Therapeutic role of dietary nitrates on cardiorespiratory function in cancer survivors

by

Vanessa-Rose Garcia Turpin

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Approved by:

Major Professor
Dr. Carl J. Ade

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Abstract

Introduction: The acute and chronic adverse physiological consequences of anticancer therapy include direct injury to the entire cardiovascular-skeletal muscle axis. As such, these patients are at an increased risk of both cancer therapy-related and age-related pathological outcomes; primary cardiovascular disease, exercise intolerance, and cancer-related fatigue. To date, however, therapeutic strategies that mitigate these negative effects within the human body have yet to be established. Previous work has demonstrated that dietary nitrate (NO_3^-) supplementation can improve cardiac, vascular and cardiorespiratory exercise parameters, highlighting its potential therapeutic use in clinical populations. Therefore, we hypothesized that NO_3^- supplementation would improve both cardiac performance and exercise capacity.

Methods: To date, 6 cancer survivors (57 ± 11 years) with a history of anticancer therapy completed a randomized, double-blind, crossover study with a single, acute-dose administration of NO_3^- or placebo (PL) [140 ml]. Transthoracic echocardiographic measures at rest were made to obtain left ventricular stroke volume. Patients performed a supine-cycling steady-state exercise test (30W) with measurements of arterial blood pressure, stroke volume, cardiac output, and a maximal-effort cardiopulmonary exercise test.

Results: As intended, there was a statistically significant increase in plasma nitrite during the NO_3^- condition compared to PL (NO_3^- 1300 ± 963 µM vs. PL 111 ± 49 µM, respectively; P = 0.02). Additionally, we observed a decrease in relative oxygen uptake (VO_2) during steady-state exercise with NO_3^- compared to PL (NO_3^- 8.46 ± 2.2 vs. PL 8.98 ± 2.4 ml/kg/min; p = 0.01; Absolute VO_2: BRJ 0.64 ± 0.10 vs. PL: 0.68 ± 0.11 L/min; p = 0.01) indicating an improved exercise efficiency. Resting and steady-state arterial blood pressure, stroke volume, and cardiac output were not different between conditions. Furthermore, we did not observe any differences

between conditions for peak relative VO_2 (NO_3^- 22.42 ± 3.86 vs. PL 23.14 ± 4.01 ml/kg/min; p = 0.23), total work done (NO_3^- 70.64 ± 29.5 vs PL 70.67 ± 30.71 kJ; p = 0.49), or for gross exercise efficiency (NO_3^- 5.23 ± 1.48 vs. PL 4.97 ± 1.41 kJ/L O_2; p = 0.14) during the maximal-effort cardiopulmonary exercise test.

Conclusions: A single, acute-dose of inorganic nitrate supplementation in cancer survivors with a history of anticancer therapy enhanced steady-state exercise efficiency, but had no effect on exercise cardiac performance or peak exercise capacity.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Annotated Bibliography

Selected articles that are critically related to the development of this thesis.

Effect of Inorganic Nitrate on Exercise Capacity in Heart Failure With Preserved Ejection Fraction. Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, Cappola TP, Poole DC, and Chirinos JA. Circulation 131: 371-380; discussion 380, 2015.

Purpose: This study tested the hypothesis that: administering inorganic nitrate supplementation would improve exercise capacity in HFpEF individuals. They also investigated the effect of inorganic nitrate on the vasculature and skeletal muscle to obtain insight into the mechanisms through which an effect on exercise tolerance may occur. Methods: Inclusion criteria included those (human subjects) who have symptomatic heart failure with a preserved ejection fraction (>50%) with a ratio of early mitral inflow velocity to septal tissue velocity and one other sign of chronically elevated filling pressures. Study was a randomized, double-blind, crossover study with single dose of inorganic nitrate from concentrated beet root juice containing 12.9 mmol NO3- in 140 ml versus placebo control. Washout was at least 5 days (range: 5-42 days). Exercise efficiency was measured (ratio of total work performed to total oxygen consumed) along with measurements of peak VO2, vasodilatory reserve during exercise (change in peripheral vascular resistance from rest to peak exercise), and skeletal muscle mitochondrial oxidative function. Arterial wave reflections were also assessed during post occlusion of the forearm. Patients performed a maximal exertion-limited exercise test using a graded exercise protocol on a cycle ergometer. Resistance started at 12.5 W for 3 minutes and then increased to 25 W for 3 minutes and then increasing every 3 minutes by 25 W. 15 minutes after this exercise is when patients underwent a 6 minutes 25 W constant resistance load. Post occlusion hyperemia test was performed to determine mVO2 recovery. Results: This study found that peak VO2, total work performed, and exercise duration were all significantly increased after NO3- supplementation. Ventilatory threshold was significantly greater after NO3- supplementation and also enhanced the reduction in SVR at peak exercise and also a significant increase in cardiac output versus placebo control. The same for an increase in stroke volume and heart rate. Steady state VO2 was not different between treatments. No difference in mVO2 between arms of placebo control and BRJ during rest but during exercise mVO2 recovery back to baseline tended to be shorter with BRJ. Brachial flow after cuff release was greater in BRJ arm. Discussion: A single dose of BRJ before exercise significantly improves peak VO2 in subjects with HFpEF. This was accompanied by a reduction in SVR, increase in cardiac output, and increase in VO2 at which ventilatory threshold occurred. Suggestive that NO3- improves exercise capacity in HFpEF by improving peripheral response to exercise and by providing greater O2 delivery to exercising muscles. Improvements in oxidative function have also been demonstrated in this study with the use of NIRS after NO3- supplementation which is suggestive of improved ATP production.

Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study
Lansley, Katherine; Winyard, Paul; Fulford, Jonathan; Vanhatalo, Anni; Bailey, Stephen; Blackwell, Jamie; DeMenna, Fred; Gilchrist, Mark; Benjamin, Nigel; and Jones, Andrew.

Journal of applied physiology 110: 591-600; discussion, 2011.

Purpose: 1) To determine whether physiological effects of BR supplementation were consequent to the high NO3- content of BR 2) Investigate the extent to which elevation of NO bioavailability through BR consumption might increase mitochondrial biogenesis and thus contribute to reported improvements in aerobic exercise performance 3) To extend previous findings from own lab in knee-extension and cycling to walking and running to broaden intervention application It was hypothesized that: 1) Relative to placebo, BR would increase plasma NO2- and reduce Blood Pressure. 2) Also, BR would reduce O2 cost of walking and running and increase exercise tolerance measured as time to task failure. 3) BR would increase muscle oxidative capacity as assed by Q max 4) NO3- depleted placebo would not alter physiological indexes relative to pre-intervention control indicating physiological effects (4-6 days) of BR are related to NO3- content. Methods: Randomized, double blind, crossover study with 9 male subjects (aged 22+- 4 years) all healthy with supplementation of either BR or placebo for 6 days with a 10 day washout period. BR supplement was 0.5 l/day containing 6.2 mmol of NO3- and placebo was 0.5 l/day of 0.0034 mmol of NO3- which was NO3- depleted BR. Incremental ramp performed prior to supplementation to determine VO2 max and GET. Day 4 and 5 were used for testing in which day 4 was two 6 min bouts of moderate (80% GET) and 1 exhaustive bout of severe intensity running (75% delta) Day 5 was two 6 min bouts of moderate (80% GET) and one 6 min bout of severe (75% delta). Day 6 of testing, subjects were required to complete an incremental, single-legged, knee-extension exercise test consisting of a 42 second bout at 81.2% +- 4.3% of individual max. Results: BR significantly reduced systolic BP compared to placebo. Baseline walking VO2 reduced by 12% compared to placebo. And absolute VO2 during last 30 sec of running moderately was significantly lower following BR supplementation. VO2 reduced by BR by 14% during walking compared to placebo and VO2 was reduced by 6% at task failure compared to placebo. All subjects had higher exercise tolerance as shown from increased time to task failure of 15% and all 9 subjects were able to exercise longer compared to placebo. Conclusion: 1) Short-term (4-6 days) dietary BR increased plasma NO3- and reduced systolic BP 2) BR reduced the O2 cost of walking and moderate and severe intensity running 3) increased the time to task failure during constant-speed severe intensity running and incremental knee-extension exercise. BR intake resulted in 12% reduction of O2 cost of walking. Steady state VO2 during moderate intensity running was reduced by 7%. Results seen within 4 days of supplementation. Findings are related to NO2- and NO mediated effects on muscle contractile function rather than changes in mitochondrial volume.

A comparison of organic and inorganic nitrates/nitrites
Omar, Sami; Artime, Esther; and Webb, Andrew.
Nitric Oxide 229-40. 2012.

Purpose: Review will compare similarities and differences between organic nitrate/nitrites and inorganic nitrates/nitrites in chemical structures, bio-activation pathways, pharmacological activity and effects. As well as future therapeutic applications for inorganic nitrate/nitrite.
Methods: Review covered both human and animal applications in terms of the use of organic nitrate/nitrite used for clinical applications for treatment of CVD. Organic nitrate/nitrites in the blood stream levels will quickly rise and have rapid onset of action (1-3 mins) but it is also clearly rapidly from the blood which gives a rapid offset (15-30 min). Chronic use may lead to endothelial dysfunction due to vascular tolerance thus triggering ROS and aggravating the tolerance.

However, inorganic nitrate/nitrite is added to cure meats as a preservative but after ingestion, can be produced in mammalian tissue by the oxidation of the NO produced from the enzymatic degradation of L-arginine. Reduction to nitrite is largely via entero-salivary circulation. Nitrate levels rise within 30 mins and a peak of 3 hours and is sustained for up to 24 hours. Nitrite rise is more gradual by 1-1.5 hours and then a plateau around 2.5 hours and remain elevated for 6 hours. If inorganic nitrate is ingested orally, its bioavailability is 95-98%. Nitrate-nitrite-NO pathway is enhanced by hypoxic and acidic conditions. The World Cancer Research Fund/American Institute of Cancer Research found no evidence linking ingestion of vegetables high in nitrate with development of cancer. Dietary Approach to Stop Hypertension (DASH) found dietary intake of fruits and vegetables for three weeks resulted in a reduction in both systolic and diastolic BP. Inorganic nitrate/nitrite consumption offers cytoprotection against I/R injury, inhibition of platelet aggregation, BP reduction, and reduction in the O2 cost during exercise. Endothelial function = eNOS to generate NO and EDRF. Results: Organic nitrates may be effective in lowering BP and used acutely for hypertensive emergencies, the effect is variable and longer-term use is limited by the rapid development of tolerance. A study in rats found continuous delivery of organic nitrate lead to an increase in infarct area however, on the other hand, a discontinuation of treatment 3 hour prior to ischaemic event caused a decrease in infarct size.
Inorganic nitrate/nitrite was found to decrease infarct size and restored cardiac function in in vitro and in in vivos models. Summary: In contrast to organic nitrates, inorganic nitrites now hold considerable promise as a treatment for myocardial infarction and is currently being used in clinical trials.

Dietary nitrate provides sustained blood pressure lowering in hypertensive patients. A randomized, phase 2, double-blind, placebo-controlled study
Kapil, Vikas; Khambata, Rayomand; Robertson, Amy; Caulfield, Mark; and Ahluwalia Amrita. Hypertension 320-7. 2014.

