

The Effect of Nitrate Supplementation by Beetroot Juice on Anaerobic Performance of Gymnasts

Literature Review and Research Plan

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By

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Abstract

Research has been conducted to investigate dietary nitrate supplementation on improvements in athletic performance. Nitrate is a polyatomic ion obtained from diet that aids in vasodilation, vessel tone, and maintaining proper endothelial function. The first purpose of this thesis is to review the literature conducted on nitrate supplementation with a focus on anaerobic exercise performance. Nitrate supplementation has been shown to improve aspects of both aerobic and anaerobic performance due to the increased cardiovascular and metabolic demands of exercise, but results in anaerobic performance have been less consistent. The second purpose of this thesis is to propose a research design developed to investigate the use of beetroot juice on anaerobic performance of collegiate gymnasts. A randomized crossover design would have been used, including six days of supplementation with either beetroot juice or low-nitrate placebo, a gymnastics-specific anaerobic performance test, and a Wingate cycle ergometer test. Blood lactate levels would have been monitored during recovery after these tests to determine how nitrate supplementation impacts post-exercise blood lactate levels. This study was canceled due to COVID-19 research restrictions, but the literature review and experimental research design may provide insights to possible physiological benefits and mechanisms of nitrate supplementation.

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Literature Review

Nitrate

Nitrate is a polyatomic ion that is essential for all living organisms. It is reduced to nitric oxide (NO) by one of two biological pathways that will be discussed later in this review. Nitric oxide plays a vital role in cardiovascular health and function and is essential in order for the body to adapt to the demands of exercise. During exercise, working tissues require more nutrient delivery, such as oxygen, and waste removal, such as lactate, for proper function. These nutrients and waste products are carried through blood vessels which require NO for vasodilation⁷ in order to keep up with the increased demands. NO also regulates mitochondrial respiration, platelet function, and leukocyte migration (7, 9) promoting ATP synthesis in the mitochondria and preventing thrombosis, or clotting, within blood vessels. It also regulates inflammation and vessel tone (9), which are important aspects of cardiovascular health. The initial stage of cardiovascular disease is endothelial dysfunction (9), so having adequate amounts of NO present to regulate the function of the endothelium is critical in maintaining proper cardiovascular health. The endothelium is the inner layer of blood vessel walls. When functioning properly, the endothelium reacts to produce vasodilation or vasoconstriction depending on the needs of the tissues those blood vessels supply to.

There are two pathways in which the body uses nitrate and its reduced form nitric oxide. The first is the L-arginine–NO synthase pathway. In this pathway, biochemical and mechanical stimuli increase cause calcium to be released from stores within the sarcoplasmic reticulum. These biochemical stimuli include thrombin, serotonin, acetylcholine, bradykinin, and adenosine diphosphate (ADP). ADP is a product of the catabolism of ATP that is especially present during exercise, and thrombin, bradykinin, and serotonin are all released during exercise. Mechanical

stimuli include cyclical strain and shear stress. These biochemical and mechanical stimuli create a high demand of NO to dilate blood vessels to working tissues. Endothelial nitric oxide synthase (eNOS) oxidizes the 5-electron oxidation of the terminal guanidine nitrogen atom of L-arginine, which is synthesized to become nitric oxide. Once synthesized, the short half-life of NO allows it to rapidly diffuse across the endothelium to relax the smooth muscle within blood vessels (9).

The second biological pathway is the Nitrate-Nitrite-NO pathway. This process begins when nitrate is ingested through dietary intake – 80% of which is accomplished through consuming vegetables such as green leafy vegetables, beetroot, and some drinking water (9). In the mouth, facultative anaerobic bacteria in saliva take nitrate from consumed food and use it as an electron receptor for oxygen, which reduces it to nitrite (5, 9). At this point, some existing nitrite is absorbed into blood plasma, and distributed through the body to become a ready source of NO for vasodilation (9). Remaining nitrite is reduced further in the acidic environment of the stomach (5). This type of environment is also found in hypoxic muscle tissue during exercise, also stimulating the breakdown of nitric into NO. The Nitrate-Nitrite-NO pathway allows for storage of NO in large amounts in the blood and body tissues, ready for use when necessary (9).

Overview of nitrate and exercise performance

Numerous studies have been conducted regarding nitrate and exercise performance. Bescos et al. (7) stated that increased levels of NO production improves blood flow to working skeletal muscle tissue. Improved blood flow is hypothesized to improve tolerance to exercise and recovery by enhancing oxygen and nutrient delivery (7). NO also enhances the body's ability to adapt to exercise by modulating muscle contraction, altering calcium homeostasis, and altering glucose uptake and metabolism, making it easier for the glucose to be absorbed and utilize by working

tissues (9). Researchers speculate that plasma nitrite concentration can be a good indicator of exercise capacity after observing an increase in natural NO production during exercise.

Nitric oxide also plays a role in cellular respiration. The molecule interacts with mitochondrial respiratory chain enzymes which enhances proper respiration (9). Mitochondrial respiration is made more efficient because fewer protons leak through the mitochondrial membrane (11), allowing for more to be used in the electron transport chain to synthesize ATP. The electron transport chain is an aerobic means of producing ATP because it requires the use of oxygen as an electron acceptor. This fact leads researchers to investigate nitrate and aerobic performance potentially to determine whether nitrate supplementation would allow for greater amounts of ATP to be produced for use during aerobic exercise. Studies involving nitrate supplementation primarily use beetroot juice (BRJ) as the supplement because of the high nitrate concentration. BRJ can be diluted or concentrated to attain the desired nitrate concentration, which is one of the benefits of using this means up supplementation.

It has been found that nitric oxide primarily improves blood flow to type II motor units (14). These are the body's fast-twitch muscle fibers that allow for explosive movement, proposing that increased NO may improve performance in power-based exercise. These muscle fibers primarily utilize anaerobic means of ATP production – the ATP-PCr energy system and anaerobic glycolysis. The ATP-PCr energy system lasts for the first several seconds of exercise and anaerobic glycolysis lasts for the first few minutes. Pyruvate is the end molecule of the anaerobic glycolytic process, and up to this point there has been no involvement of oxygen. If oxygen is still not available, pyruvate will be used to oxidize an NADH molecule, resulting in the formation of lactate from pyruvate. Until oxygen is available for use, this process continues and lactate begins to collect in the muscle tissue (26).

After several minutes of exercise, the aerobic glycolytic energy system becomes the predominant contributor to ATP production. With the presence of oxygen, the pyruvate at the end of anaerobic glycolysis can be broken down by pyruvate kinase to create Acetyl-CoA, which can be used in the Krebs cycle to create ATP (26). Promoting vasodilation to allow for more blood flow to type II muscle fibers to facilitate anaerobic glycolysis and eventually lead to aerobic glycolysis and improving metabolic efficiency is hypothesized to aid in improving exercise performance (14).

In paragraphs that follow, literature will be presented that has addressed findings of nitrate supplementation on both aerobic and anaerobic exercise performance.

Nitrate and aerobic performance

Aerobic exercise has been the primary focus of nitrate supplementation in regards to exercise performance. Aerobic energy production is reliant on the presence of oxygen, which is delivered to working tissues through blood vessels. Nitrate is reduced to nitric oxide, which aids in the regulation of vasodilation blood vessel walls (7), allowing greater blood flow carrying oxygen and other necessary nutrients to working tissues.

A number of investigators have tested the effects of nitrate supplementation on various aspects of aerobic performance. In 2009, Bailey et al. (1) conducted a study analyzing cycle ergometer tests with BRJ supplementation compared to a placebo. The subjects included eight healthy and recreationally active men that were familiar with exercise testing protocols from participation in previous studies. The study required subjects to perform a ramp incremental exercise test on a cycle ergometer to measure peak VO_2 and gas exchange threshold (GET). Subjects received supplementation for 6 days and were asked to gradually drink the beverage

throughout the day. Between the supplementation periods, the subjects had a ten-day washout period. On the last three days of the supplementation periods, the subjects underwent the exercise testing. Day 4 consisted of two moderate intensity cycle tests while days 5 and 6 consisted of both a moderate and a vigorous cycle test – each test separated by 25 minutes of passive recovery. Researchers found the BRJ group to have an extended time to exhaustion of 14%, a 19% reduction in amplitude of the pulmonary response, and a 23% reduction in the VO_2 slow component, suggesting that the subject's bodies were better able to adapt to the stress of the activity. Increased time to failure was also seen in another study involving trained cyclists performing incremental cycle tests to determine $\text{VO}_{2\text{peak}}$ after single-dose nitrate supplementation. These trained subjects saw a 16% improvement in time to exhaustion after supplementation with nitrate (39).

Another research group conducted two studies analyzing the potential effects of BRJ on aerobic performance (22, 23). Subjects in the earlier study were told to consume 0.033mmol/kg of a sodium-nitrate drink three times a day for two separate 3-day periods before testing. These tests used a combined upper- and lower-body cycle ergometers to determine the $\text{VO}_{2\text{max}}$ and time to exhaustion. Researchers found lower levels of oxygen uptake during the maximal effort exercise bouts during the cycle ergometer tests. This decrease in $\text{VO}_{2\text{max}}$ in the nitrate group was unexpected, but could be due to a variety of oxygen-consuming processes that occur during exercise that do not produce ATP, such as ion channel leakage, cellular transport of proteins, or heat production. The investigators also found a near-significant increase in time to exhaustion after nitrate supplementation. During- and post-exercise lactate values showed no significant difference between nitrate and placebo groups (23).

A follow-up study asked for just one three-day supplementation period for another cycle ergometer test, where subjects consumed the same amount (0.033mmol/kg three time a day) of a

nitrate-containing supplement for three days. The results showed reduced O_2 cost of exercise and improved metabolic efficiency. The respiratory exchange ratio (RER) of subjects decreased after nitrate supplementation, suggesting a greater reliance on the slightly more efficient use of carbohydrates as opposed to use of fatty acids for ATP production. Nitrate supplementation seemed to improve the coupling of oxidative phosphorylation and respiration. The oxidative phosphorylation efficiency (P/O ratio) of these subjects improved by 23%. This measures the amount of oxygen consumed compared to the amount of ATP produced. The 23% improvement suggests that the aerobic glycolytic energy system was able to function more efficiently for the amount of oxygen available to it (22).

A reduction in O_2 consumption was also observed in a moderate-intensity ramp incremental cycling exercise test set at 90% of the subject's gas exchange threshold (41). Eight healthy subjects consumed 500mL of BRJ (containing ~ 5.2 mmol NO_3^-) or a placebo for 15 days. Exercise testing occurred 2.5 hours after supplementation and on days 5 and 15 of supplementation, measuring both chronic and acute effects of nitrate supplementation. The researchers found increased plasma nitrite and decreased systolic and diastolic blood pressure of the brachial artery after supplementation with BRJ. The experimental group showed reduced steady-state VO_2 2.5 hours after ingestion, which was maintained through the 15-day period. Peak power was greater compared to baseline and placebo values at day 15 of supplementation. From these results, the researchers concluded that both acute supplementation 2.5 hours pre-testing and chronic nitrate supplementation 5 and 15 days after the beginning of supplementation reduced the O_2 cost of exercise. This reduction in O_2 cost can be maintained for 15 days if nitrate is chronically consumed.

In contrast to the previous acute supplementation study, a 2011 research group supplemented nine healthy, active males with $\sim 6.2\text{mmol NO}_3^-$ or a placebo for 6 days and were tested on days 4, 5, and 6 (46). On days 4 and 5, tests consisted of two 6-minute bouts of moderate intensity running (80% of the subject's gas exchange threshold, or GET) and one bout of severe (75% Δ) intensity running to exhaustion and day 6 consisted of incremental, single-leg knee extension testing. The moderate-intensity test revealed a 7% reduction in consumption of O_2 during the final 30 seconds of exercise. This reduction followed a 105% increase in plasma nitrite concentration after a nitrate-enriched BRJ supplement. As previously discussed, the acidic environment created by exercise in working muscle tissue reduces nitrite into nitric oxide (5), promoting greater blood flow and nutrient delivery to the working area. This increase in blood flow led to a 15% increase in time to exhaustion (46). The enriched BRJ was used to isolate the effect of nitrate on exercise performance and rule out whether effects were seen due to another component present in the BRJ such as the amino acid betaine, antioxidants, and polyphenols (46).

Only one study found analyzed the effect of baked whole beetroot on exercise performance (32). The researchers aimed to find whether consuming whole beetroot that would roughly equate the amount of nitrate found in beetroot juice would improve performance. In this study, subjects consumed 200g of baked beetroot, containing at least 500mg of nitrate or a cranberry and granular white sugar placebo that contained roughly the same number of calories and grams of carbohydrates. The subjects and ran a 5k time trial on a treadmill 75 minutes after consumption of the baked beetroot or placebo. Subjects that consumed the baked beetroot had 41-second faster finish times and a 5% faster finish times than subjects in the placebo group. The baked beetroot group also reported lower RPEs than that of the placebo group. This study found that nitrate supplementation is especially beneficial in the later parts of the race with 5% faster running

velocities during the last 1.1 miles (32). A similar result was found in 16 trained athletes after consuming 450 mL of BRJ (~5 mmol nitrate) 150 minutes before a 50-mile cycling test (44). These subjects had a faster finish time, especially in the last 10 miles of the test, suggesting late-race benefits of nitrate supplementation in both running (32) and cycling (44) performance.

In a crossover study in 2013, a group of researchers looked into the effects of BRJ on a double-step test (10). The researchers chose to use the double-step test because it more closely related to real competition situations where people are not always at a constant work rate. Subjects consumed 140 mL of BRJ (~8 mmol nitrate) per day for 6 days prior to the simulation. On the last three days of each supplementation period, subjects performed 4 minutes of moderate-intensity cycling at 90% of the subjects' GET immediately followed by 6 minutes of severe intensity cycling at 70% of the difference between the subjects' GET and VO_{2peak} . Researchers found faster VO_2 kinetics in the transition from moderate to severe-intensity exercise and improved exercise tolerance measured by time to task failure in the BRJ group compared to the placebo group. This improvement in exercise tolerance was also seen two other studies looking at BRJ supplementation and trained subjects – one in 2013 looking at 9 trained athletes and the other in 2014 on trained cyclists (39). The athletes in the 2014 study saw an average of 16% improvement in time to exhaustion after receiving BRJ (39).

