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## The Effect of Dietary Nitrate and Anthocyanins on Anaerobic Exercise Performance

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LOMA LINDA UNIVERSITY  
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**THE EFFECT OF DIETARY NITRATE AND ANTHOCYANINS ON ANAEROBIC  
EXERCISE PERFORMANCE**

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## Abstract

*Background:* Previously, beetroot and tart cherry supplementations have been investigated for their effects on exercise performance and recovery separately. Beetroot containing nitrate has been used to improve blood flow to enhance exercise tolerance. Tart cherry extract containing anthocyanins has been shown to lower inflammation and improve recovery time in exercise performance.

*Objective:* The purpose of this study was to investigate a possible synergistic effect of nitrate and anthocyanins on exercise performance in healthy individuals.

*Design:* This was a double-blind, randomized crossover study with a duration of twenty-nine days. Inclusion criteria included healthy individuals, able to exercise on a stationary bike, and willing to abstain from caffeine, mouthwash, and high antioxidant foods. Exclusion criteria included orthopedic injuries, use of nonsteroidal anti-inflammatory drugs (NSAIDs), known allergy to tart cherry and/or beetroot, and pregnant or breastfeeding.

*Participants:* Healthy men (n=6) and women (n=23), ranging between 19 and 35 years of age. Participants were recruited from Loma Linda University and surrounding cities.

*Intervention:* Participants were randomized into two groups after baseline. Treatment A was supplemented with 5.05g (3.62 mmol) of beetroot powder and placebo. Treatment B was supplemented with 5.05g (3.62 mmol) of beetroot powder and 480 mg tart cherry capsule for seven-days, followed by a seven-day washout period. Treatment was alternated at the end of the 14-day period.

*Main outcomes:* exercise performance (heart rate, speed, and distance)

*Secondary outcomes:* serum lactate and allantoin

*Statistics:* A mixed model analysis was used to measure changes in continuous variables. Mean and standard deviation were calculated for treatment A and treatment B.

*Results:* During the first five seconds of the 30 second sprint, heart rate of treatment A was significantly lower than treatment B. Difference in heart rate ranged from 1.6 to 2.7 BPM.

Participants in treatment B pedaled five meters farther, than treatment A. This outcome was not statistically significant. No significant differences were observed between both treatments on rpm, blood pressure, lactate, and allantoin.

*Conclusion:* Supplementation with beetroot powder and tart cherry for seven days may synergistically improve anaerobic exercise performance by maximizing potential heart rate and energy output.

# **The Effect of Dietary Nitrate and Anthocyanins on Anaerobic Exercise Performance**

## **Introduction**

Underlying inflammation and low oxygen uptake by muscles can hinder athletes from reaching peak performance.<sup>1</sup> Although inflammation is the body's protective response against oxidative stress and cell damage, prolonged exposure to inflammation can lead to pain and loss of function.<sup>1</sup>

Inflammation is a product of cyclooxygenase 2 (COX-2).<sup>2</sup> This enzyme produces eicosanoids, prostaglandin 1 (PG1) and prostaglandin 2 (PG2), which create a pro-inflammatory environment in the body.<sup>2</sup> An increase in underlying inflammation can create side effects, which include heat, pain, redness, swelling, and loss of function.<sup>2</sup>

During vigorous exercise, the body utilizes the creatine-phosphagen system to regenerate ADP back into ATP.<sup>3</sup> This mechanism provides immediate ATP for 10-30 seconds without the assistance of oxygen.<sup>3,4</sup> Muscles contain a limited amount of phosphocreatine. Therefore, anaerobic glycolysis is required to provide short-term ATP needs for up to three minutes.<sup>4</sup> This system is limited by lactic acid build-up. Trained athletes can tolerate higher levels of lactic acid before muscle fatigue. Ultimately, athletes succumb to Onset of Blood Lactate Accumulation (OBLA), which is caused by reduced effectiveness of hemoglobin to hold oxygen related to decreased PH levels.<sup>1</sup> OBLA can manifest as burning and heaviness in legs or major muscle groups.<sup>1</sup>

Anthocyanins from tart cherries and beetroot can blunt secondary muscle damage associated with local response in damaged muscle.<sup>5,6</sup> Anthocyanins are potent antioxidants found in the pigment of blue and purple colored plant foods.<sup>7</sup> Montmorency tart cherries contains high

amounts of antioxidants and have been studied to counteract inflammation by blocking COX-2 enzyme and reducing oxidative stress.<sup>2,8,9</sup>