Purpose: To explore whether once daily dietary nitrate supplementation for 4 weeks would confer sustained BP reduction in both drug-naïve and treated patients with hypertension
Methods: 68 patients entered in a double-blind, randomized, placebo-controlled trial. Aged 18-85 years old and uncontrolled BP monitoring of >130/85 mmHg. 34 patients received a placebo of 4 weeks daily of 250 mL nitrate-depleted BRJ with 0.007 mmol of nitrate daily and 34 patients received 250 mL daily for 4 weeks of BRJ with 6.4 mmol of nitrate daily. Patients recorded once daily home BP for two weeks and then a pre-intervention where ambulatory BP was taken. After this is when the patients were asked for consume their respective treatment for 4 wks and then there was a two week washout period and the patients were then asked to come back in for another ambulatory BP test. Findings: Dietary nitrate ingestion showed elevations in both nitrate and nitrite concentrations but this returned back to baseline after the 2 week washout period. Plasma nitrite levels were elevated from baseline by 2.7 fold with no change in the placebo limb of the study. Change in circulating nitrite and nitrate levels associated with 1.4 fold increase in plasma cGMP concentrations with no change in the placebo. Home BP was reduced within 1 week of dietary nitrate consumption and reduced over the entire 4 week intervention period. This did not hold true for the placebo group. Peak decreases in BP occurred at 6 weeks (last week of dietary supplementation. After washout, BP began returning to baseline. There were no changes relative to baseline or compared to placebo in HR. Pulse wave velocity was reduced after BRJ consumption compared with baseline. Conclusion: In treated hypertensive patients, PWV was reduced after

dietary nitrate and endothelial function was improved (change in FMD compared to placebo). Once daily nitrate intervention augments NO generation through pathway in patients with hypertension to lower BP. Nitrate administration over 4 weeks shows absence of lack of tolerance which is seen in organic nitrate supplementation.

Mechanisms underlying blood pressure reduction by dietary inorganic nitrate
Carlstrom, M; Lundberg, J.O.; and Weitzberg, C.
Acta Physiologica. 2018.

Purpose: Describe how dietary nitrate affects various organ systems and discuss underlying mechanisms that may contribute to the observed blood pressure lowering effects. Methods: Both rat and human models were discussed in this review. Findings: Both normotensive and hypertensive humans showed a lowering of BP with nitrate supplementation. Activity of eNOS is regulated by intracellular calcium that activates calmodulin which binds to eNOS and increases enzyme activity. When BH4 is limited, eNOS uncoupling occurs and the enzyme generates superoxide anions instead of NO. Induction of iNOS leads to higher amounts of NO which contribute to the innate immune system by killing bacteria, viruses, and fungi. INOS is highly expressed in several inflammatory states including cancer and atherosclerosis but its role in the CVD inflammatory process is still unclear. A study wherein 500 mL of BRJ was administered to a group of healthy subjects BP was reduced and BRJ protected the vascular endothelium from ischaemic injury and caused a reduction in ex vivo platelet aggregation. Dietary nitrates have the ability to reduce BP in normotensive and provide sustained reduction in BP in hypertensive patients. Infusing nitrite in brachial artery of healthy subjects caused an increase in forearm blood flow. ANG 2 induced vasoconstriction was markedly attenuated in renal arterioles from mice with dietary nitrate pre-treatment for 1 week. Dietary nitrates reduced arterial stiffness and improved endothelial function. Nitrite is a positive modulator of the Frank-Starling respond in the rat heart. Rammos and colleagues showed that 8 weeks of nitrate supplementation to old mice improved LV diastolic function, arterial compliance, and coronary flow reserve that were coupled to accelerated cardiomyocyte calcium handling which was coupled to increase cGMP signaling. Numerous studies have found that oxidative stress and subsequent NO deficiency in the kidney are critically associated with development of hypertension an increased risk of adverse CV complications. Chronic supplementation with inorganic nitrate attenuated hypertension, renal and cardiac injuries in rats with compromised kidney function and high salt diet. In mice, dietary nitrate supplementation for 2 weeks increased renal blood flow and GFR and attenuated the decline in kidney function and renal injuries that developed 2 weeks following the ischaemic event. Conclusion: Numerous pre-clinical trials and clinical studies demonstrate that inorganic nitrate supplementation is associated with favorable CV outcomes including reduced BP.

Acute administration of inorganic nitrate reduced VO2 Peak in endurance athletes
Bescos, Raul; Rodriguez, Ferran; Iglesias, Xavier; Ferrer, Miguel, Iborra, Elena; and Pons, Antoni
Medicine and Science in Sports and Exercise 1979-86. 2011.

Purpose: Aim was to assess the effect of a single dose of nitrate given before cycling to see the change in cardiorespiratory and metabolic response in endurance athletes at different intensities. It was hypothesized that nitrate supplementation would not be effective in improving the cardiorespiratory response to exercise at low to moderate intensities with highly athletic cyclists.

However, at higher intensities where acidosis and low O2 tension occur the nitrate-nitrite-NO pathway could be activated and increase tolerance to high intensity cycling which was measured as time to task failure. Methods: 11 male cyclists were enrolled in a randomized, double-blind, crossover study to receive a single dose of either sodium nitrate (10mg/kg) or sodium chloride in 250 ml of water with a 7-day washout period. Drinks were ingested 3 hours prior to exercise. First cycle ergometer test was submaximal at 2.0,2.5,3.0, and 3.5 W/kg with each load lasting 6 mins with 3 mins of passive recovery. Five minutes after the submaximal workloads, subjects performed an incremental exercise test to volitional exhaustion starting at 3.0 W/kg and the WR increased by 0.5 W/kg every minute. Four blood samples were collected for nitrate and nitrite analysis as well as capillary blood samples for lactate analysis were collected. Findings: After nitrate supplementation, the plasma levels were significantly lower after submaximal and maximal exercise compared to the peak value reached after 3 hours of supplementation. VO2 tended to be lower at the respiratory compensation point after nitrate supplementation than after the placebo. No difference in time to exhaustion compared between treatments. Conclusion: After a single dose of nitrate supplementation, there was no change in cardiorespiratory adaptation at low to moderate intensities on cycle ergometer in highly trained cyclists. However, at maximal intensity there were significant differences between groups. VO2 peak was significantly reduced without affecting the maximal attainable work, blood lactate, or other cardiorespiratory parameters. So, the second hypothesis was so confirmed as there was no significant difference in time to task failure between treatments.

Effects of dietary nitrate on oxygen cost during exercise
Larsen, F; Weitzberg, E; Lundberg, J; and Ekblom, B.
Acta Physiologica 59-66. 2007.

Purpose: If the administration of dietary nitrate would lead to increased systemic storage pools of nitrite and if this dietary strategy would have an impact on various physiological and biochemical parameters during exercise. Methods: Double-blind, placebo-controlled, cross-over design with 9 healthy, well trained males. Study consisted of a familiarization day as well as two 3-day periods separated by a 10 day washout period. Subjects started with either 0.1 mmol sodium nitrate/kg or equal amount of sodium chloride as the placebo. Testing was done on a cycle ergometer. Submaximal levels were adjusted to correspond to 45, 60, 70, 80, and 85% VO2 peak. Findings: Average resting BP was lowered in nitrate group compared with the placebo group. There was a pronounced decrease in nitrite concentrations during exercise which was more pronounced in nitrate group than in the control group. After nitrate administration, VO2 was significantly lower during the four work rates corresponding to 45-80% of VO2 peak compared with placebo. There were no differences in; HR, VE, VE/VO2, or RER during any of the submaximal work rates between groups. There was also no significant difference in VO2 peak between groups. Clear lowering of oxygen cost after nitrate diet at 45% VO2 peak. Conclusion: A significantly reduced oxygen demand at the four lowest submaximal work rates was notes after nitrate administration. Showing that dietary supplementation with inorganic nitrate, in an achievable amount through a diet rich in vegetables, results in a reduced VO2 during submaximal work and a significant increase in muscular efficiency and energy production efficiency.

Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise

Larsen, Filip; Weitzberg, Eddie; Lundberg, Jon; and Ekblom, Bjorn.
Free Radical Biology and Medicine 342-7. 2009.

Purpose: Study the effect of nitrate supplementation on maximal combined arm and leg exercise. To investigate whether dietary nitrate would also influence VO2 max during exercise with large active muscle groups and if this would have any effect on the time to exhaustion during incremental exercise. Investigators also studied the effects of nitrate supplementation on plasma nitrite kinetics during exercise and BP in the acute recovery period after exhaustive exercise. Methods: 9 (7 male and 2 female) healthy subjects participated in a randomized double-blind cross-over study with two trials and at least a 7 day washout period. Maximal exercise tests were performed using two combined cycle ergometers with one used ordinarily and then the other used as an arm ergometer. Cadence was set to 80 rpm for both arms and legs and the exercise protocol was an incremental exercise to exhaustion. Nitrate supplementation was 0.033 mmol NaNO3/kg three times daily with the last dose taken 40 minutes before exercise test was administered. Placebo consisted of equal amount of NaCl. Findings: After nitrate supplementation VO2 max was reduced. Heart rate and pulmonary ventilation were unaffected by nitrate supplementation. Time to exhaustion showed a near-significant increase after nitrate supplementation. One hour after a single dose of nitrate, VO2 was decreased but was unchanged in the placebo trials. There was no significant change in blood pressure this may be due to the time of blood pressure as it was taken 40 minutes after ingestion, weak statistical power, and lower supplementation period. Conclusion: Nitrate supplementation decreases VO2 max at maximal combined arm and leg exercise but this trended to an increased performance as shown by the increased time to exhaustion.

Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health
Kapil, V; Weitzberg E; Lundberg, J.; and Ahluwalia, A.
Nitric Oxide 38:45-57. 2014.

Purpose: Review discussed the evidence testing the utility of NO3- - NO2 – NO pathway for NO synthesis in CV health and disease. Methods: Both rat and human models were reviewed and discussed. Findings: 2/3 of absorbed NO3- is excreted unchanged in the urine. Most of the remaining NO3- is concentrated from the circulation in the salivary glands of NO3- by a sialic acid transporter and then secreted into the oral cavity as NO3- rich saliva at 10x higher than plasma. This is known as the entero-salivary circulation. In rat aortic rings, application of low NO20 (2.5 uM) whilst inactive under physiological conditions was shown to relax contracted rat aorta under acidci (6.6 pH). Cosby et al. Demonstrated that infusion of NaNO2 into the human forearm causes vasorelaxation with concomitant increased forearm blood flow – augmented during exercise. Webb et al. Used dietary supplementation in BRJ and found a reduction in BP over a 24 hour period with peak reductions 2.5-3 h after ingestion coinciding with peak elevations in plasma NO2-. Another study found that lowered BP was sustained for 15 days compared to control with no evidence of tachyphylaxis. Females may have a decreased basal NO2- level due to decreased sensitivity due to possible “saturation” of the vasodilatory effect of NO2- proposed by Dejam et al. NO3- supplementation improves BF and blood vessel density. NO2- supplementation in pre-clinical models with IR injury reduce infarct size and improve left ventricular function. Pre-treatment up to 2 hours prior to delivery of IR injury with dietary nitrate prevented IR induced endothelial dysfunction. Inorganic nitrate reduced arterial stiffness as measured by PWV 3 hours after ingestion. NO generated by eNOS has been shown to induce mitochondrial biogenesis. Wylie

et al. Showed that non-respondent highly trained athletes turned into responders of NO3- by increasing the NO3- dose. Kenjale et al. Found that dietary NO3- extended peak walk time by almost 20% in patients with PAD an effect that could be related to improved mitochondrial efficiency or an increased BF to affected limbs. Inorganic nitrate has half life of 6 hours in human plasma whereas nitrate is 15-45 minutes. Conclusion: Nitrate-nitrite-NO pathway can be manipulated to boost NO activity in vivo to benefit CVD, increase exercise capacity, and metabolism. This may be a cheap way to improve overall health in CVD patients as well as CV health for all.

Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in order adults
Kelly, J Fulford, J; Vanhatalo, A; Blackwell, J; French, O; Bailey, S; Gilchrist, M; Winyard, P ans Jones, AM
American Journal of Applied Physiology R73-83. 2012.

Purpose: Aims: Assess whether the physiological effects of dietary nitrate supplementation reported previously in young adults are also evident in older adults. And use H magnetic resonance spectroscopy (MRS) brain-scanning to determine whether NO3- can influence brain metabolites. Methods: Double-blind, randomized, crossover study with 12 older adults (6 male and 6 female) aged 64+-4 for males and 63+-2 years for females. BR supplementation was 140 ml/day or NO3- depleted BR for 2.5 days prior to each subsequent lab visit. Walking exercise treadmill tests determine pulmonary VO2 and a 6-min walk test was used to determine functional capacity. There were also 3 cognitive tests involved to assess whether BR supplementation altered brain function with key metabolites. A single-leg knee extension exercise was also competed to assess muscle PCr levels. Findings: BR supplementation elevated plasma nitrite levels by 503% relative to control and then a 418% increase compared to placebo. BR significantly reduced systolic, diastolic, and MAP compared to both control and placebo trials. There was so significant difference in VO2 between placebo and BR during the baseline walking period. And steady state VO2 was unchanged between treatments. BR supplementation did decrease the O2 deficit (15% decrease) and reduce the mean response time for VO2 compared to placebo. BR did not significantly alter functional capacity as measured by the 6 minute walk test which measured the total distance covered. Between the 3 cognitive tests assessed, there were no differences between any of the tests in terms of BR or placebo trials. Conclusion: NO3- supplementation significantly reduced BP and the VO2 mean response time in healthy older adults. BR supplementation did not significantly reduce O2 cost of walking, functional walking performance, or the muscle metabolic response to low-intensity exercise or any brain metabolite concentrations or cognitive function. Short-term NO3- supplementation results in a 4x increase in plasma nitrite levels and significant reductions in resting BP in normotensive older adults. These can result in reducing the risk for hypertension and CVD in older adults. The VO2 kinetics were increased during treadmill walking although it did not translate to enhanced performance during the 6 MWT. This may result in therapeutic interventions for older adults with hypertension risk and may improve their VO2 kinetics.

Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans

Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM.
Journal of Applied Physiology 135-48. 2010.

Purpose: Aim of the study was to determine why after NO3- supplementation there is a reduced O2 cost with submaximal exercise and an improved tolerance of high-intensity exercise. Study hypothesized that NO3- supplementation would reduce both low and high-intensity exercise O2 cost and this would be accompanied by intramuscular PCr degradation. It was also hypothesized that exercise tolerance would be enhanced following BR supplementation due to a sparing effect of PCr. Methods: Study was a randomized, double-blind, crossover study design that included 7 health male adults (28+-7) who were all recreationally active. Three MVC of quadriceps were completed along with an incremental test using two-legged knee-extensions to establish peak work rate. The load for the first step was 4 kg per leg and the load was increased by 1 kg for each subsequent increment until intolerance. Supplementation was 6 days of BR (5.1 mmol/day) given as 0.5 L per day or blackcurrant juice as the placebo. Subjects completed two 4 min bouts of low-intensity exercise and one bout of high-intensity exercise with 6 mins of passive recovery separating each exercise bout. Findings: BP and MAP was significantly reduced relative to PL. VO2 from rest to low-intensity exercise was reduced by 25% after BR supplementation. End-exercise VO2 was also reduced following BR supplementation. Work efficiency was calculated by dividing exercise work rate by the difference between the exercise energy expenditure and the baseline energy expenditure. Work efficiency was significantly reduced after BR. VO2 slow component was significantly reduced (50%) after BR. NO3- supplementation also resulted in a 36% reduction in the amplitude of PCr degradation during low-intensity exercise. Pi and [ADP] were reduced during BR trials. Mean ATP turnover rate from PCr hydrolysis over low-intensity was lower following BR. [PCr] slow component amplitude was significantly reduced (59%) after BR. All 7 subjects were able to increase their time to task failure after BR. Conclusion: BR reduced O2 cost during exercise of both low and high-intensity exercises in young healthy males. This was done by reducing ATP cost of muscle force production. NO3- supplementation also improves the tolerance of high-intensity exercise as reflected by the reduction to which finite PCr levels were reserved over time. The reduced O2 cost appears to be manifested only during skeletal muscle contractions. VO2 required for same work rate was reduced during both low and high intensity exercises with BR. PCr concentrations were reduced with BR while pH remain unchanged. There was an improved tolerance to high intensity exercise.

Cardiopulmonary Function and Age-related decline across the breast cancer survivorship continuum
Jones LW, Courneya KS, Mackey JR, Muss HB, Pituskin EN, Scott JM, Hornsby WE, Coan AD, Herndon JE 2nd, Douglas PS, Haykowsky M.Journal of Clinical Oncology 2530-7. 2012.

Purpose: To evaluate cardiopulmonary function across breast cancer continuum and its prognostic significance in women with metastatic disease. Methods: 248 women were divided into 4 cohorts of either before, during, or after (6 months – 3 years) adjuvant therapy or in the 4th cohort being a therapy group for metastatic breast cancer treatment. These results were compared to those of age and sex matched healthy controls. Subjects completed an incremental cycle ergometer test to measure VO2 peak. After a 3 min baseline, patients began cycling at 20 W and workloads increase 5-20 W/min until volitional exhaustion. Hb was measured through CBC. Findings: Resting HR was significantly higher in the during adjuvant group. SBP was highest in the after adjuvant group

compared to the other groups. Hb was significantly lower in the during treatment group. VO2 peak averaged 17.8 +- 4.3 ml/kg/min which was equivalent to 27%-17% lower compared to the matched controls. 32% of the sample had a VO2 peak less than 15.4 ml/kg/min which is the minimal aerobic capacity required for functional independence. The overall mean VO2 response was 34% less than that of the healthy sedentary matched controls. Conclusion: Breast cancer patients have a significant impairment in cardiopulmonary function over the entire continuum of the disease. 1/3 of the patients tested have a VO2 peak less than the functional independence threshold and they reached a predicted age-related VO2 peak 20-30 years on average earlier than health women without breast cancer history.

Cardiovascular reserve and risk profile of postmenopausal women after chemoendocrine therapy for hormone receptor-positive operable breast cancer
Jones LW, Haykowsky M, Pituskin EN, Jendzjowsky NG, Tomczak CR, Haennel RG, Mackey JR.
The Oncologist 1156-64. 2007.

Purpose: To examine CV function and risk profile of postmenopausal women treated with chemoendocrine therapy for hormone receptor-positive operable breast cancer. Specifically to explore potential differences between women receiving aromatase inhibitors vs. Tamoxifen. It was hypothesized that breast cancer patients would have worse CV function compared to age and sex matched controls. Methods: 47 breast cancer patients (all received chemotherapy) and 11 health age and sex matched controls were studied. Each completed an incremental exercise test on a cycle ergometer (15W increase every 2 mins) to determine cardiopulmonary measurements. Blood pressure, HR, SV, Q, cardiac power output (Q x MAP) were obtained at the end of each exercise stage. Results: HR, MAP and SBP were significantly higher in cancer patients compared to controls. Peak exercise power output, absolute VO2 peak, VS, and Q were all significantly lower in patients compared to controls. Breast cancer patients with the greatest impairment in VO2 peak had the worst CVD risk profile. CVD risk factors were consistently more unfavorable in those being treated with aromatase inhibitors than tamoxifen. VO2 peak in patients was measured at 24% below that of controls. Conclusion: Breast cancer patients being treated with chemoendocrine therapy has a lower peak VO2 as well as worse CV measurements compared to matched health controls. Results indicate that impaired VO2 may be secondary to a lower stroke volume and Q at peak exercise, thus resulting in a concomitant reduction in O2 delivery to the active skeletal muscles. Impaired LV preload or contractility is likely responsible for low SV in breast cancer patients. Study was conducted 3 years (average) following chemotherapy cessation thus suggesting that LV function may not fully recover following completion of chemotherapy and/or radiotherapy. Thus, breast cancer patients have a significantly lower cardiorespiratory fitness level and lower cardiac functional reserve compared to their age and sex matched controls.

Determinants of exercise intolerance in breast cancer patients prior to anthracycline chemotherapy
Beaudry RI, Howden EJ, Foulkes S, Bigaran A, Claus P, Haykowsky MJ, Gerche A
Physiological Reports 13971. 2019.

Purpose: Authors hypothesized that impairment of peak VO2 in breast cancer patients prior to adjuvant chemotherapy (prior to anthracycline treatment) is attributable to decreased exercise

cardiac Q despite preserved RV and LV ejection fraction. Methods: 29 breast cancer patients and 10 health age and sex matched controls completed the study which included an incremental exercise test performed on an upright cycle ergometer with a 10-25 W power output initially and then increased 10-30 W/min until volitions exhaustion. Then subjects were placed on a cycle ergometer where they had intensities of 20, 40, and 60% of peak power output (obtained from incremental test) and each stage was maintained for 1-3 minutes. It was previously determined that 66% of peak power during upright cycle corresponded to peak exercise in a supine position. Results: Peak exercise power output and VO2 were significantly lower (30% lower) in breast cancer patients compared to their healthy matched controls. RV and LV EDV index, ESV index, SV index, and cardiac index were all also lower in breast cancer patients vs controls. Conclusion: Submaximal and peak exercise RV and LV ejection fraction in breast cancer patients prior to adjuvant chemotherapy have significant exercise intolerance compared to their healthy age and sex matched controls. This is secondary their reduced RV and LV EDV.

Peak Oxygen Consumption and long-term all-cause mortality in nonsmall cell lung cancer
Jones LW, Watson D, Herndon JE, Eves ND, Haithcock BE, Loewen G, Kohman L.
Cancer 4825-32. 2010.

Purpose: To investiagte the prognostic significance of preoperative VO2 peak for long-term all-cause mortality among operable candidates with nonsmall cell lung cancer (NSCLC). Methods: 398 patients (both men and women but 61% were males) were studied and their VO2 peak was measured on a cycle ergometer. After 3 minutes of resting baseline data, patients began cycling at 20 W and workloads increased 5-20 W/min until volitional exhaustion. VO2 peak was categorized into 3 different groups with absolute values of: < 0.96 L/min, 0.96-1.29 L/min, and then >1.29 L/min. And then relatively into groups of: <13.9 ml/kg/min, 14-17.3 ml/kg/min, and then >17.4 ml/kg/min. Results: There was a 36% lower average compared to age and sex matched data from sedentary individuals. For the entire sample, the mortality rate decreased as VO2 peak was shown to increase in both absolute and relative terms. Median survival length for survival after participating in this exercise testing of each group is: group 1 - (low VO2 peak) was 13.3 months, group 2 – 16.1 months, and then group 3 - (highest VO2 peak) was 28 months. Conclusion: Data suggest a strong association between VO2 peak and all-cause mortality in patients with NSCLC as those who had a higher VO2 peak had a significant reduction in the risk of mortality by 24%. Findings indicate that aerobic training may be a therapeutic implication for NSCLC patients to improve their clinical outcomes.