In 2015, a research group studied eight recreationally active males on acute nitrate supplementation on cycling exercise tests, mainly to determine the effect of nitrate on exercise metabolism (47). Subjects consumed either a nitrate-depleted BRJ, full-nitrate BRJ (~8 mmol), or full-nitrate BRJ followed by about 2.5 hours prior to testing. The facultative anaerobic bacteria found in saliva are responsible for breaking down nitrate into nitrite, and antiseptic mouthwash has been shown to stop the increased in plasma nitrite after ingestion of nitrate. Mouthwash with

nitrate supplementation has also been shown to eliminate the reduction in blood pressure and gastro-protective effects of nitrate. The exercise test consisted of 60 minutes of cycling at 65% of the subjects' VO_{2peak} that was previously determined. The researchers measured plasma lactate every 15 minutes during the exercise bout, but found no differences between the groups. Investigators also found no difference in oxygen consumption, blood glucose, plasma insulin, plasma nonesterified fatty acids, among other muscle metabolism markers in either BRJ group. The researchers concluded that at the current dose, BRJ supplementation did not improve skeletal muscle metabolism.

Two kayak performance studies were conducted in by a research group in 2015 using 11 competitive kayaking athletes (6 national level males and 5 international level females) (48). Study A used the 6 national-level male subjects on 3 lab-based kayak ergometer sessions. The nitrate supplement contained about 4.8mmol of nitrate or a placebo was consumed 2.5 hours prior to the beginning of the warm-up. These subjects completed a 7x4min step test to determine VO_{2max} and first and second lactate thresholds. These values were used to determine intensities in later sessions. The final two sessions consisted of an 18-minute warm-up, 8-minute recovery, and a 4-minute maximal effort paddle. The subjects showed no difference in power output, heart rate, blood lactate, or RPE. However, the researchers found that the BRJ group traveled a greater average distance within the 4 minutes, and had a lower average VO_2 and improved exercise economy (48).

Study B used two field-based 500-meter time trials separated by 4 days. This study used the international female athletes, who consumed two BRJ shots, containing a total of ~9.6mmol of nitrate or a placebo about 2 hours before the trial. These subjects showed improved time trial performance after BRJ supplementation. Subjects saw a 2.0sec faster finish time, which is a 1.7%

improvement over the placebo group. The research also showed higher stroke rate and velocity and a slight increase in tail wind speed. Blood lactate, RPE, and gut sensation showed no significant differences between groups. This study was significant in that it showed benefit of BRJ in the performance of nationally or internationally competitive athletes, although the differences seen are less substantial with more highly-trained athletes (48).

Two more research groups studied the effect of nitrate supplementation on trained cyclists. One study done in 2015 used acute supplementation of a nitrate with beetroot juice (0.4g), caffeine (5mg/kg), or combined supplement with a placebo 2.5hrs prior to a 20km time trial (49). The other conducted in 2012 and used a chronic 6-day nitrate- (~8mmol) and placebo-only supplementation protocol for a 60-minute cycling bout followed by a 10km time trial⁵⁰. The caffeine and nitrate study used 14 female competitive cyclists (49), where the nitrate-only study used 12 trained male cyclists (50). The 2012 study determined the subjects' max Watts (W_{max}) on the cycle ergometer were measured prior to the beginning of the study. On day 6 of supplementation, subjects performed 60 minutes of submaximal cycling (30 minutes at 45% W_{max} , 30 minutes at 65% W_{max}) immediately followed by a 10-kilometer time trial (50). The 2015 research group had subjects consume their supplement 2.5 hours prior to the time trial then completed a 5-minute warm-up at 100W and then rested for 5 minutes before completing the 20km time trial (49).

The 20km time trial mentioned above only showed improvements in the caffeine group, including increased power output and higher heart rate, blood lactate, and respiratory exchange ratio (49). These researchers found no significant impact of nitrate supplementation on time trial performance (49). However, the 10km time trial reduced pulmonary oxygen uptake, increased exercise tolerance, improved time trial performance, and higher mean power output in the nitrate group compared to the placebo (50). Subjects also showed lower submaximal VO_2 values after

supplementation. Traditionally, the volume of oxygen consumption at a given workload is only expected to have slight fluctuations, but these fluctuations were significant. Another research group also found decreased VO_2 values in trained athletes (43). They required subjects to consume 280ml of BRJ (~6.5mmol nitrate) or a placebo for 6 days and perform a 20-minute cycle test at 50% of their $\text{VO}_{2\text{max}}$ for the first 10 minute and 0% of their $\text{VO}_{2\text{max}}$ for the last 10 minutes. The oxygen consumption of these subjects decreased by 3% after supplementation with nitrate (43). Researchers of the 2012 study are unsure of the mechanism causing this change in VO_2 values, but speculate it may be due to the role of nitrite and nitric oxide in cellular oxygen use regulation (50). Blood lactate was measured every 15min during the 60-minute cycling bout, but nitrate supplementation did not change these values (50).

Another cycling time trial study was done in 2014, using 22 recreationally active male subjects (51). These subjects underwent a 6-week training program of high-intensity cycle endurance training and received either beetroot juice (~0.07mmol $\text{NO}_3^-/\text{kg}\cdot\text{bw}/\text{day}$) in 3 doses throughout the day or a placebo. During the training sessions, blood lactate was measured at the beginning of the session, then 10 and 30 minutes into the exercise bout. The researchers found similar average power, heart rate, and blood lactate values between the nitrate and placebo groups, but the posttest blood lactate values of the nitrate group were slightly lower, but not statistically significantly at both the 20- and 30-minute marks compared to the pretest values of that group. The nitrate group also showed a slightly higher arterial oxygen saturation ($\%\text{SpO}_2$) and a greater degree of improvement in $\text{VO}_{2\text{max}}$ values compared to the placebo group. The researchers speculate that the lack of significant results in average power, heart rate, blood lactate measurements, and arterial saturation was likely due to the natural increase of NO bioavailability during exercise, making the effects of additional NO minimal.

Other studies have found that nitrate supplementation enhances aerobic performance by a variety of factors. Supplementation with beetroot juice improves exercise economy by maintaining power output while consuming less oxygen during a 10km cycling time trial (50). The increased blood flow to working tissues allows more oxygen delivery specifically to the mitochondria⁴, stimulating oxidative metabolism. Chronic nitrate supplementation has also shown to stimulate mitochondrial biogenesis (40), which may lead to less oxygen consumed at a given intensity. This improves energy production and reduces lactate buildup that is associated with fatigue (14).

Most studies looking at nitrate supplementation with aerobic exercise performance have shown benefits due to improved efficiency. Subjects showed an increase in time to exhaustion, improved pulmonary and metabolic efficiency, decreased oxygen cost, and late-race benefits leading to faster finish times. Nitrate should be taken at least 90 minutes prior to exercise in order to provide ergogenic effects. Vasodilation allows greater blood flow to working muscle tissue, especially type II muscle fibers, but does not significantly affect blood lactate values. Aerobic exercise performance has been the main focus of research with nitrate supplementation, but anaerobic performance has not seen quite as much research done.

Nitrate and anaerobic performance

The effect of nitrate supplementation on anaerobic performance has not been investigated as thoroughly as aerobic performance. Much of the literature looks at various aspects of anaerobic performance, uses different supplementation strategies, and has found controversial results, making it unclear whether nitrate supplementation improves on anaerobic performance or not. The suggested means of exercise enhancement is the idea that increased levels of plasma nitrite may decrease ATP turnover in cells, which accounts for 50% of ATP use, improving skeletal muscle

metabolism (5). These changes also may be due to elevated glycolytic activity (18), increased blood flow to type II motor units (17), enhancing motor neuron depolarization (15), decreased PCr degradation, and/or increased NO concentration (3, 17).

Researchers in 2009 used moderate and severe intensity exercise step tests to discover if any ergogenic effect of BRJ supplementation is present (3). Eight recreationally active men consumed 500mg, containing about 11.2 ± 0.6 mmol nitrate, for 6 consecutive days leading up to the step tests. They found that systolic blood pressure was decreased by about 6mmHg and plasma nitrite increased by 96%. The pulmonary VO_2 response of subjects decreased, and the absolute VO_2 decreased significantly during the last 30 seconds of moderate intensity exercise. This research group also found that VO_2 amplitude was significantly elevated during severe exercise, but VO_2 at task failure did not change, suggesting that subjects were able to more quickly adapt to the increased workload, but had the same exercise threshold. However, the time to task failure increased, showing an increase in exercise tolerance for the BRJ group. The blood lactate values of the BRJ group were not significantly different than the placebo group at task failure and values were not recorded during the recovery period. This group also found a reduced oxygen cost for submaximal exercise in the BRJ group.

In 2010, the same research group conducted a similar study attempting to identify the mechanism of how nitrate supplementation resulted in a reduced oxygen cost of moderate- and high-intensity exercise (4). Seven recreationally active men consumed 500mL per day of a BRJ supplement (5.1mmol nitrate) or a placebo (negligible nitrate content) for 6 days. Subjects performed maximum voluntary isometric contractions and incremental knee extensions to determine peak work rate. Muscle activity was measured by integrated electromyography of the right vastus lateralis muscle. The investigators found a 25% increase in time to failure after 500mg

of BRJ supplementation for 6 consecutive days⁴. Similar to the previous findings of Bailey et al. (4), the subjects had a 25% reduction in the increase of pulmonary VO_2 values from resting to low-intensity exercise (4). Phosphocreatine degradation reduced by 36% in low-intensity exercise and 59% in high-intensity exercise. This adaptation allowed more ATP to be preserved because PCr is being spared (4).

A later study was performed testing maximum number of repetitions on bench press for 12 male recreation sport players after consuming 6.4mmol of nitrate for 6 days prior to testing (30). Subjects performed three sets of maximal repetition bench press at 60% of their 1-rep max. After supplementation with nitrate, subjects lifted 19% more weight and had unspecified improvements in the number of repetitions performed. Researchers suggest this benefit is due to improved recovery in between sets and the sparing of PCr, allowing more work to be done during the session.

In another study, fifteen trained men performed a Wingate test after consuming a placebo or a 70mL nitrate, containing approximately 5.6mmol of nitrate (15). Participants consumed the supplementation 3 hours prior to the test. Blood lactate levels were measured immediately before performing the warm-up and 3 minutes after the conclusion of the test. Results of the Wingate cycle ergometer test showed significantly higher peak power values and reduced degradation of phosphocreatine in the BRJ group. Another study using CrossFit athletes also found increased peak power in a Wingate cycle ergometer test after 6 days of nitrate supplementation (20). In the acute supplementation study, post-exercise blood lactate levels were 82.6% higher after consuming the nitrate supplement (15). This finding was unexpected by the researchers, but the researchers speculate that the increased levels could be a factor of increased blood flow to type II muscle fibers, which produce the majority of lactate during exercise. This result could also be due to a higher reliance on carbohydrates for energy. The breakdown of carbohydrates for energy

production is a much more efficient method than that of triglycerides. A greater efficiency of energy production can lead to greater amounts of ATP available to the subject during exercise and would allow for more mechanical work to be done. Nitrate supplementation may have also had an impact acetylcholine on type II muscle fibers by enhancing motor neuron depolarization (15).

Another study also found effects specifically on type II muscle fibers after chronic nitrate supplementation. This research group analyzed the effect that 5 weeks of sprint interval training had on muscle fiber type distribution of 27 moderately trained participants (13). Three groups were studied – training in normoxia, training in hypoxia, and training in hypoxia with an oral nitrate supplement (~6.45mmol NaNO₃). Training in hypoxia could allow for greater benefit of supplementation due to increased oxidative stress. Subjects participated in three 30-minute training sessions per week, each session consisting of four to six 30-second sprints with four and a half minutes of recovery between each sprint. Researchers recorded each subject's VO_{2max}, 30-minute time trial performance, and 30-second sprint performance prior to the beginning of training and after training had concluded. The hypoxic nitrate group saw improvements in 30-second sprint performance at the conclusion of the training period. The hypoxic nitrate group also saw an increase of the percentage of type IIa muscle fibers from 45% to 56%. These findings can be attributed to the nitrate supplement because these improvements were not seen in the normoxia or hypoxia-only groups, leading the researchers to conclude that the nitrate supplement was the responsible factor for these changes.

Several other studies used cycle ergometers as the exercise modality to test anaerobic performance with nitrate supplementation (1, 29, 45). Three of these studies looked at nitrate supplementation 2-3 hours prior to testing on repeated 8- (29), 15-second¹, or 6-, 30-, and 60-second (45) sprints on a cycle ergometer. These studies showed opposing results. Subjects

performing repeated 8-second sprints performed 13% fewer repetitions and 17% less total work after nitrate supplementation. In the repeated 15-second sprint test, subjects performed 20% more repetitions and 18% more work after supplementing with nitrate. The 8-second sprint test required subjects to consume the nitrate supplement 120 minutes before the test (29), which is not aligned with the recommendation of supplementing 150-180 minutes prior to exercise due to plasma nitrite levels peaking 2-3 hours after consumption (16). The 15-second sprint test appropriately supplemented subjects 180 minutes before testing (1), which could account for the differentiation in results.

The third study required 10 recreation team sport players to perform 6, 30, and 60-second sprints on a cycle ergometer after consuming 8.4mmol of nitrate or a placebo for 5 days (45). Testing was done on day 5 of supplementation. The researchers found a 5% increase in average power, 1% increase in peak power in the 6-second sprint test, but a 1% decrease in average power and peak power in the 30-second sprints after nitrate supplementation. Researchers suggest that this difference is due to an increase in aerobic glycolysis for longer-duration exercise bouts to limit the increase in H^+ buildup, which would decrease pH (42).

Another cycle ergometer test that was used was the Wingate cycle ergometer test. 13 male competitive sport athletes performed a 3-second isoinertial test and a Wingate test on an isokinetic cycle ergometer after consuming 11.2mmol of BRJ 150 minutes prior to testing (37). The researchers found improvement in the pedaling cadence in the 3-second test, but no improvements in the isokinetic Wingate test. The fixed pedaling cadence of 120 RPM in the Wingate test may explain the lack of differences between the nitrate and placebo groups. NO is believed to increase the shortening velocity of muscle fibers (27, 28), but the subjects' muscle fiber shortening velocity

was fixed due to the isokinetic nature of the test (37), limiting the effect that NO could have had on power output.