Beetroot contains nearly 250 mg of nitrates (>4 mmol) per 100 g fresh weight.<sup>10,11</sup> Upon ingestion of dietary nitrate (NO<sub>2</sub>), anaerobic bacteria found in the oral cavity activate nitrate reductase enzyme and convert beetroot into nitrite.<sup>12,13</sup> Once nitrite reaches the stomach, nitrite gets converted to nitric oxide in blood capillaries through enzyme reactions.<sup>14,15</sup> Nitric oxide then serves as a vasodilator to allow more oxygen to be delivered to muscles<sup>8</sup>, thus, improving oxygen supply during aerobic exercise making exercise less demanding for the subject.<sup>16</sup> The physiological effects of beetroot take effect 30 minutes post-consumption, and the effect peaks after 90 minutes and remains elevated for 6 hours.<sup>17,18</sup> Health and performance benefits may last approximately two weeks post-consumption.<sup>8</sup> Bailey et al. have evaluated the effects of 500 millimeter (~16 oz) of beetroot juice on performance in healthy men.<sup>19</sup> In the study, six-day supplementation with beetroot juice extended time of exhaustion by 92 seconds, which represents a 2% decline in time needed to cover a set distance.<sup>19</sup> The same six-day supplementation protocol helped reduce oxygen consumption in trained runners during moderate- and high intensity running, which extended their time of exhaustion by 15%.<sup>7</sup> A follow-up study looking at beetroot consumption taken 2.5 hours prior to exercise by male cyclists before both a four km and 16.1 km time trial, separated by three days, showed similar findings. A 2-3% improvement in performance compared to placebo.<sup>8</sup>

The modified Wingate Protocol used in this study will trigger anaerobic glycolysis. Plasma lactate is a metabolite produced during muscle hypoxia which accumulates until a steady source of oxygen is replenished.<sup>20</sup> This biomarker is an indicator of lactate acidosis caused by inadequate oxygen in plasma. A point-of-care (POC) handheld device has been proven to be

active on emergency room patients who require rapid turnaround measurements.<sup>21</sup> Allantoin, a non-enzymatic product of uric acid oxidation, is an indicator of oxidative stress<sup>22,23</sup> which tends to peak in untrained individuals during subacute exercise.<sup>23</sup> Previous research by Kandaran et al. has showed that plasma allantoin had a significant increase immediately after exercise but returned to its initial level an hour post-exercise.<sup>22</sup> For this study, plasma allantoin was measured before and after exercise.

To date, there have been no studies examining the combined effects of nitric oxide with anthocyanin supplementation on exercise performance as assessed by an anaerobic bike protocol. We hypothesized that anthocyanins would augment the effects of nitric oxide from beetroot juice by reducing inflammation and oxidative stress, which may result in increased exercise performance. Therefore, the purpose of this research study was to examine the effects of dietary nitrate and anthocyanins on exercise performance among healthy individuals.

## **Methods**

**Subjects:** This research study consisted of 28 participants (22 female) recruited from Loma Linda and surrounding areas. Age of the participants ranged from nineteen to thirty-five years. To be included in the study, participants had to be healthy and able to exercise on a stationary bike, and be willing to abstain from caffeine, mouthwash, chewing gum, and high antioxidant foods during the week of supplementation. Participants were excluded if they had diabetes, salivary gland disorder, cardiovascular disease, orthopedic injuries, and allergy to tart cherry or beetroot, were pregnant or breastfeeding, or taking nonsteroidal anti-inflammatory drugs (NSAIDs).

The study was conducted according to the criteria set by the declaration of Helsinki, and each subject signed an informed consent before participating in the study. The Institutional

Review Board of Loma Linda University approved all methods and procedures. Risks of this study included a possible breach of confidentiality, slight discomfort from blood collection, and fatigue.

Treatment: Subjects were randomized to either treatment A or treatment B using simple randomization. Treatment A consumed 5.05 grams of beetroot powder and 480 milligrams of a placebo (microcrystalline cellulose), while treatment B consumed 5.05 grams of beetroot powder which equates to 3.62 mmol and a capsule containing 480 milligrams of tart cherry extract (CherryPURE®). The regimen lasted for seven days. On the seventh day of supplementation, participants arrived at the physical therapy research laboratory to perform the modified Wingate anaerobic test. Post intervention, participants underwent a seven-day washout period, after which treatments were alternated.

Anthropometrics: Body measurements were obtained at baseline and after each intervention. Participants avoided caffeine and heavy hydration before measurements. Body weight and body composition data were obtained using the InBody 370 bio-impedance scale. Height was measured to the nearest 0.1 cm using a portable stadiometer. All measurements were obtained with light clothing.