Cardiovascular function and predictors of exercise capacity in patients with colorectal cancer
Cramer L, Hildebrandt B, Kung T, Wichmann K, Springer J, Doehner W, Sandek A, Valentova M, Stojakovic T, Scharnagl H, Riess H, Anker SD, von Haehling S.
Journal of the American College of Cardiology 1310-9. 2014.

Purpose: To compare colorectal cancer (CRC) (both individuals who have received and have not received chemotherapy) to that of CHF in terms of the pathophysiology of both and then compare those two between that of healthy controls. It was hypothesized that knowing CHF pathophysiology we can better understand the impairment of exercise capacity in patients with CRC, independent of the commencement of chemotherapy. Methods: 50 CRC patients (26 being

treated with chemotherapy and then 24 not receiving chemotherapy) along with 51 CHF patients and 51 controls subjects performed a treadmill exercise test in order to obtain VO2 peak. DEXA, HRV via 24 hour electrocardiographic monitoring. Results: CRC patients had significantly higher resting HR and lower hemoglobin values compared with patients with CHF and controls. Compared with control subjects, LV ejection fraction was significantly reduced in CRC patients and in patients with CHF. LVESV index was increased for both CHF and CRC patients. LV mass was significantly increased in CHF compared with CRC and control groups. Thickened LV posterior wall was observed in CHF and CRC patients compared to health controls. CHF had significantly higher E:E'. CRC and CHF groups showed significant decreases in exercise performance (VO2 peak) and a significantly impaired breathing efficiency and anaerobic threshold. CRC patients who did not receive chemotherapy showed a significantly reduced peak VO2 peak and LV ejection fraction compared with control subjects. CRC patients had a significantly decreased lean mass value in their legs compared to the other two groups. CRC patients had significantly higher HR at rest compared to CHF and controls.Patients with CRC had a reduced exercise capacity compared with age matched controls. There were statistically significant reductions in LVEF as well as all markers of HRV. HRV was further decreased by the commencement of chemotherapy which was shown by lower peak HR and lower peak VO2 during exercise after chemotherapy initiation. Exercise capacity of CRC is severely impaired compared with controls and this effect is independent of chemotherapy. There were also decreases in sympathetic and parasympathetic function in CRC patients but high frequency was within normal range for those who did not receive chemotherapy. Thus suggestion that impairment of parasympathetic system occurs in early stages of chemotherapy. Chemotherapy patients had a decreased max HR during exercise compared to those without treatment. Conclusion: Exercise capacity in patients with CRC is significantly reduced. This held true for patients who did not undergo chemotherapy but worsened once chemotherapy was initiated.

Cardiorespiratory and neuromuscular deconditioning in fatigued and non-fatigued breast cancer survivors
Neil SE, Klika RJ, Garland SJ, McKenzie DC, Campbell KL.
Supportive Care in Cancer 873-81. 2012.

Purpose: Compare cardiorespiratory and neuromuscular deconditioning between breast cancer survivors who have cancer-related fatigue vs breast cancer survivors without cancer-related fatigue. It was hypothesized that the fatigued group would have a lower lactate threshold, lower VO2 peak, and greater decline in voluntary activation compared to the non-fatigued group. Methods: 16 breast cancer survivors with fatigue and 11 breast cancer survivors without fatigue were studied. Test day 1 consisted of the subjects undergoing a maximal incremental cycle ergometer test: 5 min warm up and then an increase of 20 W per stage (15 W for those less fit and 25W/stage for those deemed more fit). Stages were 3 minutes long and part two of the test began once blood lactate increased > 1.0 mmol/L from baseline. Part 2 stages were reduced to 1 min durations. Test day 2 consisted of dynamometer with right leg knee flexion. Subjects performed 3-s isometric MVC of quads and then a muscle fatiguing protocol of sustained isometric contractions at 30% MVC until volitional exhaustion. Results: Power at lactate threshold was significantly lower in the fatigued group. Absolute VO2 peak was also lower in the fatigued group compared to the non-fatigued. Non-fatigued group had a significantly lower voluntary activation (central fatigue) before and after sustained contraction than in the fatigue

group. Conclusion: Breast cancer survivors with persistent fatigue after treatment experience more cardiorespiratory deconditioning compared to non-fatigued breast cancer survivors.

Inorganic nitrate is a possible source for systemic generation of nitric oxide
Lundberg JO, Govoni M.
Free Radical Biology and Medicine 395-400. 2004.

Purpose: To study if ingested inorganic nitrate would result in increased plasma levels of nitrite and other related nitrogen oxides. Methods: 9 healthy subjects (5 male, 4 female) participated in the study in which they came to the lab in a fasted state and ingested 10 mg/kg of sodium nitrate in 100 ml of water. Blood was sampled prior to ingestion along with time points of 15, 30 60, and 90 min after ingestion. Saliva was collected at those same time points and urine was collected prior to and then at 1, 2, and 3 hours after ingestion. Four subjects also participated in a sub-group test where they were not allowed to swallow during the first hour after nitrate ingestion. Results: At 30 min after ingestion, salivary nitrate, nitrite, and S-nitrosothiols had all increased. Plasma nitrite increased at 15 min time point and continued to increase through the entire study duration. Conclusion: Plasma levels of nitrite increased for a sustained period of time after ingestion of nitrate and plasma S-nitrosothiols remained unchanged. The increase in plasma nitrite was abolished if the subject did not swallow thus indicating that saliva is a major source of plasma nitrite. Nitrite is a substrate for NO synthase-independent NO generation from nitrate. A reverse pathway for regeneration of NO from nitrate has been shown here.

Dietary nitrate supplementation reduced the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans
Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM.
Journal of Applied Physiology 1144-55. 2009.

Purpose: Authors hypothesized that BR supplementation would reduce the O2 cost of moderate-intensity exercise and increase exercise tolerance during severe-intensity exercise as measured by the time to task failure for a cycle ergometer test. Methods: 8 healthy males were in the study that was a random, cross-over, placebo-controlled study with BRJ and blackcurrant juice with a 10 day washout period. This was over the course of 6 day supplementation and with the ingestion of 500 ml/day. Cycle ergometer test was 20 W baseline to moderate and severe-intensity work rates with subjects completing 2 moderate bouts of exercise of day 4 and then one moderate and one intense exercise on days 5 and 6. Results: BR ingestion significantly reduced SBP by 6 mmHg across the study time period. Deoxyhemoglobin concentration amplitude was reduced follow BR suggesting the fractional O2 extraction was reduced. A 19% reduction in the amplitude of pulmonary VO2 response vs placebo was observed for the same absolute moderate intensity cycling work rate. Absolute VO2 over final 30 seconds of moderate exercise was significantly lower after BR ingestion. Amplitude of VO2 slow component was significantly smaller following BR supplementation. Exercise tolerance was enhanced as shown by the increased time to task failure (16%). O2 cost was reduced by 20% of a given increment in work rate. VO2 response to severe exercise was increased by 7% and amplitude of subsequent VO2 slow component was reduced by 23%. Conclusion: 3-day of BR supplementation (before testing but 6 total days of ingestion) significantly reduced SBP and the O2 cost of cycling for submaximal work rate and

increased the time to task failure during severe intensity exercise. VO2 was reduced by BR and it may be due in part to less O2 extraction was required consequent to a reduced aerobic energy turnover or muscle energy utilization. Since HR and VE were not significantly different between treatments, this suggests that reduction in VO2 originated in the skeletal muscles and not from alterations in the energetic cost of cardiorespiratory support processes. There was an improved O2 efficiency which may be related to reduction in mitochondrial proton leak or proton pump slippage. O2 consumption reduction may also be due to reduced ATP cost of force production. NO3- supplementation may enhance exercise performance at high-intensities.

Hypoxic modulation of exogenous nitrite-induced vasodilation in humans
Maher AR, Milsom AB, Gunaruwan P, Abozguia K, Ahmed I, Weaver RA, Thomas P, Ashrafian H, Born GV, James PE, Frenneaux MP.
Circulation 670-7. 2007.

Purpose: Authors hypothesized that nitrites would have a greater dilator effect in capacitance than resistance vessels because of lower O2 tension and that resistance vessel diation should become more pronounced during hypoxemia. While subjects were breathing room air (group A) nitrite would be a potent dilator of capacitance vessels and when subjects were breathing 12% O2 (group B) nitrite would be a potent arteriolar dilator. Methods: 40 health subjects were recruited and split into two groups, group A (24 subjects) under normoxic conditions and then group B (14 subjects) under hypoxemic conditions. Forearm venous volume was measured along with FBFR which is a ratio of infused arm: control arm. BP cuffs were inflated over both upper arms at pressures of 0, 10, 20, and 30 mmHg. In 7 subjects for group B 7.84 umol/min of nitrite was infused via intrabrachial. The other 7 subjects from group B were given 314 nmol/min. Results: Doses of nitrite at 784 nmol/min, 3.14 umol/min, and 7.84 umol/min showed large increases in forearm vascular tone. Only 3.14 and 7.84 umol/min were noticeable increases in FBFR. At the peak dose, a 40% venodilation was observed. Conclusion: Hypoxia significantly enhanced the increase in FBFR during nitrite infusion. Hypoxia enhanced nitrite-induced relaxation in both vessel types compared with relaxation under normoxia however, veins had a greater proportional relaxation than arteries. Hypoxia is a prime determinant for the response to exogenous nitrite as both vessel types responded profoundly to hypoxic conditions. Significant augmentation in FBFR during hypoxia was seen with low-dose (314 nmol/min) nitrite which implies a physiological role under hypoxic conditions.

Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise
Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, Jones AM.
American Journal of Applied Physiology 1121-31. 2010.