Several studies looked at repeated sprint performance with nitrate supplementation. In 2016, 20 male competition team sport athletes performed two repeated sprint trials, each consisting of twenty 30-meter sprints with 30 seconds of rest in between (12). In a double-blind approach, subjects consumed BRJ containing 11.4 mmol of nitrate or a placebo 150min before each trial. Researchers did not find significant results in the repeated sprint trials between supplement groups. In trial 1, investigators found a 1% decrease in average sprint time after BRJ supplementation. Trial 2 showed a 2% decrease in average sprint time and a 3% decrease in fastest sprint time of subjects. These differences may have been minimized due to the 30-second rest period between sprints, which should have been sufficient to replenish PCr levels, decreasing the ergogenic effect of nitrate supplementation (16).

Another study also looked at sprint performance after supplementing with BRJ, but in trained kayakers (31). In a single-blind randomized crossover study, 8 trained male kayakers consumed either 5mmol of nitrate or a low-nitrate placebo 180min before performing five 10-second sprints on a kayak ergometer with 50 seconds of light paddling between each sprint. These athletes saw a 4% increase in average power after nitrate supplementation in the fifth sprint, but the researchers did not find significant differences in peak power output, fatigue index, or total work between the nitrate and placebo groups in any of the performed sprints.

A couple studies have used repeated sprints to simulate competition (11, 39). One of these studies supplemented 16 recreation team sport males with 140mL per day nitrate-rich BRJ (12.8mmol nitrate) or nitrate-depleted BRJ for 7 days (39). On the last day of supplementation, subject performed intermittent sprint tests while completing cognitive tasks to reflect real

situations in a sport competition. The sprint tests consisted of two 40-minute “halves” with 6-second sprints separated by 100 seconds of active recovery and 20 seconds of rest. The researchers found a 5% increase in work volume mid-simulation, 2% post-simulation, and 4% for the whole session after supplementation with nitrate. Researchers also found improved cognitive reactivity after supplementation with BRJ, but no difference in the accuracy of the cognitive tasks (39).

The other performance study included the following using 13 female team-sport participants with nitrate and sodium phosphate (SP) supplementation (11). SP and BRJ have been studied individually and have shown benefits to exercise performance, but these researchers searched to determine whether there was any additional benefit from the combined effects. These subjects were randomly placed in one of four groups: SP + BRJ, SP + BRJ placebo, BRJ + SP placebo, or BRJ and SP placebo. SP supplements were taken by dissolving a capsule in water with PowerAde powder for 6 days, and the nitrate supplement 70mL of Beet-It, containing ~6mmol of nitrate, 3 hours prior to exercise. After completing a 10-minute standardized warm-up, the participants performed six 20-meter sprints, each separated by 25 seconds from the beginning of one sprint to the next. The simulated team-game circuit began immediately after the repeated sprints. The circuit consisted of four 15-minute quarters with 4 minutes of rest in between quarters and 10 minutes total at halftime. Subjects performed one “lap” on each minute of the quarter, consisting of two 10-meter sprints, one 20-meter sprint, 12 meters of agility, 30 minutes of striding, two periods of jogging and three periods of walking. The SP and BRJ group showed an average 3.25sec and 3.12sec faster time to complete each sprint, respectively, which is approximately a 5% improvement over the placebo group. These results suggest that SP and BRJ supplementation, especially prior to competition, will improve performance (11).

Continuously changing workloads is common in sport performance and other game-like situations. In a double-step exercise test, recreationally active subjects completed an unloaded to moderate intensity test and a moderate to severe intensity test (7). Subjects in the nitrate supplement group consumed 140mL of a BRJ that contained about 8mmol nitrate or a placebo for 6 days. The subjects' VO_{2peak} and gas exchange threshold (GET) was measured using a cycle ergometer to determine workloads for testing. On the last three days of supplementation, subjects underwent exercise testing. The fourth and fifth days of supplementation tested subjects with 3 minutes of “unloaded” work, 4 minutes of moderate-intensity work, and 6 minutes of severe intensity. These tests were used to determine VO_2 and muscle kinetics. The concentration of muscle deoxyhemoglobin increased more rapidly in the transition from moderate to severe-intensity exercise, as did phase II VO_2 kinetics after supplementation with BRJ. Exercise testing on day 6 was moderate to severe-intensity exercise until failure. The investigators used time to task failure to measure exercise tolerance, which increased after BRJ supplementation. The researchers noted that these results were from recreationally active subjects and that not enough research had been done with highly trained athletes to date.

Bailey et al. (3) noted the remarkable effects that a slight nutritional intervention can have on human performance. Reducing the oxygen cost of exercise at a given work rate by 20% is no small margin (3), especially when competitors work to find any slight advantage over their opponents. Nitrate supplementation has shown to be effective in improving anaerobic threshold, which could lead to athletic performance benefits, and some researchers presume this improvement is due to increased population of mitochondria in muscle fibers (14).

However, not all anaerobic studies have had these types of positive results. In 2018, Bernardi et al. (52) conducted a repeated sprint cycle test with ten well-trained martial arts athletes.

Each subject completed 7 days of supplementation either with BRJ (9.3mmol NO₃⁻) or a placebo. Testing consisted of a 5-minute warm up incorporating three 3-second sprints and twenty 6-second sprints followed by 24sec of recovery between each sprint on a for the testing portion of the visit. Results showed no significant differences between the nitrate group and the placebo in relative peak and mean power output. The researchers attributed the insignificant differences between supplemental groups to be due to the highly-trained status of subjects due to their higher basal plasma nitrite concentrations.

Studies conducted in 2015 also showed no significant differences in nitrate supplementation and performance (24, 24). The first studied hypoxic and normoxic conditions with BRJ supplementation (24). Eleven trained male cyclists consumed 70mL of either a 6.5mmol nitrate supplement or placebo 120 minutes before completing a 10k on a cycle ergometer in either a hypoxic or normoxic condition. Results showed no differences between hypoxic or normoxic conditions in any group (24). In a study testing a 4000m incremental exercise bout to exhaustion and a 10km time trial in hypoxic conditions, no differences were found between the BRJ and placebo groups in time to exhaustion in the incremental bout (25). In the 10km time trial, subjects showed an average of an 11-second improvement in time to completion, however this result was found to be insignificant (25). These subjects consumed ~7mmol of nitrate about 2.5 hours before exercise. Researchers also found no differences in oxygen cost, heart rate, RPE, or arterial oxygen saturation post-nitrate consumption (25). Both studies found no significant differences in hypoxic and normoxic conditions (24, 25).

Although multiple studies have found positive effects of nitrate supplementation on anaerobic performance, many of these results have not been replicated. Also, each study used various forms of exercise testing, such as cycle ergometer tests (15, 24), incremental exercise tests

(25), and repeated sprints (11) and evaluated various markers of anaerobic performance. These markers include oxygen cost (3), blood lactate (15), and exercise tolerance (3, 7), but no variable has been studied extensively. Future research should include some of the testing modalities used in previous studies, such as the Wingate cycle ergometer test or step tests. Many studies used male subjects, but few used females and most did not look at the effects of nitrate specifically on sport performance. This would be beneficial because athletes look for anything to aid in performance and give an edge over competitors. The study explained in the following section was designed to include these areas of the research.

Research Methods

Identifying specific topic

Growing up as a lifetime athlete, finding every advantage over competitors was always a focus. I had always been passionate about nutrition and looked for dietary means of improving performance. After learning of the physiological effects of nitrate, I was curious to determine whether it could help athletes, especially in my own sport.

In the fall 2019 semester, I conducted research studying the effect of nitrate supplementation on exercise performance. My thesis advisor suggested trying to find a topic within nitrate supplementation that did not have significant amounts of research done on it. This would ensure that the study would contribute to an area of exercise physiology that has information gaps or lack of information altogether. Much of the research found analyzed its effect on aerobic performance, predominantly in cycling, but few studies had been done with anaerobic performance. Many sports, including gymnastics, are predominantly reliant on anaerobic energy systems for high levels of performance.

I have been a gymnast since kindergarten and compete on the women's gymnastics team at SUNY Brockport. I would have been able to use my connection as a SUNY Brockport athlete to find subjects to participate in this study. My knowledge of gymnastics-specific elements worked well with the exercise science knowledge of both myself and my thesis advisor to determine the best course of action to study the effect of nitrate on a specific population of athletes.

This research would have been beneficial because it would provide more information in a focus of exercise science with little conclusive research done to-date. This study would have looked at nitrate and anaerobic exercise performance, specifically in sport. Very few of the current studies completed on nitrate and anaerobic performance use highly-trained athletes or female

subjects, and none look at gymnastics specifically. Gymnastics, among many other competitive sports, is highly anaerobic, so determining any potential ergogenic effects of nitrate supplementation in these highly trained anaerobic athletes could help improve performance of many more anaerobic athletes than gymnasts alone.

Overview of experimental design

This study would have used a random crossover study design using nitrate supplementation with collegiate female gymnasts. The purpose of the study would have been to analyze the impact of nitrate supplementation on Specific Aerobic Gymnastics Anaerobic Test (SAGAT) (2) performance, anaerobic performance variables of the Wingate cycle ergometer test (6), and blood lactate levels post-exercise. The subjects would have completed two 6-day supplementation periods to determine whether nitrate supplementation would have affected sport performance and post-exercise blood lactate. Each supplementation period would have been separated by a one-week washout period after the exercise testing day of the first supplementation period. Baseline testing and exercise test familiarization would have occurred prior to the first supplementation period. Researchers would have expected improved anaerobic performance in the nitrate supplementation group.

Subjects

The subjects for this study would have included 20-30 collegiate female gymnasts between 18 and 22 years old. Subjects would have been recruited during the first couple weeks of practice during the fall semester (see appendices B and C). Each athlete is required to be screened by a physician prior to participating in collegiate athletics, and clearance from the physician would have

served as clearance for this study. Subjects would have been required to sign an informed consent form (see appendix D) and fill out a health history questionnaire (see appendix I) prior to baseline testing. Participants would not have had any orthopedic limitations such as injury to ensure that they would have been able to comply with the training regimen and testing. Any subject that becomes injured would have been removed from the study.

Supplementation

Each subject would have been provided one dose of Beet-It, containing 400mg of nitrate, or one dose of the placebo per day. The supplementation period would have lasted 6 days with the sixth day being the testing day. Many studies look at acute supplementation of nitrate with aerobic or anaerobic performance (16, 25, 41), but more success has come from chronic supplementation.

The normal practice schedule for the SUNY Brockport gymnastics team has practices start at 3:30pm, and conditioning would occur about 2 to 2.5 hours later. Since plasma nitrite levels peak after 2-3 hours post-ingestion (15), subjects would have consumed their supplement between 3:00 and 3:30pm to allow peak nitrite levels to occur just before conditioning.

After the first supplementation period, the subjects would have done a one-week washout period and then will begin the next supplementation period with the opposite supplement of the one they would have previously had (i.e. subjects from the week 1 BRJ group would then consume the placebo supplement for week 2). (*Appendix F*)

Exercise testing

To measure the effect of dietary nitrate supplementation on anaerobic performance, the researchers selected to use the Specific Aerobic Gymnastics Anaerobic Test (SAGAT) and the Wingate cycle ergometer test.

The SAGAT is a gymnastics-specific anaerobic test developed by a research group in 2015 to evaluate anaerobic exercise performance in gymnasts. This test consists of gymnastics-specific movements in a repeated sprint fashion measuring the athlete's time to completion.

To perform the SAGAT, 4 marks are made on a 10m-by-10m gymnastics floor. The first mark (position A) is placed ~2m from the end of the floor. The next mark (position B) is placed ~2m from the opposite end of the floor. Then, another mark is made ~2m from position B towards position A (line 1), and ~2m from position A towards position B (line 2). The setup of this exercise test is shown in the image below.

The test begins with the athlete standing at position A. Upon instruction to begin the test, the athlete sprints from position A to touch position B. The athlete will then turn to line 1, where they will perform one tuck-jump, two push-ups, and one L-hold. After completing all three gymnastics movements, the athlete will turn back and touch position B. Once the athlete touches position B, they have completed one bout of the test. The full test consists of 2 sets of 6 bouts performed as fast as possible. Each set is separated by a 2-minute rest period. The subject's time will be measured, and the time to complete each set will be added together for the athlete's official time.

This anaerobic test has produced similar results to that of the Wingate cycle ergometer test, providing reliability of data received through the use of this test. The direct application of the SAGAT to the sport of gymnastics creates a greater understanding in how the nitrate supplementation impacted sport performance. By using the SAGAT alongside the Wingate test, the researchers would have been able to see sport performance of the gymnasts by time to complete the SAGAT and obtain other variables from the Wingate to compare results to. Mean and peak

power, as well as plasma lactate values, were similar between the SAGAT and Wingate tests. The SAGAT was tested for concurrent validity, test-retest reliability, and sensitivity (2).