Compliance, diet and sleep: Participants were provided with a compliance log to record supplementation intake for seven days, a sleep questionnaire listing the number of hours slept the night before treatment, and a 24-hour food recall before each treatment.

Blood measurements and analysis: Blood samples were obtained from participants during each phase. Two blood biomarkers, lactate and allantoin, were examined during each visit before and after bike tests. Blood lactate was measured and analyzed using a Lactate Pro Analyzer.

Allantoin was measured in a sub-sample of participants (35%). Blood samples were stabilized in



a styrofoam container after which they were centrifuged and aliquoted within an hour, then frozen at a temperature of  $-80^{\circ}\text{C}$ . Blood samples were shipped to University of Minnesota for allantoin analysis, then analyzed using an ELISA kit by MyBioSource.

**Modified Wingate Anaerobic Bike Test:** Each subject performed the modified Wingate anaerobic bike test on Monark (928E) stationary bike. The bike was programmed to include a test load equivalent to 10% of the subject's weight. The test protocol comprised of a three-minute warm-up, a 30 second sprint, and a two-minute recovery pedaling phase. Test weight was applied on the bike, which was set relative to 10% of the subject's body mass. Revolution per minute (RPM), workload (watts), and rate of perceived exertion (RPE) were examined at baseline, first and second intervention.

**Clinical measurements:** Blood pressure, heart rate, oxygen saturation, and blood pressure were examined three consecutive times before and after each bike test. Subjects were instructed to sit comfortably with both feet flat on the floor. The blood pressure cuff was placed on the left arm over the brachial artery. After the first measurement, there was a two-minute intermission before the second measurement and a one-minute break before final measurement. A PolarFT7 chest monitor was used to analyze heart rate consistency throughout the bike test. A pulse oximeter was placed on the index finger to measure oxygen saturation during warm up and the 30-second sprint bike test. Speed (rpm) and power (watts) were recorded throughout each treatment by Monark bike software. RPE Borg rating scale was measured before and after each bike test. Participants were asked to fast two hours before the bike test and wear loose clothing and comfortable shoes for accurate testing and measurements.

## **Results**

Twenty-five participants completed the study (20 females), with three participants unable to complete the research due to scheduling conflicts. Participants were asked to ingest supplement once per day around the same time for seven days, including the day of treatment. Participants who missed a day of supplementation were asked to double up the following day. Compliance of supplementation intake for treatment A was 94%, while treatment B was 98%.

Table 1. Participant characteristics at baseline (N=28).

	Treatment A	Treatment B
Gender – no. (%)		
Female	9 (64.3)	13 (92.9)
Male	5 (35.7)	1 (7.1)
Age (years)	24.2 ±2.5	24± 2.3
Height (cm)	169.5± 12.2	158.0± 4.4
Weight (kg)	67.6 ±14.3	59.1± 10.7
BMI (kg/m <sup>2</sup> )	23.5 ±3.8	23.7±4.3
Fat (%)	25.8 ±9.9	30.4 ±8.1
Free Fat Mass (kg)	50.1± 12.5	40.6 ±5.3

\*Plus-minus values are means ± standard deviation

Treatment A: beetroot powder and placebo.

Treatment B: beetroot powder and tart cherry capsule.

As illustrated in Table 2, differences between systolic blood pressure, diastolic blood pressure, rate of perceived exertion, and allantoin level in treatment A and treatment B were found statistically insignificant. The average resting systolic blood pressure was lower in treatment A (107.8 mmHg) before the bike test compared with treatment B (112.6 mmHg). After the bike test, systolic blood pressure was increased in both treatments. Treatment A exhibited a 4.6% increase in systolic blood pressure, whereas treatment B only had a 0.4% increase. The average resting diastolic blood pressure of treatment A (72 mmHg) was similar to treatment B (71.7 mmHg). Average diastolic blood pressure decreased by 0.4% in treatment A and 1.8% in treatment B. Average resting heart rate of treatment A was 68.8 bpm and 65.6 bpm for treatment B. Average heart rate after the bike test increased in both treatments, by 25.3% for treatment A

and 27% for treatment B. Average rate of perceived exertion was 11.3 in treatment A and 11.5 in treatment B, and the average was the same (15.5) in both groups after the bike test. The average lactate was 1.6 mmol/L in treatment A and 1.5 mmol/L in treatment B before the bike test, and the average lactate increased by 9.5% in treatment A and 9.7% in treatment B. Average allantoin before the bike test was 18.7 in treatment A and 15.5 in treatment B, and allantoin was reduced to 14.2 in both groups after the bike test.