Purpose: It was hypothesized that 1) the plasma concentration would be elevated and BP reduced during one BR supplementation after 2.5 hours of ingestion (0.5 L) and then again after 5 and 15 days of supplementation at 0.5 L/day. 2) the O2 cost of moderate-intensity exercise would be reduced 2.5 hours after the first dose of BR and then again 5 and 15 days after. 3) VO2 max and the GET would not be affected 2.5 hours after the first dose but would be increased after 5 and 15 days of supplementation. Methods: 8 healthy subjects (3 female) were enrolled in the study. They

were physically active but not highly trained. They participated in the study which included cycling on a cycle ergometer with 3 min unloaded pedaling and then the work rate increased 30 W/min until volitional exhaustion. Subjects performed a two-step transition from 20 W baseline to moderate-intensity cycling at 90% GET with each bout lasting 5 minutes and bouts separated by 10 mins. After obtaining this data, subjects were randomly assigned to either a BRJ or PL group (blackcurrant juice) of 0.5 L/day and it was a crossover design with a 15 day supplementation period with 10-day washout period. Testing were on days 2, 5, 8, 12, and 15 to measure NO2- and BP and exercise testing were on days 5 and 15. Results: Nitrite concentration was elevated by 36% at 2.5 hours after ingestion and was highest on day 12 (59% increase) and day 15 (46% increase). SBP was significantly lowered after BR during all time points except day 5. MAP was significantly lowered after BR all time points except day 8. VO2 at end exercise was significantly lowered after BR during all time points. Amplitude of VO2 response was significantly lower after BR compared to PL at all time points. Reduction of O2 cost (functional gain; increase in VO2 relative increase in external work) was significantly lower after BR from supplementation from baseline at all time points. A 5% reduction in steady state VO2 was observed during moderate intensity exercise. VO2 max had a significant increase at days 5 and 15 after BR. Peak power output was significantly higher in first dose (2.5 hours) and after 15 days of BR. S1 slope of delta VO2/delta work rate was significantly reduced after 15 days of BR compared to baseline and 15 days PL. SBP was significantly reduced and was most pronounced after 12 days of supplementation. After 15 days of BRJ, peak power output and work rate associated with GET were higher than baseline and PL. Relative to baseline VO2 max was increased ~140 ml/min after 15 days of nitrate supplementation. Conclusion: Lower O2 cost of moderate intensity exercise was shown after one dose of BRJ and then was maintained through a 15 day supplementation period. BR supplementation in young adults ~0.07 mmol/kg of nitrate resulted in a sustained reduction in SBP and DBP. The O2 cost of moderate-intensity exercise was significantly reduced after one dose of 0.5 L and these effects were maintained after 5 and 15 days of continued supplementation. Mechanisms that may contribute to increased VO2 max after 15-day BR include; 1) NO-mediated changes in local perfusion to skeletal muscle and possible effects on Q. 2) Could be linked to increased mitochondrial mass as a consequence of elevated NO availability. 3) Chronic exposure of mammalian cells to NO result in cGMP mediated activation of regulatory protein sirtuin (SIRT1) which upregulated transcriptional factors and nuclear respiratory factors involved in coordination of mitochondrial fusion and fission events.

Beetroot juice and exercise: pharmacodynamic and dose-response relationships
Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE, Vanhatalo A, Jones AM.
Journal of Applied Physiology 325-36. 2013.

Purpose: Investigate the effects of acute NO3- doses of 4.2, 8.4, and 16.8 mmol of concentrated beet root juice consumed in either 70, 140, or 280 ml, respectively. Measurements being looked at were blood pressure over a 24 hour period and the effects on plasma nitrite. The physiological responses to step transitions to moderate-severe-intensity exercise, as well as determine the peak time response to each dose. Methods: Ten health, young males volunteered for the study which consisted of cycle ergometer exercise testing either on one of the three doses of BR or on a nitrate depleted placebo. All subjects were given a standardized breakfast and venous blood samples were taken 1, 2, 4, 8, 12, and 24 hours after ingestion of each beverage. Subjects were divided into two

groups with one being tested for pharmacodynamics of BR response and the other for dose response. Peak VO2 was determined from a ramp incremental test. During cycle ergometer tests, work rate was increased by 30 W/min until volitional exhaustion. Results: Peak elevation in plasma nitrite occurred 2 hours after ingestion of 8.4 mmol of nitrate. SBP was significantly lowered, compared to baseline, after all treatments with peak SBP decrease occurring 4 hours after post ingestion. This changes was correlated with the changes seen in plasma nitrite levels. Diastolic BP was also significantly reduced for 140 and 280 ml of BR consumption. These changes were also correlated with the changes seen in plasma nitrite levels. MAP was significantly decreased between all three doses of BR. There was a significant dose-dependent increase in plasma nitrate following BR after 2.5 hours. End exercise VO2 was significantly lowered after 280 ml of BR and there was a trend towards significant reduction for 140 ml of BR ingestion. VCO2 at baseline was increased significantly for all three BR doses. End exercise VCO2 was significantly higher for all three doses of BR. RER increased as the dose increased, this may be indicative of the sugars within the beverage of BR. VO2 and RER measured at task failure were not altered by dose or by treatment. BR doses of 140 and 280 ml showed an increase in time to task failure compared to their placebo counterparts. Conclusion: BR doses of 140 and 280 ml resulted in an increased time to task failure during severe intensity exercise. BR consumption may help maintain or improve vascular health in young adults as studies suggest that SBP lowering by 10 mmHg may reduce the risk of ischemic heart disease by 25% and the risk of stroke by 35%.

Chapter 2 - Introduction

Advances in cancer therapy coupled with early detection strategies have contributed to the continual improvement in cancer specific mortality for many cancers including breast, lymphoma, prostate, and gastrointestinal cancers. However, in the years following anticancer therapy treatment, cancer survivors are at an increased risk of both cancer therapy and age-related pathological outcomes; primary cardiovascular disease, exercise intolerance, and cancer-related fatigue (Yeh, Tong et al. (2004). Unfortunately, there remains a paucity of pharmacological therapies that target these adverse outcomes (Shelburne, Adhikari et al. 2014).

Exercise intolerance has consistently been reported in cancer survivors treated with neoplastic anticancer therapies. A recent cross-sectional cohort study of breast cancer patients, conducted by Jones et al. (2012), evaluated the effects of anticancer chemotherapy on cardiopulmonary exercise capacity before, during, and after chemotherapy administration (Jones, Courneya et al. 2012). They reported that breast cancer patients had significant impairment of their cardiopulmonary exercise capacity, assessed via peak oxygen uptake (peak VO_2), across the entire continuum of the disease with the most adverse impairments observed during chemotherapy administration and when the disease was metastatic. Additionally, this study reported that over 30% of these breast cancer patients had a peak aerobic exercise capacity that was below what is classified for functional independence (≤15.4 ml/kg/min). Furthermore, these pathological indices were also observed after the secession of anticancer therapy and into recovery. Additionally in a separate study, Jones et al. (2007) reported that breast cancer

survivors who have undergone anticancer therapy continue to have a significant reduction in their cardiopulmonary function into survivorship (Jones, Haykowsky et al. 2007). These impairments were seen in the cancer survivors who had the worst cardiovascular disease (CVD) risk profile, suggesting the more CVD risk factors a patient has, the stronger the association with cardiopulmonary function impairments. Furthermore, the investigators found that in addition to decreases in peak aerobic exercise capacity, breast cancer survivors treated with anticancer therapies have a significantly lower peak exercise stroke volume, cardiac output, and cardiac power output compared to their healthy age-matched controls. In agreement with this work, we have recently identified decreases in cardiac function in cancer survivors following anticancer therapy treatment (Manuscript: Lovoy et al. 2018) as well as decreases in microvascular function (Sutterfield, Caldwell et al. 2018). Furthermore, we have reported that cancer survivors have an impaired exercise blood flow and microvascular oxygenation response compared to non-treated controls, which, in addition to central limitations, may alter the balance between aerobic oxidative phosphorylation and anaerobic glycolysis within the contracting muscle. (Didier, Ederer et al. 2017). These vascular abnormalities, coupled with reductions in myocardial function, may therefore result in an integrative-dependent decrease in both convective and diffusive oxygen (O_2) transport, predisposing to earlier exhaustion.

Previous work has demonstrated that dietary nitrate (NO_3^-) supplementation, through the ingestion of beet root juice (BR) or sodium nitrate, has positive effects on vascular regulation such as lowering blood pressure (Kapil, Weitzberg et al. 2014) as well as improving the oxidative capacity in young adults (Bailey, Winyard et al. 2009) (Bailey, Fulford et al. 2010) (Vanhatalo, Bailey et al. 2010) (Lansley, Winyard et al. 2011), older adults (Kelly, Fulford et al. 2013), and in heart failure patients (Zamani, Rawat et al. 2015) (Eggebeen, Kim-Shapiro et al.

2016). Furthermore, NO_3^- supplementation can increase exercise tolerance (Larsen, Weitzberg et al. 2010) (Bailey, Fulford et al. 2010). Thus, implementing a therapeutic intervention during or following anticancer therapy that includes NO_3^- as treatment may allow cancer patients suffering from cardiopulmonary impairments to have an increased quality of life (Eggebeen, Kim-Shapiro et al. 2016). Additionally, we have recently demonstrated that 7-days of NO_3^- supplementation improved diastolic function in cancer survivors through a significant increase in septal wall early diastolic velocity, strain rate during early diastolic filling, mitral valve late diastolic filling, and left ventricular longitudinal strain rate in early filling compared to placebo conditions (Manuscript: Lovoy et al. 2018). However, there remains a significant gap in knowledge for understanding the potential therapeutic use of NO_3^- supplementation for improving parameters of exercise tolerance in cancer survivors with a history of anticancer therapy. Therefore, in this proof-of-concept trial, we hypothesized that administration of a single, acute-dose of NO_3^- supplementation will improve exercise capacity and exercise efficiency in cancer survivors.

Chapter 3 - Methods

Participants

All procedures were approved by the Kansas State University Institutional Review Board for research involving human subjects; and all standards conformed to the Declaration of Helsinki. Patients were eligible for inclusion in the study if they were at least 18 years of age, had a history of cancer, and received chemotherapy as part of their treatment. All patients must have been diagnosed as cancer-free at the time of the study. Patients were excluded from the study if they had any of the following: were diagnosed with overt cardiovascular disease, diabetes, kidney

diseases, high risk of kidney stones, hemochromatosis, or were a current smoker. Patients were instructed to take all regularly prescribed medications at their normally scheduled times. If patients were taking antioxidant supplements (e.g., fish oil) they were asked to refrain from taking these upon enrollment in the study and through the 7-day duration of the study. Patients were also asked to refrain from using antibacterial mouthwash and/or chewing gum on their exercise test days, as each have been shown to attenuate the reduction of NO_3^- to nitrite (NO_2^-), which is essential in the nitrate-nitrite-nitric oxide pathway within the entero-salivary pathway (Govoni, Jansson et al. 2008). The pathways of nitric oxide (NO) production including the reduction pathway of nitrate to nitrite to nitric oxide is depicted in **Figure 1** (Jones, Thompson et al. 2018).

Experimental Procedures

All patients had three separate visits to the laboratory with the first visit being a familiarization day. During the familiarization day, patients completed the informed consent, health history questionnaire, physical activity questionnaire, and a timeline sheet that outlined their cancer diagnosis and chemotherapy treatment history. Upon completion of paperwork, patients were then familiarized with the exercise protocol that was performed on a semi-recumbent cycle ergometer. The overall study consisted of a two-day randomized, double blind, placebo-controlled crossover design, in which patients were assigned to either consume a nitrate-rich supplement in the form of a specifically formulated beetroot juice, or a nitrate-poor placebo in the form of blackcurrant juice. Patients then entered a 7-day washout period before entering the opposing arm of the study. Two-hours prior to each of the two testing sessions, patients orally consumed either 140 ml of concentrated nitrate-rich [NO_3^-] beetroot juice supplement (BRJ;

BEET IT Sport, James White Drinks Ltd, Ipswich, UK) containing [12.2 mmol NO_3^-], or 140 ml nitrate-depleted placebo supplement (PL; Blackcurrant juice with negligible NO_3^- content). The BRJ dose of 140 ml [12.2 mmol NO_3^-] was chosen for this experiment as it has previously been shown to be the most effective for lowering submaximal steady-state oxygen consumption and improving time to task failure in young, healthy adults when compared to dosages of 70 and 280 ml (Wylie, Kelly et al. 2013). Additionally, the oral consumption of either treatment two-hours prior to exercise testing was chosen as previous work has demonstrated that peak plasma NO_2^- levels have been observed 2-2.5 hours post-ingestion of BRJ (Wylie, Kelly et al. 2013). Patients were instructed to refrain from exercise at least 12 hours prior to testing as well as refraining from the consumption/usage of mouthwash, chewing gum, alcohol, or any type of food at least three hours prior to their scheduled laboratory visit time during their testing days.