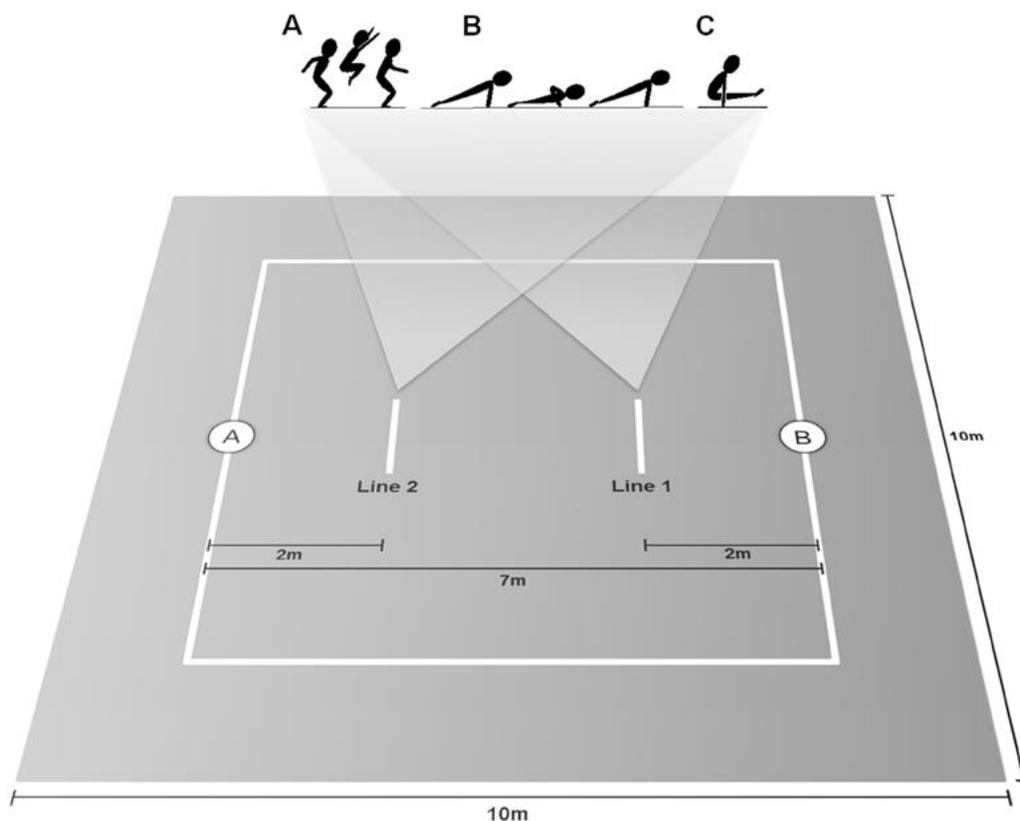


Image from Alves et. al.

The Wingate cycle ergometer test is a 30-second supramaximal test. It requires maximal energy production from the two anaerobic energy systems which makes this test a great indicator of anaerobic fitness. Studies have found that up to 85% of ATP used during this test came from the phosphagen and anaerobic glycolytic energy systems⁶. It can be assumed that type II muscle fibers play the dominant role in this test due to their correlation with mean anaerobic power, providing an explanation for the primary use of the anaerobic energy systems (6).

The Wingate test begins with a 10-minute warm-up on a stationary bike, pedaling at 60-70 RPM at about 100-125W. The first two minutes will be steady-state pedaling and the next four

will use sprint intervals, sprinting for 10 seconds with 40 seconds of recovery between sprints. Following the sprints, the subject would have pedaled comfortable for 5 minutes between 100-125W.

Just before the beginning of the test, the subject would have undergone an acceleration period where the subject begins to approach their maximal speed as the prescribed load (7.5% of the subject's body weight in kg) is automatically applied to the bike. The subject would have then completed the full 30-second maximal effort test. At the end of the 30-second period, the load will be automatically taken off and the subject will continue pedaling at a comfortable pace for 5 minutes as a cool-off period.

The Wingate test would have automatically measure peak power, average power, and fatigue index by the computer system used, which the researchers would have then documented. The Wingate cycle ergometer test is a widely used and accepted test for anaerobic performance with repeatable, reliable results. Multiple variables are measured, which may have provided the researchers with a clearer picture on the subject's anaerobic fitness and capacity. (*Appendix H*)

Lactate

Lactate is produced as a byproduct of energy production in the absence of oxygen (anaerobic glycolysis). Anaerobic glycolysis is the initial energy system used during activities lasting longer than about 6 to 10 seconds. If oxygen is not present at the end of anaerobic glycolysis, the end molecule, pyruvate, is converted into lactate which builds up in the working muscle tissue.

Lactate can be cleared from muscle tissue in three areas: the liver, heart, or type I muscle fibers transported through blood vessels. Once lactate reaches the liver, heart, or type I muscle

fibers, it will be oxidized to become a neutral pH. The body can become more efficient at clearing lactate from working tissues, which allows more mechanical work to be done.

The main idea of this study would have been to determine whether vasodilation from nitrate reduces blood lactate levels post-exercise. The researchers speculated that lower post-exercise lactate levels would suggest that nitrate supplementation aids in clearing lactate, likely through vasodilation to allow more blood flow into and out of the working muscle tissue.

To measure blood lactate levels, the researchers would have used a LM5 Lactate Analyzer. First, an alcohol wipe would be used to sterilize the finger and allowed to air-dry. A fresh lancet would have been used to prick the finger while the researcher applies slight pressure. The first drop of blood would have been wiped away with fresh gauze and several drops of blood would have been collected in a centrifuge tube to be transferred with a pipet into the LM5 Lactate Analyzer.

Experiment methodology

Week 1	Recruitment, informed consent forms, and exercise test familiarization
Weeks 2-4	Training acclimation period, dietary recall final 3 days
Week 5	Begin supplementation week 1, exercise testing on day 6
Week 6	Begin washout week, dietary recall final 3 days
Week 7	Begin supplementation week 2, exercise testing on day 6
Completion	Study Completed & Subject Compensation Distribution

Previously anticipated experiment outline.

Testing Day Schedule

Subjects would have been spaced out for time slots on testing day to allow for greater efficiency of testing. Subjects would arrive to the testing site and begin with a standardized 10-minute warm-up on a stationary bike. After completing the warm-up, subjects would have completed the SAGAT. One researcher would have timed the subject for both sets and the 2-minute rest in between sets.

Next, subjects would have had a 20-minute active recovery period where they would have walked around the testing site. Blood lactate levels would have been measured immediately after finishing the SAGAT and at the 5, 10, 15, and 20-minute marks during the recovery period.

At the conclusion of the recovery period, subjects would have been seated on the stationary bike to complete the Wingate cycle ergometer test. This test would have taken about 15 minutes in total from warm-up to cool-down. Blood lactate measurements would have been taken immediately after completing the test and 3 and 10 minutes after the test.

Subject Compensation

All subjects would have received a \$25 Barnes & Nobel gift card at the completion of the study. Only participants that completed the entire study would have been eligible for compensation.

Applying for IRB approval

5/7/2020	Study created in PACS system
5/8/2020	Initial submission
5/20/2020	First full board review & revisions requested
6/9/2020	Second submission
6/13/2020	Revisions requested & third submission
6/17/2020	Revisions requested
7/7/2020	Fourth submission
8/12/2020	Set up date for follow-up full board review
9/9/2020	Planned full-board review & final decision made by return to research – study cancelled

See Appendices A-I

Applying for funding

Estimations of costs were calculated to total about \$1,400 to include the Beet-It Beet Juice Sport Shots, compensation of subjects, and centrifuge tubes for blood lactate analysis. The following sources are how we intended to fund the study.

I applied for two different research grants to fund the study – the Honors Research Grant and the Student Research Mini Grant. I received the application for the Honors Research Grant in June 2020. Along with the application form, I was required to submit a letter to explain how the funds would be used for the study, a letter of support from Dr. Heidi Byrne, Ph.D., and a copy of my Degree Works degree audit. In the letter, I described the need for funds to provide both the experimental and placebo supplements as well as funding subject compensation.

I reached out to the head of the Undergraduate Research program at Brockport to get more information about the Student Research Mini Grant. We were unsure whether the pending status of approval from the IRB would impact the application for this grant. I began the application for this grant on Brockport MachForms in September 2020, but did not submit it due to the cancellation of the study from COVID-19 restrictions.

For the remaining funds needed for the study, research faculty planned to utilize their annual travel funds to support the study. These three areas of funding would have covered the entire cost of the study. (*Appendices J-L*)

Conclusion

This study would have provided insight on nitrate supplementation in anaerobic sport performance, specifically in female athletes. Females are not commonly used in nitrate supplementation studies, so this would have shown a different perspective. Also, using a sport-specific exercise test and the Wingate cycle ergometer test would have shown whether nitrate supplementation could have beneficial effects specifically in the sport of gymnastics and general anaerobic performance. Measuring blood lactate levels could have aided in determining how nitrate affects anaerobic energy system contribution and potential recovery mechanisms post-exercise. We hoped that this study would provide valuable insight on nitrate supplementation and sport performance and now hope that it may serve to help guide future research.

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Appendix A



The College at BROCKPORT

STATE UNIVERSITY OF NEW YORK
Institutional Review Board

Protocol # [number will be provided by IRB after pre-review]

Human Participants Research Review Form

Instructions:

Each question is very specific; please respond ONLY to the question being asked. Mark any sections that do not apply to your protocol as N/A.

As of July 1, 2018, proposals will only be accepted via the online PACS system. Please upload each supporting document separately onto the designated SmartForm page.

Expanded guidelines for completing the proposal form are available on the IRB site at https://brockport.edu/support/institutional_review_board/

PLEASE NOTE: You may not begin any part of your project—including recruiting participants—until you receive notification of approval from the IRB office.

SECTION ONE: SUMMARY INFORMATION

1. Basic Information:

Principal Investigator:	Amy Eck
Phone:	(571)442-4131
Email:	Aeck3@brockport.edu
Status:	<input checked="" type="checkbox"/> Undergraduate Student <input type="checkbox"/> Graduate Student <input type="checkbox"/> Faculty/Staff
Department:	KSSPE
Advisor Name & Department:	Heidi Byrne, KSSPE
Advisor Email:	hbyrne@brockport.edu
Research Category:	<input type="checkbox"/> Exempt <input type="checkbox"/> Expedited <input checked="" type="checkbox"/> Full Board Review

2. Project Title:

The Effect of Nitrate Supplementation by Beetroot Juice on Anaerobic Performance Measures of Gymnasts

3. How do you intend to use the information gathered?

(e.g., thesis, campus presentation, conference presentation, possible publication, etc.):

Senior Honors Thesis with conference presentation and publication.

4. Consultants or Co-Investigators, Institutional/Department Affiliations & Status:

Dr. Craig Mattern, Associate Professor, KSSPE; Dr. Justin Faller, Assistant Professor; KSSPE

4a. If working with a co-investigator external to The College at Brockport, you must include the following supporting document:

CITI or NIH certification for any external collaborator who will have direct contact with participants

5. Research assistants (if any):

N/A

6. Study Timelines:

Estimated start date:	September 2020
Will this project be completed within one calendar year?	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO

7. Setting—List all facilities/sites where you will be collecting data. Include physical address(es).

Tuttle North Athletic Complex (350 New Campus Drive, Brockport, New York 14420); Exercise Physiology Lab and Tuttle North Gymnastics Gym

7a. If research is taking place at an external institution or agency (e.g., not within the College at Brockport), you must include the following supporting documents in the appendix:

- Letter, e-mail or verbal script used to solicit support from external institution or agency
- Form H—Letter of Support from External Institution or Agency

SECTION TWO: PROTOCOL NARRATIVE/INSTRUMENTS

8. Describe the purpose, specific aims, or objectives of your research project such that it can be understood by a reviewer outside of your academic discipline. (1-2 paragraphs)

The purpose of this research project is to determine the effect of nitrate on anaerobic performance, specifically in gymnasts. Nitrate is reduced to nitric oxide and helps to dilate blood vessels, promoting greater blood flow. During exercise, muscle tissue has an increased oxygen demand, requiring greater blood flow to meet its needs. Nitrate supplementation is aimed to help blood vessels open to allow more oxygen- and nutrient-rich blood to reach the working muscle tissues. Many studies have shown benefits of nitrate supplementation in aerobic activities, or longer-duration activities that require oxygen for energy production. Anaerobic activities, like gymnastics, are higher-intensity activities that utilize other energy sources because energy production by oxygen has delayed-onset and is not immediately available. Without oxygen, lactic acid builds up in tissues and causes fatigue. More recent studies have looked into nitrate supplementation for anaerobic activities mainly focused on promotion of lactate removal.

This research project will use volunteers from a team of gymnasts that follow the same training regimen. They will be asked to drink a beetroot juice supplement prior to training for one week and a placebo for one week, with a washout week in between the two trial periods. The subjects will not know whether or not they are taking the supplement or the placebo during the study. Prior to the beginning of the study, they will be familiarized with a Wingate cycle ergometer test, which is a 30-second sprint test on a stationary bike, as well as a gymnastics-specific anaerobic mat test that will be done on the gymnastics floor (Specific Aerobic Gymnastics Anaerobic Test - SAGAT). They will perform these tests at the end of each trial week and their values will be compared between their own post-supplement performance values and post-placebo performance values. These values will include mean anaerobic power, lactate clearance, peak anaerobic power, fatigue index, and time to complete the mat test.

9. State the research question(s) or the hypothesis to be tested.

How will nitrate supplementation by beetroot juice affect the fatigue index, lactate clearance, mean and peak anaerobic power, and time to completion of a SAGAT test in collegiate gymnasts?

We hypothesize that nitrate supplementation will enhance performance markers including mean anaerobic power, peak anaerobic power, lactate clearance, fatigue index, and time to complete the SAGAT.

10. Summarize relevant existing data, literature, past and ongoing studies, and how your study ties in with these. Use in-text citations where appropriate. (2 paragraphs minimum)

Nitrate is a compound taken in through diet, mainly from vegetables. It is reduced within the body to nitric oxide (NO) and has many critical roles in cardiovascular physiology. NO lies between the lumen, or opening, and smooth muscle cells within blood vessels. When stimulated, it promotes vasodilation to allow greater amounts of blood flow and oxygen delivery to tissues (1). NO is known to significantly lower blood pressure and increase mitochondrial respiration (5). It also prevents thrombosis (clots), proliferation (growths of plaque), and atherosclerosis (pathological hardening of arteries from plaque formation), further promoting efficient blood flow (1).

Nitric oxide levels naturally increase during physical activity to increase vasodilation, delivering greater amounts of oxygen and nutrients by increasing blood flow to working muscles. NO alters glucose uptake and metabolism to make it more accessible for the muscles (1). The ability to

better deliver oxygen, glucose, and other essential nutrients is critical to maintaining exercise intensity (1), which is why nitrate supplementation has become a topic of interest for exercise physiologists. Many have looked into aerobic exercise performance due to nitrate's vasodilating properties and aerobic energy production's oxygen-dependency. Studies have found a lower rating of perceived exertion (RPE), faster end speed of an aerobic test or event (8), increased rates of gas exchange, improved blood flow to working muscles, and improved exercise tolerance. One study provided physically active males either a beetroot juice (BRJ) supplement or placebo and found improvements in their final speed and RPE during a 5k run. The subjects were able to maintain a higher exercise intensity later in the event when the placebo group started showing signs of fatigue, which was attributed to the steadily increasing nitrite levels throughout the 5k (8).