Table 2. Means and percent change in blood pressure, heart rate, RPE, lactate and serum allantoin before and after the bike test (N=28).

	Treatment	Pre	Post	% Change	p-value
SBP	A	107.8	112.4	4.60	0.129
	B	112.6	113.0	0.4	
DBP	A	72.0	71.6	-0.4	0.531
	B	71.7	69.9	-1.8	
Heart Rate	A	68.8	93.9	25.3	0.613
	B	65.6	93.5	27	
RPE	A	11.3	15.5	5.2	0.589
	B	11.5	15.5	4	
Lactate (mmol/L)	A	1.6	11	9.5	0.652
	B	1.5	11.2	9.7	
Allantoin	A	18.7	14.2	-24.10	0.274
	B	15.5	14.2	-8.50	

Treatment A: beetroot powder and placebo.

Treatment B: beetroot powder and tart cherry capsule.

RPE: rate of perceived exertion

Results from Figure 1a show the average heart rate (bpm) of both treatments during the 30-second sprint with a significant difference in the first five seconds ( $p < 0.05$ ). The difference ranged from 1.6 to 2.7 bpm. There were no significant differences after the first five seconds during the 30-second sprint. The mean heart rate at 30 to 55 seconds after the sprint was significantly higher in treatment B than in treatment A ( $p < 0.05$ ). Estimated difference in heart rate during this interval ranged from 0.94 to 2.04 bpm (Figure 1b). The mixed model polynomial trend indicated no significant differences in speed (rpm) between the two treatments at any time

during the 30-second sprint (Figure 2a). There were no significant differences in power (watt) between the two treatments during the first nine seconds of the 30-second sprint (Figure 2b). However, average power in treatment A was significantly higher than those in treatment B from 10 to 25 seconds ( $p < 0.05$ ). Estimated difference in power during this interval ranged from 15.3 to 19.0.