Measurements

Plasma Nitrite and Complete Blood Count

Upon arrival to the laboratory, trained phlebotomists drew blood samples from the patients into lithium heparin vacutainers (Becton Dickenson, NJ) for measurement of plasma NO_2^- levels. Immediately following venous blood draws, the blood samples were centrifuged for 10 minutes at 3300 RPM, plasma was then collected and immediately frozen in a –80° C freezer for later NO_2^- analysis. Measurements of [NO_{2-}] were performed within 30 min after samples had thawed via chemiluminescence with an Ionic/Sievers NO analyzer (NOA 280i, GE, Boulder, CO) in triplicate after instrument calibration (Ferguson, Holdsworth et al. 2016). Additional venous

blood samples were drawn for a complete blood count (CBC) test to determine blood hemoglobin and hematocrit values. CBC tests were completed by Clinical Laboratory Scientists in Lafene Health Center at Kansas State University and results were collected once exercise testing was complete.

Cardiac and Arterial Blood Pressure Measurements

Transthoracic echocardiography was performed at rest by an experienced sonographer, according to the standards of the American Society of Echocardiography using a commercially available system (Vivid S6 BT12; GE Healthcare) with a 1.5 to 4.3-mHz phased array transducer. Briefly, the left ventricular outflow tract (LVOT) diameter was obtained from the parasternal long axis and used to calculate LVOT cross-sectional area. Doppler velocity-time integral (VTI) of the LVOT was obtained from the apical 5-chamber view. Doppler VTI measures the distance blood travels during a period of flow. Measurements were performed over a 10 second period and used to calculate stroke volume (SV) and cardiac output (CO).

$$SV = \pi \times \frac{LVOT^2}{2} \times LVOT\ VTI$$

$$CO = \frac{SV \times HR}{1000}$$

Continuous beat-by-beat blood pressure measurements of systolic, diastolic, and mean arterial pressures were obtained by calibrated finger photoplethysmography (Finometer Pro; Finapress

Medical Systems, Amsterdam, The Netherlands). All blood pressure measurements were performed and monitored after the patients were positioned on the semi-recumbent cycle ergometer at rest and throughout both exercise tests. During exercise, cardiac output (Q) was calculated as heart rate (HR) × stroke volume (SV), with SV calculated using the ModelFlow method (Finometer Pro; Finapress Medical Systems) after correction for age, sex, body mass, and stature. These were corrected at rest using the echocardiography derived measurements of SV, which was time aligned to the arterial pressure recordings. Systemic vascular resistance (SVR) was calculated as the quotient of MAP and Q. The Finometer Pro has previously been validated to evaluate MAP, Q, and SVR responses in younger and older populations (Schutte, Huisman et al. 2004). Arterial oxygen saturation was measured via pulse oximeter on the finger (Datex Ohmeda S5 Light Monitor; GE Health Care, Milwaukee, WI).

Constant-Intensity Cardiopulmonary Exercise Test

Upon completion of the echocardiographic examination, each patient performed a constant-intensity cardiopulmonary exercise test on a hyperbolic Lode semi-recumbent cycle ergometer. Following a 2-min resting baseline, subjects pedaled at a self-selected range of 40–55 rpm for 6 minutes at a constant workload of 30 Watts. Pedal rate along with leg length adjustment for the cycle ergometer were recorded and reproduced in subsequent tests in order to remain consistent with previous work (Wylie, Kelly et al. 2013). Breath-by-breath pulmonary ventilation and gas exchange data were continuously measured (Ultima CPX, Medical Graphics Corp., MN, USA). Steady-state oxygen uptake (VO_2) was defined as the average VO_2 during the final 60 seconds of exercise (Balady, Arena et al. 2010).

Peak Cardiopulmonary Exercise Test

Approximately 5 minutes after the constant-intensity cardiopulmonary exercise test, all patients performed a maximal exertion-limited incremental exercise test on the same semi-recumbent cycle ergometer using a ramp protocol. For five of the six patients, the ramp exercise protocol followed a computer programmed linear increase of 10 W/min as to maintain consistency with similar investigations (Eggebeen, Kim-Shapiro et al. 2016). One patient was an avid cyclist; thus, we altered the ramp increase to 15 W/min as recommended by American College of Sports Medicine (American College of Sports Medicine 2012). All ramp incremental tests were performed until volitional exhaustion. The exercise test was terminated if any of the American Heart Association absolute indications for test termination were met (Gibbons, Balady et al. 1997). These included ST-segment elevation (1.0 mm) in leads without preexisting Q waves because of prior myocardial infarction, drop in systolic blood pressure > 10 mmHg; despite an increase in workload, when accompanied by any other evidence of ischemia, moderate-to-severe angina, central nervous system symptoms (ataxia, dizziness, near syncope), signs of poor perfusion (cyanosis or pallor), sustained ventricular tachycardia (VT) or other arrhythmia, including second or third-degree atrioventricular (AV) block; that interferes with normal maintenance of cardiac output during exercise, technical difficulties in monitoring the ECG or systolic blood pressure, the patient's request to stop, if there was an exaggerated blood pressure response (systolic blood pressure >250 mmHg or diastolic blood pressure >115 mmHg), or if pedaling rate fell at least 10 rpm below the initial self-selected rate as followed by previous studies (Wylie, Kelly et al. 2013) (Eggebeen, Kim-Shapiro et al. 2016). Breath-by-breath pulmonary ventilation and gas exchange data were continuously measured, with peak VO_2

determined as the average value during the final 30 seconds of exercise. Maximal effort was confirmed by attainment of at least three criteria: (1) unable to maintain cadence; (2) a respiratory exchange ratio >1.1; (3) heart rate >90% of age-predicted maximum; (4) a plateau of $\dot{V}O_2$ defined as no expected increases (<150 ml min^{-1}) in $\dot{V}O_2$ from the previous test stage.

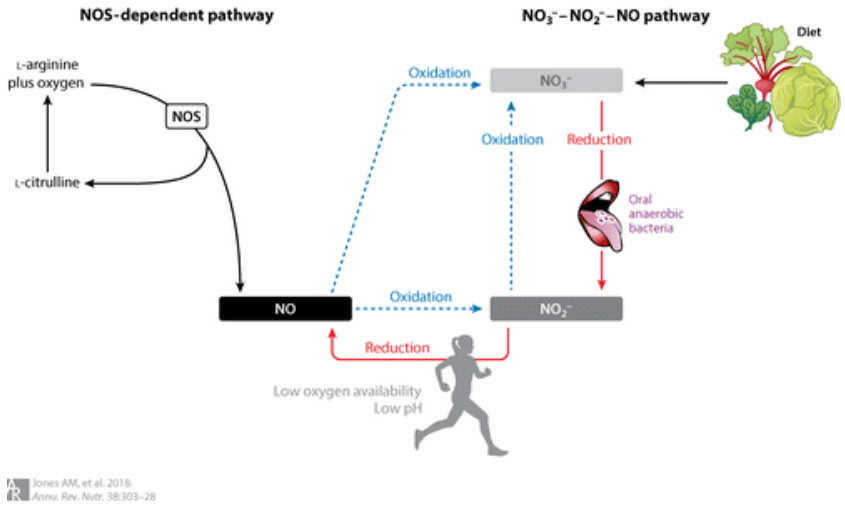

Figure 1 - Schematic of pathways leading to the production of nitric oxide (NO) within the human body including the nitrate (NO_3^-) – nitrite (NO_2^-) –nitric oxide (NO) reduction pathway. (Jones, Thompson et al. 2018).

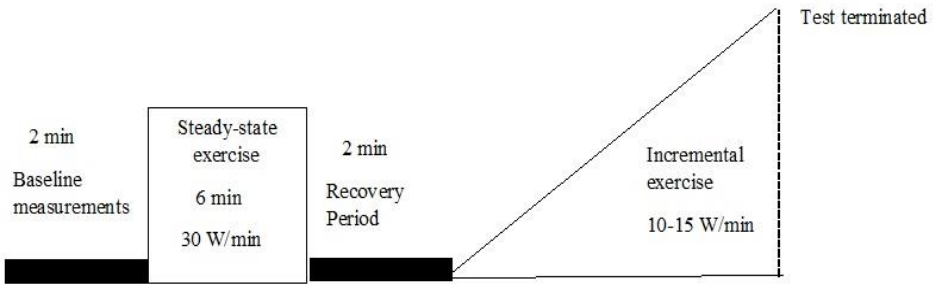

Figure 2 - Schematic illustration of exercise test protocol depicting both steady-state exercise followed by incremental exercise testing.

Statistical Analysis

Data were analyzed with commercially available statistical software package (Sigmaplot; version 12.5, Systat software, San Jose). All continuous variables are reported as mean ± SD unless otherwise stated. Study outcomes between the BR and PL measurements were compared by use of the paired t-test. Differences were considered statistically significant when $p \leq 0.05$. Given the sample size and need to detect the smallest meaningful physiological differences, effect size comparisons were also made via Cohen's d with threshold values for small, moderate, and large effects as 0.2, 0.5, >0.8 respectively.

Chapter 4 - Results

A total of sixteen cancer survivors were screened from previous study participant lists with eight initially interested in completing the resting echocardiograph and the cardiopulmonary exercise tests. Two patients were excluded from the peak cardiopulmonary test due to one having hip

surgery, and another patient having adverse health concerns consisting primarily of a history of atrial fibrillation. Thus, six patients completed all tests and were included in our final analyses.

Study Participants

Six older adults (4 female and 2 male; n=4 breast cancer, n=2 lymphoma) who were cancer survivors with a history of anticancer therapy treatment completed the study. The mean age of the patients was 57 ± 11 years, height 169.5 ± 9.14 cm, weight 80.78 ± 27.23 kg as shown in **Table 1**. At the date of enrollment, all patients were diagnosed as cancer free for an average of 4.83 ± 4.75 years, as indicated by the date of their last anticancer therapy treatment.

Oral ingestion of NO_3^- supplementation, through consumption of BRJ, significantly increased plasma nitrite levels (1300 ± 963 µM) compared to the nitrate-poor placebo supplementation (111 ± 49 µM; P = 0.02). This data suggests that there was successful reduction of NO_3^- to NO_2^-, which is consistent with both our previous findings (Manuscript: Lovoy et al 2018) and the reports of others (Wylie, Kelly et al. 2013) (Vanhatalo, Bailey et al. 2010) (Eggebeen, Kim-Shapiro et al. 2016) (Kelly, Fulford et al. 2013) (Larsen, Weitzberg et al. 2010) (Larsen, Weitzberg et al. 2007) (Bescos, Rodriguez et al. 2011) (Kapil, Khambata et al. 2015) (Zamani, Rawat et al. 2015).

