instead of free fatty acids for ATP generation. Participants not engaging in beta-oxidation would have increased RER values. Therefore, an incorrect reading from the heart rate monitor during the ramping protocol could have led to the high RER values in participants.

Each participant was given a 70-mL shot of BRJ containing 400mg of nitrate 2.5 hours before their experimental protocol. Throughout the absorption of nitrite, it is found that only 25% of nitrite is absorbed into the bloodstream, and the remaining nitrite is secreted from the kidneys in urine (Jones et al. 2018). If only 25% is absorbed into the blood, there is a chance that even less makes it to the mitochondria. This being said, there may not have been enough absorbed nitrite to create a significant difference in oxygen consumption. It was not certain that even 25% made it into the bloodstream, and 75% or more of the absorbed nitrite may have gone to the kidneys to be secreted (Jones et al. 2018).

The stationary bike (NEO Tacx Bike Smart) used allowed control of the participants' power resistance and observation of the rpm values. Although the power was controlled to a participant's personalized 30% max power output, there is

uncertainty that participants were at the exact power value for the entire bout of exercise. As participants exercised, they were instructed to cycle at 70 rpm. The participants' rpm values constantly fluctuated around 70 ± 5 rpm. Variance in the rpm values can not guarantee the power was consistent throughout the entire bout of exercise. The power output could also have fluctuated as the rpm values fluctuated, like in a homeostatic balance. Therefore, because the work rate varying with changes in rpm, it is uncertain that participants were continuously at their target power output.

Conclusion

This study analyzed the effect of nitrate-containing beverages on OCE in adults. Based on the data obtained, acute ingestion of beetroot juice containing a recommended dose of 400mg of nitrate did not significantly impact VO_2 or RER during moderate-intensity aerobic exercise. Despite initial hypotheses suggesting a potential decrease in VO_2 following nitrate consumption, the results contradict such expectations. The contradiction may be due to the described error of the spirometer pod hose discussed previously, however, this outcome aligns with findings from previous studies, such as Betteridge et al.'s study (2016), which also reported no acute effects of nitrate on VO_2 during similar exercise intensities.

The absence of significant change in OCE challenges the popular idea of nitrate's influence on exercise physiology in the fitness industry, particularly in the context of aerobic metabolism. RER also showed no difference, following the expected results, as well as other literature (Bailey et al. 2009). While a few studies have emphasized the potential ergogenic benefits of nitrate supplementation, particularly in enhancing exercise performance (Macuh and Knap 2021), this study's findings suggest no significant relationship between nitrate intake and physiological responses during moderate-intensity exercise.

Future Research Ideas

These results demonstrate the importance of further exploration and refinement of the understanding of nitrate's effects on exercise metabolism. Future research could delve deeper into factors influencing individual OCE variability in response to nitrate supplementation and the potential influence of varying exercise intensities and durations on nitrate's effects on OCE. Such insights could contribute to developing more targeted and effective strategies for optimizing athletic performance and enhancing aerobic capacity through nutritional supplementation.

Equations

Equation 1

$$HR_{max} = 220 - Age$$

Equation 2

$$VO_2 = V_E\ x\ (\%O_{2\ air} - \%O_{2\ expelled})$$

Equation 3

$$VCO_2 = V_E\ x\ (\%CO_{2\ expelled} - \%CO_{2\ air})$$

Equation 4

$$RER = \frac{VCO_2}{VO_2}$$

References

Ahmad M, Wolberg A, Kahwaji CI. 2023. Biochemistry, Electron Transport Chain. StatPearls Publishing. [accessed 2024 Mar 29]. https://www.ncbi.nlm.nih.gov/books/NBK526105/.

Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM. 2010. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans.

Journal of Applied Physiology. 109(1):135–148. doi:10.1152/japplphysiol.00046.2010.

Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. 2009. Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. Journal of Applied Physiology. 107(4):1144–1155. doi:10.1152/japplphysiol.00722.2009.

Basu S, Azarova NA, Font MD, King SB, Hogg N, Gladwin MT, Shiva S, Kim-Shapiro DB. 2008. Nitrite Reductase Activity of Cytochrome c*. Journal of Biological Chemistry. 283(47):32590–32597. doi:10.1074/jbc.M806934200.

Betteridge S, Bescós R, Martorell M, Pons A, Garnham AP, Stathis CC, McConell GK. 2016. No effect of acute beetroot juice ingestion on oxygen consumption, glucose kinetics, or skeletal muscle metabolism during submaximal exercise in males. Journal of Applied Physiology. 120(4):391–398. doi:10.1152/japplphysiol.00658.2015.

Blackie SP, Fairbarn MS, McElvaney NG, Wilcox PG, Morrison NJ, Pardy RL. 1991. Normal Values and Ranges for Ventilation and Breathing Pattern at Maximal Exercise. Chest. 100(1):136–142. doi:10.1378/chest.100.1.136.

Brand MD, Pakay JL, Ocloo A, Kokoszka J, Wallace DC, Brookes PS, Cornwall EJ. 2005. The basal proton conductance of mitochondria depends on adenine nucleotide translocase content. Biochemical Journal. 392(2):353–362. doi:10.1042/BJ20050890.