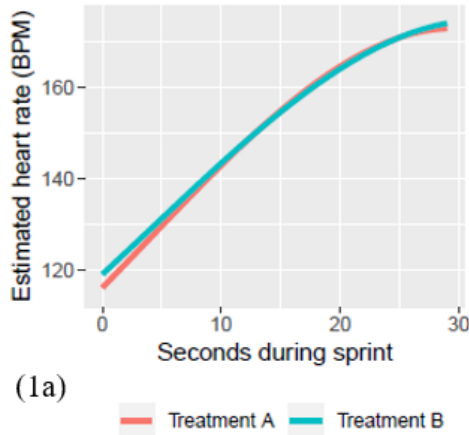


Figure 1a. Average heart rate during 30-second sprint

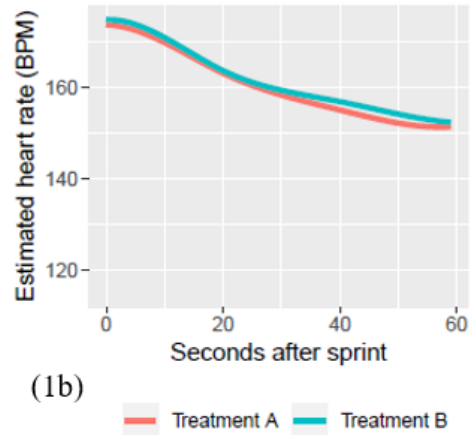


Figure 1b. Average heart rate 60-second after sprint

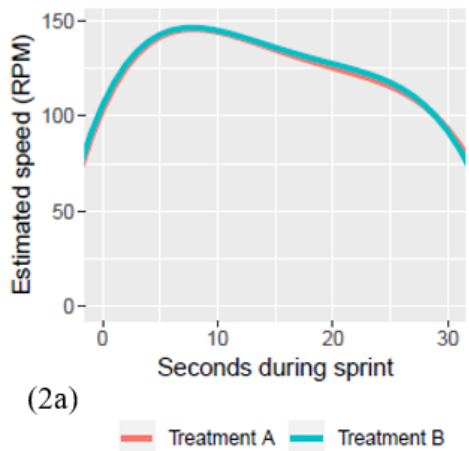


Figure 2a. Average speed during 30-second sprint

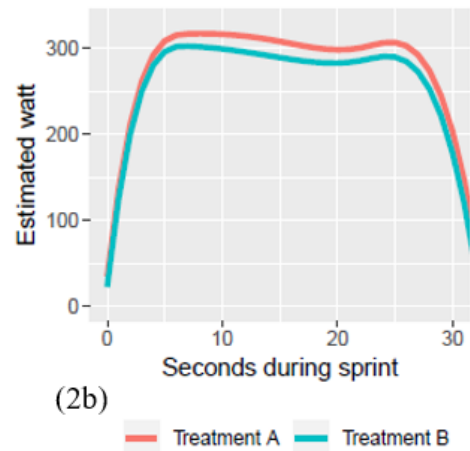


Figure 2b. Average power during 30-second sprint

Treatment A traveled 383 meters during the 30-second sprint, and treatment B traveled 388 meters during the 30-second sprint. Treatment B had a mean of five meters greater distance traveled than treatment A ( $p = 0.25$ ). Treatment B traveled farther than treatment A by 1.3%.

Table 3. Mean distance traveled (meters) and percent change

Treatment	Distance (m)	% Change	p-value
A	383	1.3	0.247
B	388		

## Discussion

Bike results of improved distance traveled reiterated the purpose of this research, suggesting a synergistic effect between dietary nitrate and anthocyanins. According to Lansley et al., anaerobic activity with six-day beetroot supplementation at a higher concentration (6.2 mmol) showed reduced oxygen cost and improved exercise intensity.<sup>24</sup> Lansley et al. conducted another study with the same supplementation protocol on professional cyclists, which resulted in a significant decrease in systolic blood pressure.<sup>25</sup> On the other hand, research on tart cherry with 30 millimeter supplementation twice a day for eight days on high-intensity cycling activity revealed reduced exercise-induced inflammation.<sup>2</sup> Therefore, we investigated the combined supplementation for seven days of 3.6 mmol of beetroot and 480 mg of tart cherry to investigate vasodilation and antioxidant effects on exercise performance during anaerobic activity.

Our primary findings indicated a synergistic effect of nitrate and anthocyanins on improving exercise performance (heart rate, speed, and distance) in healthy individuals. In this study, nitrate and anthocyanins were provided in powder and capsule form, which were both well-tolerated by participants. Results indicated that treatment B had a higher heart rate than treatment A during the first five seconds of the 30-second sprint. A higher heart rate may lead to an increase in cardiac output which will improve overall blood flow to muscular tissue.<sup>26</sup> An increase in maximal cardiac production improves an individual's capacity to circulate oxygen, which increases mitochondrial efficiency for energy production.<sup>26</sup> For this study, the heart rate of treatment B was 1.6 to 2.7 bpm higher than treatment A during the first five seconds of the

sprint. Duration of the supplementation periods could have attenuated the heart rate because past studies typically lasted for three days.<sup>27</sup> However, recent studies in nitrate supplementations on submaximal (45-80%  $\text{VO}_2$ ) exercises using cycle ergometers have shown that extended periods of supplementation do not affect heart rate.<sup>19,28</sup> A study conducted by Bailey et al. recruited eight recreational trained male cyclists and administered a treatment dose of 5.5 mmol nitrates in beetroot juice for six days.<sup>19</sup> Results by Vanhatalo et al. revealed that 15 days of supplementation with 5.2 mmol of nitrate in beetroot juice on eight untrained males and females also did not attenuate heart rate in moderate exercise.<sup>29</sup> In this study, 3.2 mmol of nitrates was given for seven days to an untrained general population with the addition of anthocyanins or placebo. Similarities in methodology, in addition to an extended period of supplementation, suggest that there is a synergistic effect between nitrate and anthocyanins. Increase in heart rate is significant in that it may improve exercise performance. A combination of beetroot and tart cherry supplements used in treatment B may enhance exercise performance in competitive sports.

With respect to total distance traveled, treatment B traveled a mean distance of 388 meters when compared to 383 meters traveled by treatment A, which showed that participants in treatment B traveled five meters or 16.4 feet farther than treatment A. This could suggest that the combination of nitrate and anthocyanins may synergistically reduce disruption of skeletal muscle metabolism due to hypoxia. Increased hypoxia can contribute to exercise intolerance and fatigue owing to excess blood lactate accumulation.<sup>30,31</sup> Previous studies have shown that nitrate supplementation reduces oxygen and ATP cost in low-intensity exercise in addition to preserving phosphocreatine during high-intensity exercise, resulting in increased exercise tolerance.<sup>19,28</sup>