Constant Intensity Exercise Protocol

While under the BR condition, we observed significantly decreased relative **(Figure 3)** and absolute VO_2 values during steady-state exercise compared to PL (Relative VO_2: BRJ 8.46 ± 2.24 vs. PL: 8.98 ± 2.40 ml/kg/min; p = 0.01; (Absolute VO_2: BRJ 0.64 ± 0.10 vs. PL: 0.68 ± 0.11 L/min; p = 0.01). No differences were observed for VCO_2 during constant-intensity exercise during BRJ (0.60 ± 0.07 L/min) compared to PL conditions (0.61 ± 0.094 L/min; p = 0.36) or respiratory exchange ratio (RER) (BRJ: 0.94 ± 0.08 vs. PL: 0.90 ± 0.075; p = 0.12). Cardiovascular measurements during steady-state exercise were not different between conditions including, HR (BRJ: 87 ± 8 vs. PL: 88 ± 8 bpm; p = 0.33), cardiac output (BRJ: 7.7 ± 2.9 vs. PL: 8.7 ± 4.1 L/min, p = 0.15), SV (BRJ: 87.4 ± 27.7 vs. PL: 96.3 ± 40.5 ml/beat; p = 0.18), systolic blood pressure (BRJ: 149 ± 10 vs. PL: 153 ± 13 mmHg; p = 0.23), diastolic blood pressure (BRJ: 84 ± 9 vs. PL: 83 ± 9 mmHg; p = 0.44), left ventricular ejection time (BRJ: 329 ± 24 vs. PL: 333 ± 16 milliseconds; p = 0.26), or rate pressure product (BRJ 13049 ± 1659 vs. PL 13491 ± 1803 beats/min mmHg; p = 0.21).

Peak Cardiopulmonary Exercise Test

We did not find any significant difference between BRJ and PL conditions during the incremental exercise test for relative VO_2 (BRJ: 22.4 ± 3.9 vs. PL: 23.1 ± 4.0 ml/kg/min; p = 0.23) or absolute VO_2 (BRJ: 1.77 ± 0.50 vs. PL: 1.86 ± 0.68 L/min; p = 0.20) **(Figure 4A)**. This lack of difference between conditions held true for exercise time (BRJ: 14.8 ± 4.0 vs. PL: 14.9 ± 4.3 minutes; p = 0.31), total work done (BRJ: 70.64 ± 29.5 vs. PL: 70.67 ± 30.71 kJ; p = 0.49) **(Figure 4B)** and gross exercise efficiency (BRJ: 5.23 ± 1.48 vs. PL: 4.97 ± 1.41 kJ/L O_2; p = 0.14) **(Figure 4C)**. Similarly, we did not observe any differences between conditions for VCO_2

(BRJ: 2.17 ± 0.57 vs. PL: 2.22 ± 0.77 L/min; p = 0.36), RER (BRJ: 1.23 ± 0.09 vs. PL: 1.22 ± 0.12; p = 0.37), HR (BRJ: 135 ± 22 vs. PL: 139 ± 38 bpm; p = 0.36), or cardiac output (BRJ:12.3 ± 5.8 vs. PL: 14.2 ± 7.8 L/min; p = 0.18). It is noteworthy to state that our cardiopulmonary function exercise tests were assessed at peak VO_2 as recommended for clinical populations as opposed to VO_2 max tests (American Thoracic and American College of Chest 2003).

Table 1 - Patient characteristics. Values are average ± SD or number of patients (%); n = 5 for values.

Table 1. Patient Characteristics

Variable	
Age, mean (SD), y	57 (10.8)
Male, n (%)	2 (33)
Height, mean (SD), m	169.5 (9.1)
Weight, mean (SD), kg	80.8 (27.2)
Body mass index, mean (SD), kg/m^2	28.2 (9.9)
Current smoker, n (%)	0 (0)
Hypertension, n (%)	1 (20)
Diabetes, n (%)	0 (0)
Drug Therapy, n (%)	
B-Blocker	0 (0)
ACE inhibitor/ARB	1 (20)
Calcium channel blocker	0 (0)
Statin	0 (0)
Diuretic	0 (0)
Aspirin	0 (0)
Cancer Type, n (%)	
Breast	4 (67)
Lymphoma	2 (33)
Prostate	0
Chemotherapy, n (%)	
Alkylating Agent	6 (100)
Anthracycline	2 (33)
Antimicrotubule	5 (83)
Aromatase Inhibitor	0
Monoclonal Antibody	5 (83)
Radiation, n (%)	3 (50)

ACE indicates angiotensin-converting enzyme;
ARB, angiotensin receptor blocker;

Table 2 - Pulmonary VO_2 during constant intensity and incremental (peak) exercise intensity following dietary supplementation with either placebo (PL) or nitrate (BRJ; beetroot juice).

Table 2. Effect of NO_3^- Versus Placebo

	Effect of Placebo	Effect of BRJ	p Value
Constant Intensity Cardiopulmonary Exercise Test			
VO_2, ml·kg^{-1}·min^{-1}	8.98 (2.4)	8.46 (2.24)	0.01
VO_2, ml·min^{-1}	680 (113)	640 (99)	0.01
VCO_2, ml·min^{-1}	610 (94)	600 (70)	0.36
Respiratory exchange ratio	0.90 (.07)	0.94 (0.08)	0.12
Heart rate, beats·min^{-1}	88 (8)	86 (11)	0.15
Cardiac output, L·min^{-1}	8.7 (4.1)	7.7 (2.9)	0.15
Stroke volume, ml	96 (41)	87 (28)	0.18
SBP, mmHg	153 (13)	149 (10)	0.23
DBP, mmHg	83 (9)	84 (9)	0.44
Left Ventricular Ejection Time, ms	333 (16)	329 (24)	0.26
Rate pressure product, beats·min^{-1}·mmHg	13491 (1803)	13049 (1659)	0.21
Peak *Cardiopulmonary* Exercise Test			
Exercise time, min	14.9 (4.3)	14.8 (4)	0.31
Total work done, kJ	70.67 (30.71)	70.64 (29.5)	0.49
Total Exercise efficiency, kJ/L O_2	4.97 (1.41)	5.23 (1.48)	0.14
VO_2, ml·kg^{-1}·min^{-1}	23.1 (4.0)	22.4 (3.9)	0.23
VO_2, ml·min^{-1}	1860 (683)	1770 (504)	0.20
VCO_2, ml·min^{-1}	2220 (769)	2170 (568)	0.36
Respiratory exchange ratio	1.22 (0.12)	1.23 (0.09)	0.37
Heart rate, beats·min^{-1}	140 (38)	135 (22)	0.36
Cardiac output, L·min^{-1}	14.3 (7.8)	12.3 (5.8)	0.18
Peak work rate, W	147.2 (39.1)	146 (36.1)	0.34

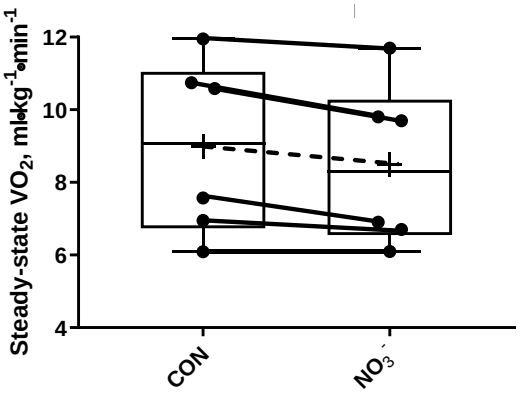

Figure 3 - Steady-state VO_2 following either placebo or nitrate (NO_3^-) supplementation.

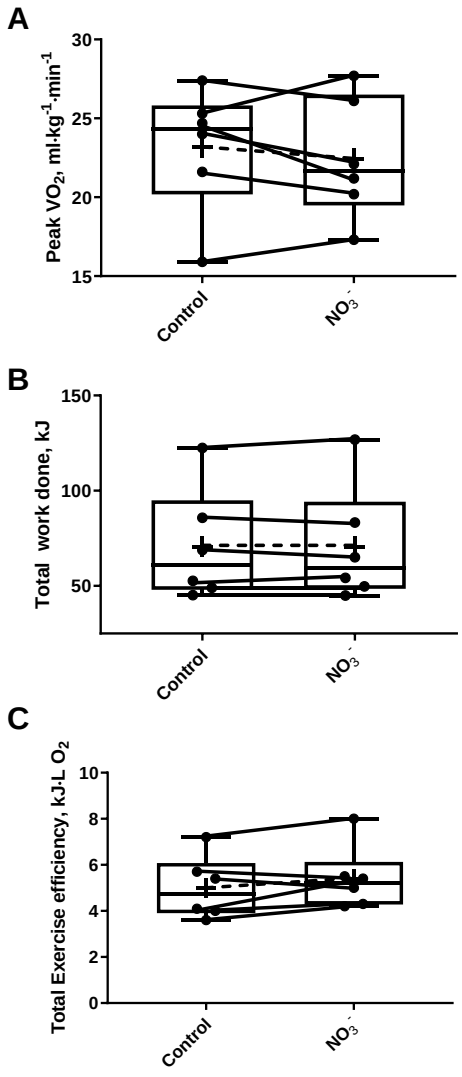

Figure 4 - (A) Peak VO2 following either placebo or nitrate (NO3-) supplementation.
(B) Total work done under either placebo or NO3- supplementation.
(C) Total efficiency under either placebo or NO3- supplementation.

Chapter 5 - Discussion

The primary aim of this study was to investigate the therapeutic effects of inorganic dietary nitrate supplementation on the cardiorespiratory function of cancer survivors with a history of anticancer therapy. We have previously examined the effects of inorganic dietary nitrates on the cardiovascular system through a week long, randomized, double-blind, crossover study in which cancer survivors were supplemented with NO_3^- for a one week duration. We found that compared to the nitrate-depleted placebo condition, cancer survivors who consumed nitrate-rich beetroot juice had a significantly improved left ventricular diastolic function (Manuscript: Lovoy et al. 2018). However, we did not examine cardiopulmonary exercise capacity in this previous study. A critical finding of the present study was that during a single, acute-dose of NO_3^- supplementation, through ingestion of 140 ml of beet-root juice (BRJ), there was a statistically significant decrease in relative and absolute VO_2 during steady state exercise as reported in **Table 2** and **Figure 3**. This is in line with our hypothesis as well as previous studies in healthy populations that have utilized NO_3^- supplementation to observe changes in cardiopulmonary responses (Bailey, Fulford et al. 2010) (Wylie, Kelly et al. 2013) (Vanhatalo, Bailey et al. 2010). However, we did not observe any differences in the submaximal stroke volume or cardiac output for these cancer survivors. Furthermore, we did not observe any significant differences between BRJ and PL conditions for relative or absolute peak VO_2 during the incremental ramp exercise test. Consistent with this finding, we did not observe differences in peak stroke volume, cardiac output, or arterial-venous oxygen difference between conditions. In total, these findings suggest that strategies that target NO bioavailability may provide a means of improving submaximal exercise economy in cancer survivors. The lack of a significant effect for cardiac function during

submaximal and peak exercise suggests that the mechanisms for this improved submaximal exercise efficiency is related to improved peripheral responses, not central cardiac performance.