Gallardo EJ, Coggan AR. 2019. What Is in Your Beet Juice? Nitrate and Nitrite Content of Beet Juice Products Marketed to Athletes. Int J Sport Nutr Exerc Metab. 29(4):345–349. doi:10.1123/ijsnem.2018-0223.

Govoni M, Jansson EÅ, Weitzberg E, Lundberg JO. 2008. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide. 19(4):333–337. doi:10.1016/j.niox.2008.08.003.

Hinkle PC. 2005. P/O ratios of mitochondrial oxidative phosphorylation. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1706(1):1–11. doi:10.1016/j.bbabio.2004.09.004.

Jones AM, Poole DC. 2005. Oxygen Uptake Dynamics: From Muscle to Mouth—An Introduction to the Symposium. Medicine & Science in Sports & Exercise. 37(9):1542. doi:10.1249/01.mss.0000177466.01232.7e.

Jones AM, Thompson C, Wylie LJ, Vanhatalo A. 2018. Dietary Nitrate and Physical Performance. Annual Review of Nutrition. 38(1):303–328. doi:10.1146/annurev-nutr-082117-051622.

Koike A, Yajima T, Adachi H, Shimizu N, Kano H, Sugimoto K, Niwa A, Marumo F, Hiroe M. 1995. Evaluation of Exercise Capacity Using Submaximal Exercise at a Constant Work Rate in Patients With Cardiovascular Disease. Circulation. 91(6):1719–1724. doi:10.1161/01.CIR.91.6.1719.

Lancaster JR, Hibbs JB. 1990. EPR demonstration of iron-nitrosyl complex formation by cytotoxic activated macrophages. Proceedings of the National Academy of Sciences. 87(3):1223–1227. doi:10.1073/pnas.87.3.1223.

Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E. 2011. Dietary Inorganic Nitrate Improves Mitochondrial Efficiency in Humans. Cell Metabolism. 13(2):149–159. doi:10.1016/j.cmet.2011.01.004.

Macuh M, Knap B. 2021. Effects of Nitrate Supplementation on Exercise Performance in Humans: A Narrative Review. Nutrients. 13(9):3183. doi:10.3390/nu13093183.

Nitrate/Nitrite Toxicity: What Is the Biologic Fate of Nitrates and Nitrites in the Body? | Environmental Medicine | ATSDR. 2023 May 25 [accessed 2024 Feb 28]. https://www.atsdr.cdc.gov/csem/nitrate-nitrite/biologic_fate.html

O'Neill M, Watt MJ, Heigenhauser GJF, Spriet LL. 2004. Effects of reduced free fatty acid availability on hormone-sensitive lipase activity in human skeletal muscle during aerobic exercise. Journal of Applied Physiology. 97(5):1938–1945. doi:10.1152/japplphysiol.01135.2003.

Palmer RMJ, Ferrige AG, Moncada S. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature. 327(6122):524–526. doi:10.1038/327524a0.

Pignatelli P, Fabietti G, Ricci A, Piattelli A, Curia MC. 2020. How Periodontal Disease and Presence of Nitric Oxide Reducing Oral Bacteria Can Affect Blood Pressure. International Journal of Molecular Sciences. 21(20):7538. doi:10.3390/ijms21207538.

Powell FL, Hopkins SR. 2004. Comparative Physiology of Lung Complexity: Implications for Gas

Exchange. Physiology. 19(2):55–60. doi:10.1152/nips.01469.2003.
Poole DC, Richardson RS. 1997. Determinants of Oxygen Uptake. Sports Med. 24(5):308–320. doi:10.2165/00007256-199724050-00003.
Ricquier D, Bouillaud F. 2013. Uncoupling Proteins. In: Lennarz WJ, Lane MD, editors. Encyclopedia of Biological Chemistry (Second Edition). Waltham: Academic Press. p. 482–487. [accessed 2024 Mar 29]. https://www.sciencedirect.com/science/article/pii/B9780123786302001626.
Shen W, Hintze TH, Wolin MS. 1995. Nitric Oxide. Circulation. 92(12):3505–3512. doi:10.1161/01.CIR.92.12.3505.
Selinger JC, Donelan JM. 2014. Estimating instantaneous energetic cost during non-steady-state gait. Journal of Applied Physiology. 117(11):1406–1415. doi:10.1152/japplphysiol.00445.2014.
Stanaway L, Rutherfurd-Markwick K, Page R, Wong M, Jirangrat W, Teh KH, Ali A. 2019. Acute Supplementation with Nitrate-Rich Beetroot Juice Causes a Greater Increase in Plasma Nitrite and Reduction in Blood Pressure of Older Compared to Younger Adults. Nutrients. 11(7):1683. doi:10.3390/nu11071683.
van Loon LJC, Greenhaff PL, Constantin-Teodosiu D, Saris WHM, Wagenmakers AJM. 2001. The effects of increasing exercise intensity on muscle fuel utilisation in humans. The Journal of Physiology. 536(1):295–304. doi:10.1111/j.1469-7793.2001.00295.x.
Zhao Y, Brandish PE, Ballou DP, Marletta MA. 1999. A molecular basis for nitric oxide sensing by soluble guanylate cyclase. Proceedings of the National Academy of Sciences. 96(26):14753–14758. doi:10.1073/pnas.96.26.14753.