Tart cherries contain a high concentration of anthocyanin, cyanidin-3-glucoside<sup>9</sup>, which has been suggested to increase endothelial nitric oxide synthase expression (eNOS) and decrease inducible nitric oxide synthase (iNOS). Expression of eNOS aids in the catalyzation reaction of L-arginine into nitric oxide, while the inhibition of iNOS decreases the production of peroxynitrite.<sup>32</sup> Conversely, other studies have shown that tart cherry supplementation is independent of eNOS activity and such activity could instead occur as a result of antioxidant effect of polyphenols.<sup>8,33</sup> It should be noted from this study, however, that eNOS activity may already be optimal in trained endurance cyclists. In this case, additional tart cherry supplementation would have minimal effects.<sup>8</sup>

Our research anticipated that increased oxygen supply to muscle would delay lactate formation from anaerobic glycolysis. However, lactate levels did not change significantly. Our findings were similar to reported lack of significant change in blood lactate.<sup>19</sup> According to Kander et al., allantoin can be used as a marker for oxidative stress.<sup>22</sup> Allantoin is produced when uric acid, the end product of purine metabolism, reacts with reactive oxygen species as an antioxidant.<sup>22</sup> We hypothesized that there would be less allantoin produced after the bike exercise following tart cherry supplementation. However, our results were not significant, likely due to blood collected at the 30-second mark rather than at the 10-minute mark.<sup>22</sup>

To the best of our knowledge, this is the first study to examine the synergistic effect of beetroot and tart cherry supplementation on exercise performance in untrained individuals. This study is strengthened by adequate power and high compliance of subjects. Limitations of this study include a lower dose of beetroot supplementation and reliance on self-reported data. We recommend that future studies increase the dietary dosage of supplements and utilize sensitive

biomarkers to understand the mechanism effect of dietary nitrate and anthocyanins on exercise performance.

In conclusion, supplementation with beetroot and tart cherry for seven days in untrained individuals may synergistically improve anaerobic exercise performance by maximizing potential cardiac output and consequently lead to increased energy output.



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# Appendix A

## IRB



LOMA LINDA UNIVERSITY  
School of Allied Health Professions

### INFORMED CONSENT

**TITLE:** THE EFFECTS OF DIETARY NITRATE AND ANTHOCYANINS ON ANAEROBIC EXERCISE PERFORMANCE

**PRINCIPAL INVESTIGATOR:** Edward Bitok, DrPH, MS, RDN, Assistant Professor, Department of Nutrition and Dietetics, School of Allied Health Professions

**GRADUATE STUDENT INVESTIGATOR:** Yun Chieh Huang, Paul Tsai, and Aurora Sanchez

### WHY IS THIS STUDY BEING DONE?

The purpose of this graduate student research is to examine the effect of dietary nitrate and anthocyanins on anaerobic exercise performance among Loma Linda University students.

Anthocyanins (from tart cherries) are strong antioxidants that reduce inflammation. Nitric oxide is a chemical made in the body to expand blood vessels and can also be found in plant foods like beetroot.

You are among 50 individuals invited to participate in this study because you are a healthy 21 to 35-year-old Loma Linda University student who is willing to abstain from caffeine, mouthwash, chewing gum, and high antioxidant foods during the weeks you are taking the supplements (14 days). A list of high antioxidant foods to be avoided will be provided to you.

You will not be eligible to participate in the study if you are diabetic, have a salivary gland disorder, cardiovascular disease or orthopedic injuries, pregnant or breastfeeding, currently taking NSAIDS, have an allergy to anthocyanins(tart cherries) and/or beetroot.

Your participation in this study will last for 6 visits over 29 days with each visit lasting approximately 30 minutes.

### HOW WILL I BE INVOLVED?

Participation in this study involves the following:

#### Visit 1 (Day 0)

- You will be provided with more information on the study.

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- If you choose to participate in the study, you will sign an informed consent.
- A student investigator will go over study procedures and compliance.
- You will complete brief demographic and physical activity questionnaires.
- You will be given a compliance check list to follow and a list of high antioxidant foods to avoid for your next visit.

Visit 2 (Day 1)

- You will wear exercise clothes and shoes.
- Your body weight and composition will be measured
- Your blood pressure will be measured.
- A student investigator will obtain a lactate fingerstick measurement.
- A certified phlebotomist will draw 10 milliliters of blood (2 teaspoon) with an IV from a random subsample (35%) of participants
- Your heart rate and oxygen saturation will be monitored.
- You will be given instructions on an assigned stationary bike.
- You will be given three minutes to warm up and get familiar with the assigned equipment, and then you will do a 30 second warm up sprint.
- Following the warm up, you will pedal at a comfortable pace for one minute and take a 2-minute break on the bike without pedaling.
- After the break, you will cycle 10 seconds and then sprint 30 seconds as fast as possible. Then, you will pedal at a comfortable pace again for 2 minutes.
- A student investigator will again do a lactate fingerstick measurement.
- A certified phlebotomist will draw another 10 milliliters (2 teaspoon) of your blood from the same vein.
- Your blood pressure will be measured.