We found a significant decrease in steady-state VO_2 after a single dose of NO_3^-. This is consistent to what has been observed in younger, healthy subjects, but in contrast to those with heart failure. In agreement with our findings, Lansley et al. (2011) conducted a randomized, double-blind, crossover study in which young, healthy, physically active males were supplemented with 0.5 L/day of BRJ [6.2 mmol NO_3^-] or 0.5 L/day of PL for 6 days. The investigators observed a 14% decrease in steady-state VO_2 during a constant speed (4 km/hour) walking protocol while subjects were under the BRJ condition compared to PL (Lansley, Winyard et al. 2011). Additionally, previous work by Bailey et al. (2009) conducted a randomized, double-blind, crossover study with healthy, young, adult males. The subjects were also supplemented with either 0.5 L/day of BRJ [6.2 mmol NO_3^-] or 0.5 L/day of PL. Exercise testing consisted of a step protocol from low-moderate (80% GET) and moderate-severe intensity exercise on a cycle ergometer. In accordance with our observations, the investigators reported a 19% reduction in the amplitude of the VO_2 response from the step increment of moderate-severe cycling during the BR supplementation compared to PL. A significant reduction in absolute VO_2 was also observed during the final 30 seconds of moderate intensity exercise following BRJ consumption compared to that of PL (Bailey, Winyard et al. 2009). Contrary to our findings as well as those previously presented by Lansley et al. and Bailey et al., work conducted with heart failure patients reported no difference in submaximal steady-state VO_2 between BRJ and PL conditions (Eggebeen, Kim-Shapiro et al. 2016). These findings were

reported for both acute (single dose of NO_3^- through BRJ ingestion) and with one week of NO_3^- supplementation.

We believe that the significant reduction in O_2 cost during steady-state exercise (**Table 2** and **Figure 3**) was primarily due to an increase in peripheral responses, specifically oxidative phosphorylation efficiency. Mitochondrial oxidative phosphorylation efficiency is the amount of O_2 consumed per ATP molecule produced (Hinkle 2005). Initially, NO_3^- is reduced to NO_2^- within the oral cavity where nitrate-reducing bacteria are found (Weitzberg and Lundberg 1998). From here, NO_2^- is further reduced to bioactive nitric oxide (NO) within blood and tissues through several different pathways (Benjamin, O'Driscoll et al. 1994) (Modin, Bjorne et al. 2001) (Millar, Stevens et al. 1998). One reduction pathway is a non-enzymatic reduction of inorganic nitrite to NO which is seen during acidic conditions where tissue pH is reduced to levels seen during hypoxic conditions and ischemia (Modin, Bjorne et al. 2001). Interstitial pH decreases during exercise and gradually continues to decline during graded exercise tests (Street, Bangsbo et al. 2001). This is suggestive that during exercise testing, interstitial pH would decrease in our patients thus causing an acidic environment which would allow plasma NO_2^- to be further reduced to bioactive NO causing peripheral vasodilation. A crucial role for bioactive NO is modulating muscular contractions and increasing blood flow to working muscles (Stamler and Meissner 2001). This dilation allows an increase in blood flow resulting in more O_2 carried to active muscles and the delivery of O_2 from the blood to the active muscle is governed by mitochondrial O_2 consumption (Korthuis 2011). This mechanism may explain why we observed a decrease in steady-state VO_2 for cancer survivors.

From a molecular perspective, mammalian cytochrome c oxidase is the final enzyme in the mitochondrial electron transfer chain (Li, Park et al. 2006) which is part of the inner mitochondrial membrane in eukaryotes (Capaldi 1990). Cytochrome c oxidase has been regarded as a major regulation site for oxidative phosphorylation (Kadenbach, Huttemann et al. 2000). In the rat model, it has been shown that NO induces a kinetic constraint on cytochrome oxidase which decreases energy waste and increases oxidative phosphorylation efficiency (Clerc, Rigoulet et al. 2007). This mechanism works by NO binding to cytochrome c oxidase leading to partial mitochondrial respiration inhibition (Brown and Cooper 1994). These works suggest that NO is a physiological regulator of cytochrome oxidase and thus can cause inhibition of the enzyme which leads to an increase in oxidative phosphorylation.

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Mechanistically, we believe our results indicate that nitrate supplementation through a single, acute dose of beetroot juice, enhanced mitochondrial efficiency within the cancer survivor population. Reactive oxygen species (ROS) targets mitochondrial DNA that encodes several proteins essential for the function of the mitochondrial respiratory chain (Gogvadze, Orrenius et al. 2008). Additionally, most anticancer therapies elicit increases of ROS to damage cancer cells and consequently healthy tissue. This has been shown to induce mitochondrial DNA damage and impair mitochondrial function (Tocchetti, Cadeddu et al. 2019). This can lead to proton leakage in the electron transport chain which, in turn, leads to a reduction in the production of ATP. Work by Larsen et al. (2011) found that NO_3^- supplementation [0.1 mmol/kg/day for 3 days] increased the respiratory control ratio, the ratio of respiration with amounts of ADP and respiration with amounts of substrates when ADP has been phosphorylated to ATP, compared to PL (NO_3^- 8.5 ± 0.7 vs. PL 6.5 ± 0.7) (Larsen, Schiffer et al. 2011). Additionally, they found that

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there was an increased amount of ATP produced as well as a reduction in the amount of oxygen consumed under NO_3^- supplementation compared to placebo. This lead to an increase in the P/O ratio, which is indicative of mitochondrial efficiency — further indicating that NO_3^- supplementation enhances oxidative phosphorylation efficiency and reduces VO_2 during steady-state exercise.

In the present study, we saw no effect of NO_3^- supplementation on submaximal cardiac function or arterial blood pressure. This is contrary to our previous work in cancer survivors treated with anticancer therapy in whom we observed a significant increase for resting parameters of left ventricular strain rate in early filling, early mitral septal wall annular velocity, and mitral A-wave velocity (Manuscript: Lovoy et al. 2018). Increases in these cardiovascular measurements are indicative of improved left ventricular diastolic function following BRJ supplementation compared to PL but do not appear to have translated to improved cardiac performance during exercise. However, the lack of difference in blood pressure between conditions is consistent with that seen in aforementioned work by Lansley et al. (2011) for young, healthy males (Lansley, Winyard et al. 2011). In contrast to our findings and those of Lansley et al., previous studies have reported a decrease in blood pressure after BRJ supplementation compared to PL for young, healthy (Bailey, Fulford et al. 2010) (Vanhatalo, Bailey et al. 2010) (Wylie, Kelly et al. 2013), older, healthy (Kelly, Fulford et al. 2013), and heart failure patients (Kapil, Khambata et al. 2015) (Eggebeen, Kim-Shapiro et al. 2016).

We did not observe a change in peak VO_2 or total work performed during the peak cardiopulmonary exercise test following NO_3^- supplementation. In contrast to our findings, the

aforementioned work by Lansley et al. (2011) reported a decrease in peak VO_2 for young, healthy adults following a 6 day NO_3^- supplementation period (Lansley, Winyard et al. 2011). However, despite the decrease in peak VO_2, they observed an increased time to task failure (15%) under the BRJ condition compared to PL (Lansley, Winyard et al. 2011), highlighting an increased exercise efficiency with NO_3^- supplementation. Additionally, it has been reported that heart failure patients show an enhanced peak VO_2 when supplemented with BRJ compared to PL and they observed an increase in total amount of work done (Zamani, Rawat et al. 2015). The reason behind our lack of changes in peak VO_2 or total work performed following BRJ supplementation is unknown, but several possibilities exist. First, we did not observe any changes in cardiac performance or the arterial-venous oxygen difference, which suggests our dose of NO_3^- did not have any effect on our measured parameters of the O_2 transport pathway. However, it should be noted that these parameters did not provide detailed insight into other aspects of convective and diffusive O_2 transport (i.e. muscle blood flow or O_2 diffusing capacity). Secondly, previous work has reported a significant age-related decline in cardiorespiratory fitness for both men and women with a classification VO_2 value of 15.4 ml/kg/min being reported for functional independence for older individuals aged 55-85 years (Paterson, Cunningham et al. 1999). Meaning that cardiorespiratory function exercise tests may not be a true representation for maximal effort for older individuals and thus lead to a lack in identifying the effects of BRJ. However, our group of cancer survivors were physically active and had a peak aerobic capacity above what is classified for functional independence. This observation may be partially explained by data collected from our health history and physical activity questionnaire reporting that all of our patients in this study were meeting the Physical Activity Guidelines for Americans of at least 150 minutes/week of moderate-intensity exercise

(US Department of Health and Human Services 2018). All patients engaged in structured moderate-intensity exercises an average of 3 days of the week and each exercise session lasting approximately one hour in duration. Additionally, our patients engaged in structured walking an average of 5 days of the week with each session lasting approximately one hour in duration. The majority of our patients (67%) also regularly engaged in strength training exercises each week. Being physically active and meeting the American physical activity guidelines is vital to overall health and quality of life. Huang et al. (1998) has reported that older individuals who are unfit are associated with more functional limitations however, this trend reverses for those who engage in physical activity (Huang, Macera et al. 1998). This suggests that older individuals who regularly engage in physical activity will have more functional independence and thus a better quality of life (Paterson, Cunningham et al. 1999). Lastly, we administered a single and uniform amount (140 ml) of NO_3^- [12.2 mmol NO_3^-] which may not have been enough to elicit a cardiopulmonary response during peak exercise as indicated by the lack of difference in VO_2 responses between conditions. In contrast, Bescos et al. (2011) have reported a decrease in peak VO_2 during a maximal exertion cycle ergometer test after an acute dose of NO_3^- supplementation (Bescos, Rodriguez et al. 2011). However, these investigators administered NO_3^- supplementation that was dose dependent on their subjects' body mass at a ratio of 10 mg: 1 kg of body weight. In the present study, we administered a dose of 140 ml [12.2 mmol NO_3^-] of BRJ to all patients regardless of body weight however, there was a range of 65 kg between our patients.

Limitations

A major limitation to our study was the small sample size (n = 6) of cancer survivors. However, the cross-over study design allowed us to reduce variability within our measurements and allowed observation of differences between BRJ and PL conditions (Zamani, Rawat et al. 2015). Additionally, the patients that completed our study were relatively healthy with all patients regularly participating in physical activity. We believe this is partially explained by the length of time in which our patients were diagnosed as cancer free with the average time being 4 years and 9 months and the longest time duration of 14 years. Thus, our sample may not accurately reflect that of the entire cancer survivor population, specifically those who have recently completed anticancer therapy treatment and are adjusting back to normal life activities while handling adverse side effects from their treatments.

Conclusion

In conclusion, a single, acute-dose of inorganic nitrate supplementation significantly reduced the oxygen consumption required during steady-state exercise for cancer survivors. Our findings suggest that NO_3^- supplementation may be a beneficial therapeutic strategy for cancer survivors who have undergone chemotherapy as some of the side effects of chemotherapy have been shown to be irreversible and thus, have life-long lasting effects. Future studies are warranted to investigate the mechanisms that elicit a reduction in VO_2 following longer term NO_3^- supplementation specifically for clinical populations.

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