Studies analyzing the effects of nitrate supplementation on anaerobic exercise performance are not as numerous. Some anaerobic studies have found altered contractile function and metabolic and vascular control in type II, or fast-twitch, muscle fibers (2). It has also been shown to improve contractile force, rate of force development, and calcium release, as well as increasing both the relative and absolute blood flow to contracting type II muscle fibers (2). In addition to increasing contraction capacities and efficiency of type II muscle fibers through increased mitochondrial efficiency, one study found that nitrate supplementation even increased the proportion of type II muscle fibers (3).

The most widely seen results of BRJ supplementation on anaerobic performance are increased time to exhaustion and increased exercise tolerance (7). This means that subjects were able to work for a longer period of time before reaching fatigue, and they were able to work at a higher intensity. At about 70-75% maximal intensity activity, subjects in the BRJ were able to work for 14-16% longer than those in the placebo group before reaching exhaustion (5). This was also seen in trained cross-fit individuals (6) and cycling tests (9), as well as active, but not highly trained individuals (2, 10). Palveo's cycling test even found less post-test lactic acid accumulation during high workloads after supplementation. A Wingate test found final lactate levels being 82.6% higher in the BRJ group than the placebo group, attributing to greater amounts of lactate clearance from muscle tissue, as well as their peak power being significantly higher (4).

Although the relationship between increase nitrate consumption and aerobic performance has been thoroughly studied, the limited studies that have been conducted on potential anaerobic benefits of BRJ supplementation have had controversial results. Many studies struggle to find concrete evidence as to which variable of anaerobic performance may or may not be benefitted by BRJ supplementation. Much of the controversy lies within the dosage and timing of supplementation, as well as specific tests to accurately analyze any effects (4). Each of the studies have varied in concentration, dosage, and time supplementation was consumed prior to testing. The trend towards higher dosages and chronic consumption have shown the most promising results, but exact values have not been determined (7).

This study will be using "Beet-It" beetroot juice shots as the supplementation given to the participants. They will take one shot per day for 6 days prior to testing. The supplement or placebo will be taken 2 hours before training each day. Each "Beet-It" shot contains 400mg of nitrate in a 70mL bottle. The present study is interested in recording the effects beetroot juice has on peak and mean power, lactic acid clearance, fatigue index, and specific performance markers for gymnasts. Although much of the research has been focused on aerobic performance, recent studies have focused on anaerobic sport performance benefits of nitrate.

11. Provide a list of the references cited above.

1. Bondonno, C.P., Croft, K.D., Hodgson, J.M. Dietary Nitrate, Nitric Oxide, and Cardiovascular Health. *Critical Reviews in Food Science and Nutrition*. 2016;56(12). DOI: 10.1080/10408398.2013.811212.
2. Breese, B.C, McNarry, M.A., Marwood, S., Blackwell, J.R., Bailey, S.J., Jones, A.M. Beetroot juice supplementation speeds O₂ uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. *American Journal of Physiology – Regulatory, Integrative, and Comparative Physiology*. 2013;305(12):R1441-R1450. DOI: 10.1152/ajpregu.00295.2013.
3. De Smet, S., Deldicque, L., James, R., Slae, C., Bishop, D.J., Hespel, P. Nitrate Intake Promotes Shift in Muscle Fiber Type Composition during Sprint Interval Training in Hypoxia. *Frontiers in Physiology*. (2016):7. DOI: 10.3389/fphys.2016.00233.
4. Domínguez, R., Garnacho-Castaño, M.V., Cuenca, E., García-Fernández, P., Muñoz-González, A., de Jesús, F., Lozano-Estevan, M.D.C., da Silva, S.F., Viega Herreros, P., Maté-Muñoz, J.L. Effects of Beetroot Juice Supplementation on a 30-s High-Intensity Inertial Cycle Ergometer Test. *Nutrients*. 2017;9(12):1360. DOI: 10.3390/nu9121360.
5. Kelly, J., Vanhatalo, A., Wilkerson, D. P., Wylie, L. J., & Jones, A. M. (2013). Effects of Nitrate on the Power–Duration Relationship for Severe-Intensity Exercise. *Medicine & Science in Sports & Exercise*, 45(9), 1798–1806. DOI: 10.1249/mss.0b013e31828e885c.
6. Kramer SJ, Baur DA, Spicer MT, Vukovich MD, Ormsbee MJ. The effect of six days of dietary nitrate supplementation on performance in trained CrossFit athletes. *Journal of the International Society of Sports Nutrition* 2016;13(1).
7. Kokkinoplitis, K., Chester, N. The effect of beetroot juice on repeated spring performance and muscle force production. *Journal of Physical Education and Sport*. 2014;14(2):242-247.
8. Ormsbee, M.J., Lox, J., Arciero, P.J. Beetroot juice and exercise performance. *Nutrition and Dietary Supplements*. 2013;5:27-35. DOI: 10.2147/NDS.S52664.
9. Palevo G., Williams, N., Harp, A., Barring, E., Mize, L.B. High Concentrated Beetroot Juice Supplement Improves Cycling Power, VO₂, Time to Exhaustion, Heart Rate and Anaerobic Threshold in Trained Cyclists. *Journal of Exercise Physiology Online*. 2019;22(4):39-45.
10. Perez, J., Dobson, J., Ryan, G., & Riggs, A. (2019). The Effects of Beetroot Juice on VO₂ max and Blood Pressure during Submaximal Exercise. *International Journal of Exercise Science*, 12(2), 332–342.

12. Research Design: Quantitative

Qualitative

13. Specify the research design (e.g., experimental, correlational, case study, etc.):

Experimental, crossover

14. How will this research design answer your research questions(s)? (1-3 sentences)

Direct manipulation of supplements consumed by subjects will allow us to determine whether improvements in performance can be attributed to the dietary manipulation and not outside sources. We will analyze performance measures to determine if the supplement

will improve the subjects' power and rate of fatigue. We will also measure blood lactate levels and compare them between the values while consuming the nitrate supplement and the placebo to determine the effectiveness of the supplement in lactate removal.

15. Will deception procedures be used in this study? Yes No

If yes, attach Form G in appendix.

16. Will data collection be audio-/video- recorded? Audio Video No

16a. Alternative data collection methods if participants do not consent to audio- or video-recording:

Click or tap here to enter text.

17. Instruments:

- N/A *secondary analysis of existing data
- Created by researcher / Interview
- Existing instrument in public domain
- Existing instrument not in public domain
- Adapted from existing instrument
- Created by researcher / Interview

18. Please list each instrument with a description and citation, if applicable. Attach the specific instruments as well as permission to use each instrument in the appendix.

Health History Questionnaire. This will only be used for screening purposes. Procedures for the SAGAT and Wingate Tests are attached on a separate document.

19. Link to instrument if using online platform (e.g., Qualtrics):

N/A

20. For research taking place in a classroom or educational setting ONLY: Describe in detail the activities planned for non-participants (if applicable) and explain where both participants and non-participants will be during the research activities.

N/A

SECTION THREE: PARTICIPANT SELECTION & RECRUITMENT

21. Participant Information and Recruitment Process

Indicate the maximum total number of participants that will be recruited or records that will be reviewed.	<u>35</u>
Bear in mind that all recruited participants must have an equal opportunity to participate in the study.	
Age Range of Participants (check all that apply):	<input type="checkbox"/> Under 18 <input checked="" type="checkbox"/> 18 or over <input type="checkbox"/> N/A

22. Indicate whether you will specifically target any of the following vulnerable populations in your study:

- | | |
|--|---|
| <input checked="" type="checkbox"/> N/A | <input type="checkbox"/> Non-English speaking individuals |
| <input type="checkbox"/> Students of principal investigator (PI) or staff/research team | <input type="checkbox"/> Limited or non-readers |
| <input type="checkbox"/> Students (K-12) in an educational setting (in class or at school) | <input type="checkbox"/> Economically/educationally disadvantaged individuals |
| <input type="checkbox"/> Employees supervised by PI, research member or research sponsor | <input type="checkbox"/> Wards of the state (e.g., foster children) |
| <input type="checkbox"/> Prisoners | <input type="checkbox"/> Institutionalized patients/residents |
| <input type="checkbox"/> Refugees | <input type="checkbox"/> Individuals with impaired decision-making capacity |
| | <input type="checkbox"/> Other – Explain below: |

Click or tap here to enter text.

23. If you checked any of the boxes above, describe the additional precautions that will be taken to protect these individuals from coercion or undue influence during the recruitment and/or consent process:

N/A

24. Describe any criteria that define who will be *included* in your study and provide a rationale:

Participants in my study will be collegiate gymnasts between the ages of 18 and 22. This will ensure that all subjects will be on the same training regimen and reduces any potential age factor. Included participants will have no orthopedic limitations such as injury to ensure that they will be able to comply with the training regimen and testing. All participants will have received the necessary health history clearance from the school in order to participate in intercollegiate athletics.

25. Describe any criteria that define who will be *excluded* from your study and provide a rationale:

Anyone with an orthopedic limitation such as an injury will not be permitted to participate in the study because they will not be able to comply with training or be able to complete the tests. Anyone 17 or younger or 23 or older will be excluded from the study. Anyone that does not receive clearance from the health history and/or athletic trainers will be excluded from the study.

26. Describe screening procedures for determining participants' eligibility, if applicable. Screening refers to determining if prospective participants meet the inclusion and exclusion criteria described above.

All student-athletes are required to be screened by a physician before they are allowed to participate in collegiate sports. Investigators will also provide a pre-screening health history questionnaire to ensure no present issues at the start of the study.

27. Check all recruitment methods/materials you plan to use. Attach all recruitment materials in the appendix.

- N/A *secondary analysis of existing data
- Recruitment letter
- Recruitment e-mail
- Flier
- Verbal script
- Tabling

Social media posting (specify platform)

Other materials/methods—explain below:

Click or tap here to enter text.

28. Describe when, where and how potential participants will be recruited. Be specific.

At the beginning of the academic semester, Amy Eck will have a meeting with the gymnastics team in the Tuttle North Gymnastics Gym to explain the study and ask for volunteers to participate. She will provide a handout explaining the entire study and explain all of the procedures and risks associated with participation and answer any questions that potential participants may have. She will allow them 1 week to review the responsibilities and then will present the informed consent form to volunteers.

SECTION FOUR: INFORMED CONSENT/MINOR ASSENT

29. For expedited and exempt studies, you may request to waive documented/signed consent if one of the following options applies:

- **Option 1:** The only record linking the participant and the research is the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each participant will be asked whether they want documentation linking them with the research and their wishes will govern. The research is not subject to FDA regulations.
- **Option 2:** The research presents no more than minimal risk of harm to participants and involves no procedures for which written consent is normally required outside of research context.

Click or tap here to enter text.

Is a waiver of documented/signed consent requested?

YES

NO

29a. If YES, which of the above option applies? 1 2 3

30. Informed Consent Process—Check all methods you plan to use:

- N/A *secondary analysis of existing data
- Online consent (e.g., clicking to indicate consent, electronic signature, digital signature)
- In person
- By regular mail, including interoffice mail
- By e-mail
- By phone
- Other method—explain below:

Click or tap here to enter text.

31. Describe in detail how, when and where you will seek informed consent from participants (or from participants' legally authorized representative), keeping in mind that exempt studies still require a statement of consent to be shared with prospective participants. Attach consent documents in appendix.

Once volunteers have agreed to participation in the study, they will be assigned a time slot for familiarization of testing procedures. When they arrive at the first visit, they will be provided an informed consent form to fill out prior to starting the familiarization process.

32. Minor Assent Process—If working with children, describe in detail how, when and where you will seek minor assent. Attach assent documents in the appendix.

N/A

33. In special cases, you may request to waive or alter the informed consent process if ALL of the following apply:

- Research is no more than minimal risk;
- Research could not be carried out without requested waiver/alteration;
- Waiver/alteration will not adversely affect the rights and welfare of participants;
AND
- When appropriate, additional information will be provided to participants after they have completed the study.

Is a waiver or alteration of informed consent requested? YES NO

33a. If YES—explain below:

Click or tap here to enter text.

SECTION FIVE: METHODS & PROCEDURES

34. Provide a detailed description of the methods and procedures to be followed during this research project, including the role(s) of the researcher and research assistants.

As applicable, provide: a) data collection timeline, b) number and duration of study visits, c) overall follow-up time, d) total time participants will be enrolled in the study, and e) procedures for member-checking. You may wish to include a table or chart showing the research timeline.

Provide enough detail so that a reviewer outside of your discipline will understand the research. Depending on the nature of your research, response length may range from 1-2 paragraph to multiple pages.

There is no need to include detailed participant recruitment, screening or consent procedures in your response, as these have already been addressed within the proposal form.

After filling out the informed consent form at the initial visit, they will be led through the Specific Aerobic Gymnastics Anaerobic Test (SAGAT) and Wingate Test as described in the informed consent form, lasting about 1 hour and 15 minutes. No supplementation will take place prior to the first visit. Subjects will be given 3-4 weeks to become reacclimated to the team's training regime, then the study will proceed with supplementation. Participants will complete a

3-day dietary recall using the “My Fitness Pal” app before supplementation begins. They will be asked to consume roughly the same diet throughout the study, and they will be asked to complete another 3-day dietary recall before the second treatment period. Subjects will be randomly assigned a confidential number for group assignment and data collection. The first round of supplementation will last 6 days prior to testing. The subjects will be randomly assigned either the nitrate supplement or placebo for that week and will take it approximately 2 hours prior to resistance or cardiorespiratory training.

At the end of the week, subjects will be assigned a time slot to come perform the SAGAT and Wingate test. This visit will last approximately 1 hour and 15 minutes. A baseline blood lactate measurement will be taken, then they will be led through a standardized warm-up. All investigators are trained to collect fingerstick blood samples and will aid in collecting samples. To take a blood lactate sample, the investigator will be required to wear gloves. The investigator will thoroughly clean the subject’s finger with an alcohol wipe and allow the finger to dry. Putting slight pressure on the finger, the investigator will use a new lancet to pierce the skin on the tip of the finger. The first drop of blood will be wiped away, and a fresh drop of blood will be gently pushed out. With a new lactate strip in the Analox LM5 Lactate Analyzer, the end of the strip will be carefully place in the new drop of blood until the strip is filled. The analyzer will show a blood lactate reading, which will be recorded. The investigator will then remove the initial strip and repeat the lactate reading with a fresh strip. The second value and the average of these values will be recorded. The participant should expect a range of 1 to 9 finger pricks during each testing visit.