Randomization

- You will randomly be assigned into one of two groups (group A or B).

Washout period

- You will rest for 7 days, abstaining from heavy exercises per instruction restrictions.
- You will abstain from consuming high antioxidant foods (a list of foods to avoid will be provided to you)

<b>Recommended exercises</b>	<b>Exercises to avoid</b>
Walking	Lunges
Jogging	Deadlifts
Stretching	Leg press
Yoga	Knee extension
Swimming	Running
	Biking

- Two days before your next visit, a reminder will be sent via email and/or text to pick up the supplements with a list of instructions.

Visit 3 (Day 9)

- You will arrive at the lab to collect your supplements. The supplements you will ingest will either be nitrate (beetroot) and anthocyanins (tart cherries) or nitrate and placebo.
- Only the principal investigator will know which supplement you are taking.
- You will ingest one dose in front of the student investigator for compliance.
- You will also ingest a daily dose of assigned supplements for 6 concurrent days

Visit 4 (Day 15)

- You will visit the study lab
- You will repeat the same procedure as visit 2.

Washout period

- You will rest for 7 days, abstaining from heavy exercises and high antioxidant foods as in previous washout
- Two days before your next visit, a reminder will be sent via email or text to pick up the supplements with a list of instructions.

Visit 5 (Day 23)

- You will repeat the same procedure as visit 3. However, this time you will be provided with the other supplement.

Visit 6 (Day 29)

- You will repeat the same procedure as visit 2 and 4.

**WHAT ARE THE REASONABLY FORESEEABLE RISKS OR DISCOMFORTS I MIGHT HAVE?**

This study poses no greater risk to you than what you routinely encounter in day-to-day life. However, there may be a possible breach of confidentiality, exhaustion, and slight pain after the blood draw.

All records and research materials that identify you will be held confidentiality and will not be shared with third parties. Any publications resulting from this study will not disclose your identity without your permission. Information identifying you will only be available to the study personnel and no one else.

To minimize the risk of breach of confidentiality, we will store all the data and identifiers in a locked cabinet of a secure office. During the physical activity, you will be given two minutes of warm up to reduce the risk of injury and a one-minute recovery period after each 30 seconds of exercise. Ice packs will be provided to reduce the discomfort on the site of blood draw.

**WILL THERE BE ANY BENEFIT TO ME OR OTHERS?**

Although you may not personally benefit from this study, your participation will help practitioners gain better insights into the role of phytonutrients in sports and exercise

performance. You will receive your anthropometric information after completion of the study which include: body fat, and blood pressure/heart rate. Furthermore, we may also learn about the potential benefits of anthocyanins and nitrate.

#### **WHAT ARE MY RIGHTS AS A SUBJECT?**

Your participation in this study is entirely voluntary. You may refuse to participate or withdraw once the study has started. Your decision whether to participate or terminate participation at any time will not affect your current or future academic standing with the researchers. You do not give up any legal rights by participating in this study.

#### **WHAT COSTS ARE INVOLVED?**

There is no cost to you for participating in this study. The supplements will be provided to you at no cost.

#### **WILL I BE PAID TO PARTICIPATE IN THIS STUDY?**

You will be given a \$50 gift card at the completion of the entire study period.

#### **WHO DO I CALL IF I AM INJURED AS A RESULT OF BEING IN THIS STUDY?**

If the situation is a medical emergency, call 911 or go to the nearest emergency room. Then, notify the study staff as soon as you can. For a non-emergency injury or illness, notify your study staff as soon as you can. Appropriate medical treatment will be made available to you. However, you and your insurance company will be billed at the usual charge for the treatment of any research-related injuries, illnesses, or complications. You might still be asked to pay whatever your insurance does not pay. Also, no funds have been set aside nor any plans made to compensate you for time lost for work, disability, pain, or other discomforts resulting from your participation in this research. By participating in the study, you do not give up any of your legal rights.

#### **WHO DO I CALL IF I HAVE QUESTIONS?**

Call 909-558-4647 or e-mail [patientrelations@llu.edu](mailto:patientrelations@llu.edu) for information and assistance with complaints or concerns about your rights in this study.