First, they will perform the SAGAT, for which they will be asked to perform a series of sprints, tuck jumps, push-ups and L-holds and will be timed for completion. After this period, subjects will get a 20-minute recovery period. During this period, another blood lactate measurement will be performed immediately after, 5 minutes after, 10 minutes after, 15 minutes after, and 20 minutes after completion. After the 20-minute recovery period, subjects will be led through a standardized warm-up for the Wingate, then will perform 30 seconds of maximal effort, followed by a cool-down period. Peak and mean anaerobic power and fatigue index will be measured by a computer system and recorded. Blood lactate measurements will be recorded immediately after, 3 minutes after, and 10 minutes after completion.

The week following the first testing period will be a wash-out week, in which subjects will continue the training regimen without supplementation.

During the final week of the study, the subjects that received the nitrate supplement will take the placebo, and vice versa. Supplements will be taken 2 hours before resistance or cardiorespiratory training for 6 days prior to testing. The final day of testing will last about 1 hour and 15 minutes and will be conducted the same as the second visit/first testing day.

Participants will be enrolled in the study for a total of 3 weeks, with 1 day prior to familiarize testing procedures.

Amy Eck will be conduction the majority of data collection. At least one Brockport faculty member, Dr. Heidi Byrne, Dr. Craig Matter, or Dr. Justin Faller will be present on each day of testing. Brockport faculty will aid in data collection where needed. All investigators are trained with the Wingate Cycle Ergometer test and Amy Eck is familiar with the SAGAT.

SECTION SIX: COMPENSATION & RISK MANAGEMENT

35. Compensation/Incentives – Form of compensation:

- N/A
 Cash
 Check
 Gift card/gift certificate

- Voucher
 Raffles/lotteries
 Course/extra credit
 Other – explain below:

Click or tap here to enter text.

36. Describe compensation and how participants should be compensated. Include how you will document compensation.

Participants will be paid for their involvement in the study. Each participant that completed the training and all three testing sessions will receive \$25 at the conclusion of the study. Participants that are unable to complete the study will receive no compensation.

37. Risks—Indicate possible risks for participants, including the minimal risk of time spent participating and potential risks to privacy and confidentiality:

Maximal exertion exercise can result in lightheadedness and nausea, as well as potential orthopedic injury, but risks are minimal. Beetroot juice supplementation can result in red urine and feces from undigested red pigment (betanin) in the beets, as well as gastrointestinal issues such as stomach aches, constipation, bloating, and gas, but the risks are also minimal. There is a minimal risk of anaphylactic shock with an allergic reaction. Discomfort may be experienced from taking blood samples and during training and testing. There is a very small risk of infection during blood sampling. Blood sampling will only be taken during visits 2 and 3. Samples will be taken upon arrival, in 5-minute intervals after completing the SAGAT, and at the 3- and 10-minute mark after the Wingate test. We will likely not need to re-prick participants' fingers at each measurement, but will re-prick when we are unable to obtain an adequate amount of blood for lactate readings. This will likely only be needed for the initial measurement after each test. Participants will take beetroot juice supplements for two six-day periods. Each dose will be 2.4 ounces of beetroot juice, containing 400mg of nitrate.

37a. Describe precautions to be taken to minimize or eliminate these risks:

To combat risks associated with exercise (lightheadedness, nausea, and injury), participants will be given a proper warm-up, cool-down, and will be closely monitored by research technicians and Brockport faculty. There will also be an AED at the training and testing site. During blood sampling, researchers will follow strict sterile protocols. Participants will be given numbers corresponding to their experimental group (supplement or placebo) and their data and will only be accessible to study investigators. The participants will not be identified in the manuscript, and data files will be shredded at the conclusion of the study.

37b. Benefits—State any direct personal benefits to participants—if any—keeping in mind that most social and behavioral research does not offer any direct personal benefits:

Although there are no direct benefits for participants, there is a potential for benefits in this field of study. Information obtained from this study may help the exercise, and specifically athletic community to determine the most appropriate exercise enhancement and recovery techniques. This will help others learn ways in which they can enhance their performance through dietary interventions.

SECTION SEVEN: PRIVACY & CONFIDENTIALITY

38. Privacy and Confidentiality—List any personal identifiers to be collected, including IP addresses: [Note: Some online survey platforms can provide data without IP addresses]

Identifiers include the age, gender, height, and weight of subjects.

39. Coding—If using pseudonyms, numbering or another coding system, explain how a master list connecting codes to participant names will be protected (check as many as apply):

- N/A Not using coding
- N/A Using coding, but not maintaining master list connecting codes to names
- In password-protected file on password-protected device (using different passwords)
- Encrypted file
- In locked drawer (separate from data storage)
- Other—explain below:

Click or tap here to enter text.

39a. Explain how non-investigators will be prevented from accessing participants' identifiable information.

All information will be password-protected and only accessible to researchers.

40. Results will be reported:

Individually, using coding or pseudonyms

As aggregate data

Other—explain below:

Click or tap here to enter text.

41. Will identifiable data be retained once data collection is complete? YES
 NO

If yes—explain below:

Click or tap here to enter text.

42. Provide *physical location* where the identifiable data and IRB documentation will be kept during the research and after the study has been closed. The repository should include, at minimum, copies of IRB correspondence as well as signed consent documents. This documentation should be maintained for a minimum of 3 years after the study has been closed.

Document type:	<u>Storage location (street address, building, room number, etc.):</u>
<u>Identifiable data & signed consent forms</u>	<input type="checkbox"/> N/A <i>While study is active:</i>

	Data and forms will be locked in the Tuttle North Exercise Physiology Lab (TN230). <i>After study has been closed:</i> Data and forms will be deleted or shredded.
Copies of IRB correspondence/documentation (approval letters, continuation/modification approvals, approved protocol with all attachments)	<i>While study is active:</i> Data and forms will be locked in the Tuttle North Exercise Physiology Lab (TN230). <i>After study has been closed:</i> Data and forms will be deleted or discarded.

43. Method of destroying identifiable data and IRB documentation (after a minimum of 3 years per SUNY regulations):

<u>Identifiable data & signed consent forms</u>	<input type="checkbox"/> N/A <input checked="" type="checkbox"/> Delete files <input checked="" type="checkbox"/> Shred paper <input type="checkbox"/> Other—explain below:
Copies of IRB correspondence/documentation (approval letters, continuation/modification approvals, approved protocol with all attachments)	<input checked="" type="checkbox"/> Delete files <input checked="" type="checkbox"/> Shred paper <input type="checkbox"/> Other—explain below:

44. Timeline for destroying identifiable data and IRB documentation:

<u>Identifiable data & signed consent forms</u>	<input type="checkbox"/> N/A <input checked="" type="checkbox"/> 3 years (minimum per SUNY regulations) <input type="checkbox"/> Other—explain below:
Copies of IRB correspondence/documentation (approval letters, continuation/modification approvals, approved protocol with all attachments)	<input checked="" type="checkbox"/> 3 years (minimum per SUNY regulations) <input type="checkbox"/> Other—explain below:

SECTION EIGHT: SUPPORTING DOCUMENTS

Check the supporting documents included in protocol submission:

- Recruitment notices, letters, e-mails, fliers, scripts, etc.
- Form A—Informed consent/assent document(s)
- CITI certification for College at Brockport PI, co-PI(s) and research assistants
- CITI or NIH certification for any external collaborators
- Any survey instruments, psychological tests, interview forms, interview protocols, etc.

- Written permission to use testing instrument
- Instructions to participants for use of instrument

Forms C-J:

- C—Minor assent document, if applicable
- D —Research Using Specialized Equipment
- E—Research Involving Psychological Intervention
- F—Research Involving Physiological Intervention
- G—Research Involving Deception
- Letter, e-mail or verbal script used to solicit support from external institution or agency
- Form H—Letter of Support from External Institution or Agency
- Form J—Student as Principal Investigator
- Other—explain below:

Form M – Recruitment Letter for Adult Participants (18 years or older)
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SECTION NINE: INVESTIGATOR'S PLEDGE

By submitting this protocol, you certify that you accept responsibility for and will follow the ethical guidelines set forth by the Belmont Report, Declaration of Helsinki, the Nuremberg Code and the Ethical Principles of the American Psychological Association (if applicable), as well as by The College at Brockport. You have the requisite funding, credentials and training, if needed, to carry out all procedures involved in this protocol.

Submitting the protocol also affirms that the information you have provided concerning the procedures to be taken for the protection of human participants is correct; no other procedures will be used in this project; you will seek and obtain prior approval from the IRB for any modification in this project; and you will promptly report any unexpected or otherwise significant adverse effects encountered in the course of this study to the IRB.

Appendix B*Recruitment Letter*The Effect of Nitrate Supplementation by Beetroot Juice on Anaerobic Performance Measures of Collegiate Gymnasts

Dear Colleagues,

I am currently an undergraduate student at The College at Brockport. I am pursuing a bachelor's degree in exercise science and am a member of the Brockport Honors College. One requirement of the Honors College is to complete a senior thesis. For my thesis, I wish to study the effect of nitrate supplementation on anaerobic performance measures of gymnasts.

Before I am able to conduct any research, I need to obtain informed consent from you. I will attend one practice in which I will discuss details about the study. I will answer questions and provide informed consent forms to everyone willing to participate. Please read the consent form and contact me with any questions or concerns. If you would like to participate in the study, please contact me, and we will schedule you for your first visit.

You can reach me by email at aeck3@brockport.edu or by phone at (571)442-4131.

Best,
Amy Eck

Appendix C

Recruitment Script

Hi. My name is Amy Eck and I am pursuing a degree in exercise science and am a member of the Brockport Honors College. One of the requirements for the Honors College is to complete a senior thesis. For my thesis, I wish to study the effect of beetroot juice on anaerobic performance measures of gymnasts.

I want to study this because I have grown an interest in nutrition on exercise and athletic performance. Beetroot juice contains high levels of nitrate, which helps to open blood vessels to working muscles during exercise. Dilation of blood vessels can help with delivery of oxygen and other nutrients to the muscles, and clean out any waste products, such as lactic acid. The hope of my study is to determine whether supplementing with beetroot juice aids in lactate clearance after exercise, which could help to enhance gymnastics performance.

I am here today to ask you if you would like to become a participant for my study. The study will compose of an initial testing day and two separate 6-day supplementation periods followed by one day of anaerobic testing. Each day of testing will be about 1 hour and 15 minutes. During the first visit, you would be asked to complete a Specific Aerobic Gymnastics Anaerobic Test (SAGAT), composed of a series of sprints, tuck jumps, push-ups, and L-holds. This test lasts about two minutes. After a 20-minute recovery period, you would be asked to complete a Wingate Cycle Ergometer Test. This test is a 30-second maximal effort test on a stationary bike. Including the warm-up and cool-down periods, this test takes about 20 minutes total.

At the beginning of the supplementation periods, you would be randomly assigned to either the nitrate drink or placebo group. For the nitrate group, you would be consuming a 2.4-ounce dose of beetroot juice, containing 400mg of nitrate. You would consume one dose around 3:00 for 6 days prior to testing. During these 6 days, you would be asked to complete specified training for four of the days. For testing, you would be asked to come into either the Tuttle North Exercise Physiology lab upstairs in room 230 or here in the Tuttle North Gymnastics Gym. You would complete the same procedure as your initial visit with the SAGAT and Wingate tests. During these visits, we will also be collecting blood samples from a small finger prick for blood lactate measurements. You can expect a range of 1-9 finger pricks. These will be collected immediately after each test, at the 5-, 10-, 15-, and 20-minute mark after the SAGAT and at the 3- and 10-minute marks after the Wingate test. We will not likely have to re-prick your finger for each of these samples. Often times, we are able to get enough blood from the original finger prick for several samples. The most likely times we will need to prick your finger would be when you arrive to the testing and after each test.

The beetroot juice supplement may give you some gastrointestinal issues such as stomach pains, constipation, bloating, and gas and may give a reddish coloration to your urine and/or feces. Potential discomforts from the tests include nausea, lightheadedness, and dizziness, as they are maximal-exertion tests. Brockport faculty will always be present during testing, as well as an AED and you would be given standardized warm-ups and cool-downs to minimize the risk of these adverse events.

As compensation for participation in the study, you would receive a gift card after the conclusion of the study. You would receive \$25 for completing the training, as well as all three days of testing. Participants that are unable to complete the study will receive no compensation.

You can ask me questions anytime in person, or you can call, text, or email me. My email is [aek3@brockport.edu](mailto:aeck3@brockport.edu) and my phone number is (571)442-4131.

Thank you.

Appendix D



The College at
BROCKPORT
 STATE UNIVERSITY OF NEW YORK

Institutional Review Board

Form A—Statement of Informed Consent For Adult Participants
THE EFFECT OF NITRATE SUPPLEMENTATION BY BEETROOT JUICE ON ANAEROBIC PERFORMANCE MEASURES OF GYMNASTS
KEY INFORMATION:

- You are being asked to be in a research study of the effect of nitrate supplementation on anaerobic performance of gymnasts. As with all research studies, participation is voluntary.
- The purpose of this study is to determine whether chronic nitrate supplementation leads to a significant improvement in post exercise lactate clearance as well as improvements in mean and peak anaerobic power, fatigue index, and time to completion for the Specific Aerobic Gymnastics Anaerobic Test.
- A maximum of 35 people will take part in this study. The results will be used for completion of an honors thesis project and possible publication.
- If you agree to take part in this study, you will be involved in this study for a total of three weeks with one additional introductory session. There will be 3 days of testing, and each visit will be 1 hour and 15 minutes. I will be taking the supplement for two separate 6-day periods, in which I will also be required to complete specified training. No follow-up information will be collected after the conclusion of the study.
- If you choose to participate, you will be asked to complete a dietary recall, come to the Tuttle North Exercise Physiology Laboratory (Tuttle North Room 230) for three separate testing sessions, and attend 4 training sessions per week for three weeks in the Tuttle North Gymnastics Gym.
- It is important to realize that you will be asked to perform maximal exertion and can lead to nausea and lightheadedness. Precautions to combat these risks include completing a proper warm-up, cool-down, being closely monitored by research technicians, having Brockport faculty present, and having an AED present. You should also understand that supplementation of beetroot juice may result in red coloration of urine and feces, but that the discoloration is due to the color of the supplement. Supplementing beetroot juice may also result in gastrointestinal discomforts such as gas, bloating, constipation, and stomach aches. You may

experience red coloration in urine and feces from undigested betanin, which is the red pigment in beets. You will be asked to attend three individual visits for testing. Each visit will take about 1 hour and 15 minutes, adding to a total of 3 hours and 45 minutes of total testing time. You will also be asked to comply with the training regimen of 1 hour of training for four days in the week prior to testing.

- Although there are no direct benefits for participants, there is a potential for benefits in the field of study. Information obtained from this study may help the exercise, and specifically athletic community to determine the most appropriate exercise enhancement and recovery techniques. This will help others learn ways in which they can enhance their performance through dietary interventions.

You are being asked to be in a research study of beetroot juice supplementation on gymnastics performance. This study is being conducted in the Tuttle North Exercise Physiology Lab (Tuttle North room 230 and the Tuttle North Gymnastics Gym. This study is being conducted by: Amy Eck in the Kinesiology, Sport Studies, and Physical Education (KSSPE) department at The College at Brockport.

You were selected as a possible participant because you are a member of the Brockport Gymnastics Team and have no outstanding medical or orthopedic limitations that would affect your performance in the study.

Please read this consent form and ask any questions you have before agreeing to be in the study.

PROCEDURES:

If you agree to be in this study, you will be asked to do the following:

I will be asked to complete a dietary recall and come to the Tuttle North Exercise Physiology Laboratory (Tuttle North Room 230) and the Tuttle North Gymnastics Gym four times per week for three weeks and three separate occasions for testing. The details of each testing visit are provided below.

Visit 1 – (1 hour 15 minutes)

All aspects of the study will be explained to me and I will be given an opportunity to ask questions. During this visit I will be asked to complete a health history form and my height and weight will be measured.

My baseline mean and peak anaerobic power, fatigue index, time to completion for a mat test, and blood lactate values will be measured. I will be asked to perform a Specific Aerobic Gymnastics Anaerobic Test (SAGAT) and Wingate cycle ergometer test. I will be given a standardized warm up before beginning the tests. For the SAGAT, I will be asked to do a series of sprints, tuck jumps, push-ups, and L-holds 6 rounds and repeat the process for a total of 2 sets and will be timed for completion.

Next, I will perform the Wingate cycle ergometer test. I will be given an acclimation period of sprint intervals followed by recovery periods. At the end of the acclimation period, I will be given a 5-minute recovery period where I will continue cycling at a set workload. I will then be asked to sprint for the entirety of the 30-second test. At the conclusion of the test, I will be allowed a standardized cool-down for 5 minutes.

Visit 2 (1 hour 15 minutes)

Prior to visit 2, I will have supplemented with the nitrate drink or placebo for 6 days and completed a standard training routine. Placement in the placebo or nitrate group will be decided by random assignment. The chance of being placed in either group is 1:1.

Upon arrival a finger stick blood sample will be performed in order to determine my baseline values of blood lactate. Standard sterile technique will be employed and only 4-5 drops of blood will be required. This will provide 2 acceptable blood lactate values. I can expect a range of 1-9 finger pricks for each testing visit (Visits 2 and 3).

I will be given the same standardized warm-up from visit 1. I will begin with the SAGAT, performing a series of sprints, tuck jumps, push-ups, and L-holds for 6 rounds. I will be given a 2-minute recovery period and will perform the series again.

I will be given a 20-minute recovery before performing the Wingate cycle ergometer test. During this recovery period, lactate values will be measured every 5 minutes (immediately following exercise, 5 minutes after, 10 minutes after, 15 minutes after, and 20 minutes after). The same method to obtain these values as were used for baseline values.

Next, I will perform a Wingate test as completed in Visit 1. After completion of the cool-down period, my blood lactate measures will be recorded immediately after completion, 3 minutes post-test, and 10 minutes post-test. During this time, I will be walking around the facility to prevent fluid accumulation in my lower extremities.

Visit 3 (1 hour 15 minutes)

This visit will be identical to visit 2. However, if I received the nitrate supplement for the 6-day training period, I will receive the placebo during the second 6-day training period and vice versa. The week following visit 2 will be a wash-out week, in which I will not be taking any supplements. I will begin supplementation again after the wash-out week has concluded.

COMPENSATION/INCENTIVES:

You **will** receive compensation. You will receive a \$25 gift card to Barnes & Noble after the completion of the study. The \$25 will only be given to participants that complete the training and all three testing visits. Participants that are unable to complete the study will receive no compensation.

CONFIDENTIALITY:

The records of this study will be kept private and your confidentiality will be protected. In any sort of report the researcher(s) might publish, no identifying information will be included.

Research records will be stored securely and only the researcher(s) will have access to the records. All data will be kept in a locked file cabinet in the Exercise Physiology Laboratory (TN 230) by the investigator(s). All study records, including approved IRB documents, tapes, transcripts, and consent forms, will be destroyed by shredding and/or deleting after **3** years.

VOLUNTARY NATURE OF THE STUDY:

Participation in this study is voluntary and requires your informed consent. Your decision whether or not to participate will not affect your current or future relations with The College at Brockport or with Amy Eck, Dr. Heidi Byrne, Dr. Craig Matter, or Dr. Justin Faller. If you decide to participate, you are free to skip any question that is asked. You may also withdraw from this study at any time without penalty.

CONTACTS AND QUESTIONS:

The researchers(s) conducting this study: Amy Eck; Heidi Byrne, Ph.D.; Craig Matter, Ph.D.; and Justin Faller, Ph.D. If you have questions, **you are encouraged** to contact the researcher(s) at:

Amy Eck	Heidi K. Byrne, Ph.D.	Craig Mattern, Ph.D.
(571) 442-4131	(585) 395-2601	(585)395-5343
aeck3@brockport.edu	hbyrne@brockport.edu	cmattern@brockport.edu

Justin Faller, Ph.D.
 (585) 395-5340
jfaller@brockport.edu

If you would like to talk to someone other than the researchers, please contact The College at Brockport IRB compliance officer at (585) 395-2779 or IRB@brockport.edu.

STATEMENT OF CONSENT:

I am 18 years of age or older. I have read and understood the above information. I consent to participate in the study.

Signature: _____ Date: _____

Signature of Investigator: _____ Date: _____

Please keep the second copy of this informed consent for your records.

Appendix E



The College at
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Institutional Review Board

Form D—Research Using Specialized Equipment

If the participant(s) in your proposed research will be in contact with any mechanical, electronic, electrical or other equipment which might subject him/her to the possibility of accidental harm or injury, please provide the information requested in items 1-5 below. The use of any such equipment must be approved by the IRB prior to use in any research.

1. Identify and describe the equipment to be utilized. Use manufacturer's names and submit copies of manufacturer's literature on the equipment when available. Submission of schematics of electrical equipment will facilitate approval.

Monark Cycle Ergometer Model 828E; Velotron Cycle Ergometer (velotron.com); Analox LM5 Lactate Analyzer; mats in gymnastics room.

2. Identify and describe how the participant will interact with the equipment.

Monark Cycle Ergometer will be used for standardized warm-ups and cool-downs. Veletron Cycle Ergometer will be used for the Wingate protocol. Analox LM5 Lactate Analyzer will be used before and after tests to measure blood lactate levels. Mats in the gymnastics room will be used for the Specific Aerobic Gymnastics Anaerobic Test.

3. Indicate the exact location of the equipment.

The Monark Cycle Ergometer, Velotron Cycle Ergometer, and Analox LM5 Lactate Analyzer are kept in the Tuttle North Exercise Physiology Lab (TN230), but will be moved down to the Tuttle North Gymnastics Gym for testing. The mats are also located in the Tuttle North Gymnastics Gym.

4. Indicate the names and qualifications (with regard to the safe use of the equipment) for all individuals authorized to use the equipment for this proposal.

Amy Eck; Heidi K. Byrne, PhD.; Craig Mattern, PhD.; and Justin Faller, PhD are all qualified and trained in the use of this equipment and know how to calibrate and operate

the equipment. This equipment is part of the school's Exercise Physiology lab and is regularly used by faculty and students.

5. Describe the specific steps that will be taken to assure the proper operating and maintenance of the equipment.

All investigators are qualified and trained to use this equipment and know how to calibrate and operate the equipment.

Appendix F



The College at
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Institutional Review Board

Form F—Research Involving Physiological Intervention

If the participant(s) of the proposed research will be exposed to any physiological treatments or intervention upon the body by mechanical, electronic, chemical, biological or any other means, please provide the information requested in items 1-7 below.

1. Identify and describe the physiological intervention in detail:

Subjects will be asked to consume a nitrate supplement or placebo for 6 days prior to testing. After a 1-week washout period, the subjects will receive either the nitrate supplement or the placebo, whichever they did not get for the first trial period, for another 6 days before testing. Nitrate is reduced in the body to nitric oxide, which dilates blood vessels. Nitrate supplementation is aimed to increase blood flow throughout the body, specifically to muscle tissue during exercise.

2. Identify and describe the means used to administer the intervention in detail:

Subjects will be provided the necessary dosage of the supplement or placebo and they will be kept in the refrigerator in the team room of the Tuttle North Gymnastics Gym. Subjects will consume the supplement before the beginning of practice, about 3:00p.m., which is about two hours before the beginning of resistance or cardiorespiratory training. The supplements will be organized by one of the researchers, and subjects will not know whether they receive the nitrate supplement or placebo.

3. Identify and describe the behavior expected of participants and the behavior of the investigator during the administration of the physiological intervention in detail:

The participants will be expected to be responsible regarding their supplementation, ensuring that they take it as close to 3:00pm as possible. They should aim to consume roughly the same diet as they provided on their dietary recall during each supplementation period. They will also be expected to ensure that they complete all of the training in time with their supplementation. The investigators will keep all data and supplementation groups confidential until the conclusion of the study. All researchers except one will be

blind to which subjects are receiving the nitrate supplement and which are receiving the placebo, and they are not to share that or any information regarding study results with anyone.

4. Describe how data resulting from this procedure will be gathered and recorded:

Data will be collected electronically and will be stored on a password-secure computer only accessible to study investigators.

5. Identify anticipated and possible physiological, psychological, or social consequences of this procedure for the participants:

Subjects may find a red color in their urine or feces from the beetroot supplement. Orthopedic injuries are possible with the training regimen, but proper warm-ups will be used to minimize risks. Participants may experience nausea or lightheadedness association with maximal-exertion activity.

6. Describe the specific steps that will be taken to assure the proper operation and maintenance of the means used to administer the intervention. Give particular attention to prevention of accidental harm or injury to the human participants. Note that for use of specialized equipment, Form D must also be completed.

One investigator will know which subjects receive the nitrate supplement or the placebo. This investigator will be responsible for providing the proper dosage of each drink and ensuring its availability at the time of consumption. Each subject will have a cup with her name on it prior to daily training.

7. Indicate the investigator's competence and identify his/her qualifications (by training and experience) to conduct this procedure. Provide name, title, department, address, and phone number of the individual(s) who will supervise this procedure.

The investigator – Amy Eck, Undergraduate Exercise Science Student, (571)442-4131 – is CITI Certified. She will be overseen by faculty members at The College at Brockport – Heidi K. Byrne, PhD., Associate Professor, KSSPE, TN 305, (585)395-2601; Craig Mattern, PhD., Associate Professor, KSSPE, TNB308, (585)395-5343; Justin Faller, PhD., Assistant Professor, KSSPE, TNB320, (585)395-5340.

Appendix G



Institutional Review Board

**Form J—Advisor
Responsibility for Student**

 The College at
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 STATE UNIVERSITY OF NEW YORK

**Certification of
Researcher**

This form must be completed and signed by the student's faculty research project advisor, then scanned and submitted to the IRB as a supporting document. By completing this form, the advisor certifies that the student researcher has sufficient training and experience to conduct this study in accordance with the research protocol.

SECTION ONE: STUDENT INFORMATION

<u>Principal Investigator:</u>	Amy Eck
<u>Email:</u>	Aeck3@brockport.edu
<u>Date:</u>	May 6, 2020
<u>Status:</u>	<input checked="" type="checkbox"/> Undergraduate Student <input type="checkbox"/> Graduate Student

SECTION TWO ADVISOR INFORMATION

<u>Advisor Name & Department:</u>	Heidi K. Byrne
<u>Advisor Phone:</u>	(585) 395-2601
<u>Advisor Email:</u>	hbyrne@brockport.edu
<u>Expiration Date of Advisor's CITI Certification:</u>	10/14/2021
<u>Advisor Signature</u>	Heidi K. Byrne

SECTION THREE: ADVISOR RESPONSIBILITIES

By checking the boxes below, the student PI's faculty research advisor indicates to take responsibility for student research.	
X	I have thoroughly reviewed this IRB application and verify it is complete and the research is appropriate for student research in topic, scope and design.
X	I will meet or communicate with the student researcher to monitor progress.

X	I assume the roles and responsibilities required to oversee the conduct of this research, prevent harm to subjects and foster benefit to the subjects.
X	I ensure that any unanticipated problems, adverse effects, or incidents which may affect this project will be promptly reported to the IRB.
X	I ensure that this project adhere to all requirements for project modifications and continuing review.
X	If I will be unavailable (e.g., sabbatical leave, vacation, sick leave, etc.), I will arrange for an alternate faculty advisor to assume responsibility during my absence, and I will advise the IRB, in writing, of such changes.

Appendix H

Physiological Testing Protocols

Wingate Cycle Ergometer Test

Before beginning the Wingate Cycle Ergometer Test, the investigator will put the subject's name, height, and weight into the computer. They will adjust the height of the bike seat so that the subject has about ten degrees of knee flexion at the lowest point of the revolution. They will also adjust the seat and handlebars forwards or backwards based on the comfort of the subject.

For the warm-up, subjects will pedal at 60-70rpm with between 100 and 125 watts of resistance. Two minutes into the warm up, the subject will begin sprint intervals, sprinting for 10 seconds with 40 seconds of recovery between sprints. The subject will complete a total of 4 sprints during the warm up. The warm-up will take a total of 5 minutes and 20 seconds. After completion of the warm up, the subject will have a 5-minute recovery phase, steadily pedaling at 100-125 watts of resistance.

After 5 minutes of recovery, the investigator will hit "Start" on the computer to begin test preparation. This will begin the acceleration period. During the first 20 seconds of this period, the subject will pedal at 60-70rpm with about one third of the resistance that will be applied during the test. After 20 seconds, the subject will begin pedaling at a near-maximum speed while the prescribed load is automatically applied. Immediately following the acceleration period begins the 30-second Wingate test. The appropriate load (7.5% of the subject's body weight) is automatically applied to the bike. The subject will sprint during the entire 30 seconds of the test, trying to maintain the highest rpm possible.

At the end of the 30-second test, resistance will be automatically removed and the subject will transition directly into the cool down period. The subject will pedal for 5 minutes at 65 watts of resistance. After the cool down period, the subject will walk around the gym to prevent fluid accumulation in the lower extremities.

Steps:

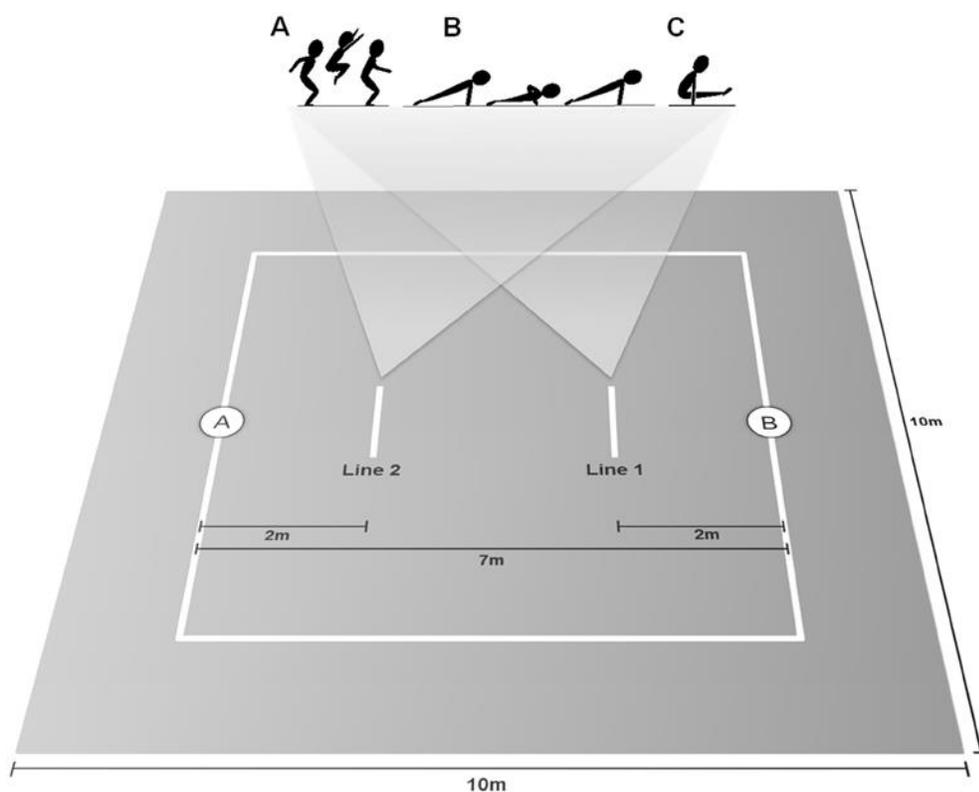
- 1.) Input subject's name, height, and weight.
- 2.) Adjust bike seat height to allow 10 degrees of knee flexion at the lowest point of the revolution.
- 3.) Adjust seat and handlebars forwards or backwards based on the comfort of the subject.
- 4.) Subject will pedal at 60-70rpm with 100-125W resistance for two minutes.
- 5.) Begin sprint intervals. 4 sprints, 10 seconds each with 40 seconds of recovery between sprints.
- 6.) Subject will pedal comfortably for 5 minutes for recovery period with 100-125W of resistance.
- 7.) Begin acceleration period.
- 8.) Pedal at 60-70rpm and about one-third of the prescribed resistance for the first 20 seconds of the acceleration period.
- 9.) Subject will pedal at speeds approaching their max as remaining load is applied.
- 10.) Begin 30-second Wingate test of maximal-effort cycling.
- 11.) After the 30-second test, resistance will be lowered to 65W and subject will have a 5-minutes cool-down period.

- 12.) Subject will walk around gym after the cool-down to prevent fluid accumulation in the lower extremities.

Specific Aerobic Gymnastics Anaerobic Test (SAGAT)

To perform the SAGAT, 4 marks would be made on a 10-meter by 10-meter floor. Position A lies about 2.5m from the end of the floor. Position B is 7m straight across from position A. Lines 1 and 2 will be placed between positions A and B. Line 1 will be 2 meters from position B (5 meters from position A), and line 2 will be 2 meters away from position A (5 meters from position B).

At the start of the test, the subject will start at position A. When prompted to start, the time will start and the subject will touch the floor with their hands and run to position B and touch the floor. They will then turn towards and touch line 1 and perform one tuck-jump (A), two push-ups (B), and one L-support (C). The subject will run back to position B and touch the floor, finishing the first bout. Then, the subject will immediately run to position A and touch the floor, turning to run to and touch line 2 to complete another round of tuck jumps, push-ups, and an L-hold. They will repeat this sequence until they complete 6 bouts as fast as possible (6 bouts = 1 set), and the time will stop. After a 2-minute recovery period, the subject will repeat the test. The sum of both sets will be recorded as the subject's performance score.



Alves et al., 2015.

Alves, C.R.R., Borelli, M.T.C., Paineli, V.d.S., Axevedo, R.d.A, Borelli, C.C.G., Lancha Junior, A.H.J., Gualano, B., Artioli, G.G. Development of a Specific Anaerobic Field Test for Aerobic Gymnastics. *PLoS ONE*. (2015);10(4). DOI: 10.1371/journal.pone.0123115.

Steps:

- 1.) Set up on a 10-meter by 10-meter floor. About 2.5 meters from the edge of the floor is position A. 7 meters from position A is position B. 2m from position B towards position A is line 1. 2m from position A towards position B is line 2.
- 2.) Subject will start at position A.
- 3.) After given the start command, the time will start and the subject will sprint 7m to position B.
- 4.) Subject will touch position B and turn to run to line 1.
- 5.) At line 1, subject will perform one tuck jump, two push-ups, and an L-hold.
- 6.) After completing all three skills, subject will run to and touch position B. This finishes the first bout of the set.
- 7.) Subject will turn around to sprint towards and touch position A.
- 8.) Subject will run to line 2 and repeat the same three exercises (one tuck jump, two push-ups, and an L-hold).
- 9.) Subject will run to and touch position A. This finished the second bout of the set.
- 10.) The subject will continue this pattern until 6 bouts have been completed.
- 11.) After completing the set of 6 bouts, the subject will have a 2-minute recovery.
- 12.) Following the recovery, the subject will repeat the pattern for another set of 6 bouts.
- 13.) The sum of the times of both sets will be recorded as the subject's score.

Appendix I

The College at Brockport, SUNY
Department of Kinesiology, Sports Studies, and Physical Education

HEALTH HISTORY QUESTIONNAIRE

Date _____

I. PERSONAL DATA:

Name _____ Age _____ Gender _____

Address _____

City _____

State _____ Zip _____

Phone: Home (____) _____ Cell (____) _____

E-Mail _____

Occupation _____

Height _____ Weight _____

In case of emergency during testing, contact: Name _____

Relation _____

Phone _____

.....

II. MEDICAL - SURGICAL HISTORY: Check (✓) if answer is yes.

Have you ever had (if so, indicate date):

	Date		Date
() Rheumatic heart disease	_____	() Accidents	_____
() Heart Murmur	_____	() Chest pains	_____
() High Blood Pressure	_____	() Tightness in chest	_____
() Gout	_____	particularly during exercise	
() Varicose Veins	_____	() Shortness of breath	_____
() Lung Disease	_____	() Heart palpitations	_____
() Injuries to back	_____	() Excessive cough	_____
() Epilepsy	_____	() Stroke	_____
() Diabetes	_____	() Heart Attack	_____
() Heart Surgery	_____	() Difficulty sleeping	_____
() Other Operations	_____	() Fatigue	_____
() Kidney Disease	_____	() Calf pain or cramps	_____
() Stomach Ulcers	_____	with exercise	
() Arthritis	_____	() Nervousness	_____
() Hospitalizations	_____	() High Cholesterol	_____
() Cardiac Catheterization	_____	() other problems	_____

Please explain any checked answers and describe any illnesses, surgeries or diseases not listed above:

III. MEDICATIONS:

Please list medications that you are presently taking.

Drug	Dose	Reason for taking
_____	_____	_____
_____	_____	_____
_____	_____	_____

IV. PRESENT HEALTH

What do you consider your present overall state of health to be?_____

If your overall health is not good what is your major complaint or problem and when did the symptoms begin?_____

Have you ever sprained, strained, severely bruised, dislocated, broken, or had chronic pain to any of the following bones or joints? (please circle all that apply)

Jaw	Neck	Shoulder	Elbow	Wrist
Back	Hip	Knee	Ankle	Foot
Shin/calf	Thigh	Arm		

Explain all circled answers:_____

Do you smoke? Yes _____ No _____ If yes, _____ per day for _____ years.

Do you follow a special diet? If yes, please describe_____

Do you drink alcoholic beverages? If yes, _____ per week.

Do you have any known allergies to foods or other drugs?

V. FAMILY MEDICAL HISTORY:

To your knowledge, have any of your relatives had any of the following?:

	Yes	No	Relative (Who?)
Cancer			
Diabetes			
Heart Disease			
High blood pressure			
High Cholesterol			
Stroke			
Peripheral vascular disease			

VI. CURRENT EXERCISE REGIMEN:

Briefly describe any regular cardiovascular/aerobic exercise that you participate in:

Type of exercise	Number of times/week	Number of minutes/session
------------------	----------------------	---------------------------

Briefly describe any regular resistance training (weight lifting) that you participate in:

Do you use machines (e.g., Nautilus, Cybex) or free weights? (Please circle one or both)

Appendix J*Honors Research Grant Application*

The Honors College Thesis Fund was established to support Honors students' thesis research.

Eligible students

must be juniors or seniors in good academic standing in the Honors College. Priority will be given to applicants

who have completed HON 395, Thesis Practicum, or an approved equivalent thesis preparation course.

Appropriate uses of this fund include equipment, materials for field research or creative works, office supplies, print

materials, survey costs and/or travel funds directly related to a student's senior thesis project.

The maximum award

a student may apply for is \$200 (USD). Applications may be submitted any time during the academic year. Students

who are awarded funding are required to submit the reimbursement request with itemized receipt(s).

Name: _____ Banner ID:

(Last) (First) (MI)

Brockport Address:

Permanent Address:

E-mail Address:

Phone Number(s): (_____) _____ (_____) _____

(_____) _____

(Cellular) (Local) (Permanent)

Academic Major(s):

Indicate thesis preparation course (HON 395 or substitute) and semester/year completed:

Thesis Subject:

Anticipated graduation semester/year: _____ Amount of funding requested (\$200 maximum): \$ _____

Please submit the following three items with this completed form:

1. A letter explaining how you will use the proposed amount of funding to conduct your thesis research.

2. A letter of support from the faculty member who serves as your Honors Thesis Director.

3. A current copy of your Degree Works degree audit.

SUBMIT THE COMPLETED APPLICATION WITH SUPPORTING DOCUMENTATION

TO:

Honors College Office

The College at Brockport

133 Albert W. Brown Building

Brockport, NY 14420

For office use only: Institutional GPA: _____ Major GPA: _____ Minimum Credits
Completed: _____
(09/10/19)

Honors College

Thesis Fund

Thesis Project Application Form

Appendix K*Honors Research Grant Student Letter*

Dear Honors Grant Committee,

I appreciate you taking the time to consider my thesis for the Honors Grant.

For my study, I would like to analyze the effect of nitrate supplementation by beetroot juice (BRJ) on performance of gymnasts. The study requires supplementation of Beet-It concentrated beetroot juice. We plan to provide our participants with one dose of BRJ for six days prior to testing in one of the two supplementation periods. During the other six-day period, participants will be given a placebo. The Beet-It supplement is sold for \$50 for 15 bottles of the beetroot juice. We will require a maximum amount of 180 bottles of Beet-It (6 days of supplementation for a maximum of 30 subjects), requiring us to purchase 12 packs of Beet-It. This will total \$600.

Each participant will be given \$25 at the conclusion of the study, given they were able to complete all three days of testing and the two training and supplementation periods. With a maximum of 30 participants for the study, the maximum amount of money required for participant compensation will be \$750.

Any money received from the Honors College will aid in funding for either the supplementation or the participant compensation.

I appreciate your time to review my application.

Sincerely,

Amy Eck

Appendix L*Honors Research Grant Advisor Letter*

The College at
BROCKPORT
STATE UNIVERSITY OF NEW YORK

Kinesiology, Sport Studies and Physical Education

July 28, 2020

Dear Honors College:

I am writing to request research funding for Amy Eck, an excellent student in the Exercise Science Major. I am happily serving as Amy's thesis advisor and we are hoping that we can begin her data collection this fall. The research study is focused on the use of high-nitrate level Beetroot juice the anaerobic performance of female college gymnasts. We are waiting on IRB final approval and will then apply for COVID-19 "return to research" approval.

Amy has done an excellent job with the study design and has worked very hard to get the documents ready and submitted to the IRB. It is our plan to eventually publish the study in a peer-reviewed journal. The funding is needed to purchase the Beetroot juice supplement and/or to provide a small gift card to each participant at the conclusion of the data collection.

Thank you for your financial support of this project. If you would like any further information, please feel free to contact me at hbyrne@brockport.edu.

Sincerely,

Heidi Byrne, Ph.D.
Associate Professor, Exercise Science
SUNY-Brockport