#### **SUBJECT'S STATEMENT OF CONSENT**

- I have read the contents of the consent form and have listened to the verbal explanation given by the investigator.
- My questions concerning this study have been answered to my satisfaction.
- Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities.



- I may call Edward Bitok during routine office hours at (909) 558-1000 ext. 59820 if I have additional questions or concerns.
- I hereby give voluntary consent to participate in this study.
- I understand I will be given a copy of this consent form after signing it.

\_\_\_\_\_  
Signature of Subject

\_\_\_\_\_  
Printed Name of Subject

\_\_\_\_\_  
Date

**INVESTIGATOR'S STATEMENT**

I have reviewed the contents of this consent form with the person signing above. I have explained potential risks and benefits of the study.

\_\_\_\_\_  
Signature of Investigator

\_\_\_\_\_  
Printed Name of Investigator

\_\_\_\_\_  
Date

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

## Appendix B

### PPARQ+

# 2018 PAR-Q+

## The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

### GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition <input type="checkbox"/> OR high blood pressure <input type="checkbox"/> ?	<input type="checkbox"/>	<input type="checkbox"/>
2) Do you feel pain in your chest at rest, during your daily activities of living, <b>OR</b> when you do physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
3) Do you lose balance because of dizziness <b>OR</b> have you lost consciousness in the last 12 months? Please answer <b>NO</b> if your dizziness was associated with over-breathing (including during vigorous exercise).	<input type="checkbox"/>	<input type="checkbox"/>
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? <b>PLEASE LIST CONDITION(S) HERE:</b> _____	<input type="checkbox"/>	<input type="checkbox"/>
5) Are you currently taking prescribed medications for a chronic medical condition? <b>PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:</b> _____	<input type="checkbox"/>	<input type="checkbox"/>
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer <b>NO</b> if you had a problem in the past, but it <i>does not limit your current ability</i> to be physically active. <b>PLEASE LIST CONDITION(S) HERE:</b> _____	<input type="checkbox"/>	<input type="checkbox"/>
7) Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

**If you answered NO to all of the questions above, you are cleared for physical activity. Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.**

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age ([www.who.int/dietphysicalactivity/en/](http://www.who.int/dietphysicalactivity/en/)).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

**PARTICIPANT DECLARATION**  
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

*I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness centre may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.*

NAME \_\_\_\_\_ DATE \_\_\_\_\_

SIGNATURE \_\_\_\_\_ WITNESS \_\_\_\_\_

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER \_\_\_\_\_

**If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.**

**⚠ Delay becoming more active if:**

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at [www.eparmedx.com](http://www.eparmedx.com) before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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## FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. **Do you have Arthritis, Osteoporosis, or Back Problems?**  
If the above condition(s) is/are present, answer questions 1a-1c      If **NO**  go to question 2
- 1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)      YES  NO
- 
- 1b. Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?      YES  NO
- 
- 1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months?      YES  NO
- 
2. **Do you currently have Cancer of any kind?**  
If the above condition(s) is/are present, answer questions 2a-2b      If **NO**  go to question 3
- 2a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?      YES  NO
- 
- 2b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?      YES  NO
- 
3. **Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm**  
If the above condition(s) is/are present, answer questions 3a-3d      If **NO**  go to question 4
- 3a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)      YES  NO
- 
- 3b. Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)      YES  NO
- 
- 3c. Do you have chronic heart failure?      YES  NO
- 
- 3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?      YES  NO
- 
4. **Do you have High Blood Pressure?**  
If the above condition(s) is/are present, answer questions 4a-4b      If **NO**  go to question 5
- 4a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)      YES  NO
- 
- 4b. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)      YES  NO
- 
5. **Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes**  
If the above condition(s) is/are present, answer questions 5a-5e      If **NO**  go to question 6
- 5a. Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?      YES  NO
- 
- 5b. Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.      YES  NO
- 
- 5c. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?      YES  NO
- 
- 5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?      YES  NO
- 
- 5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?      YES  NO

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6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*  
If the above condition(s) is/are present, answer questions 6a-6b If **NO**  go to question 7
- 6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? **YES**  **NO**   
(Answer **NO** if you are not currently taking medications or other treatments)
- 6b. Do you have Down Syndrome **AND** back problems affecting nerves or muscles? **YES**  **NO**
- 
7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*  
If the above condition(s) is/are present, answer questions 7a-7d If **NO**  go to question 8
- 7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? **YES**  **NO**   
(Answer **NO** if you are not currently taking medications or other treatments)
- 7b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? **YES**  **NO**
- 7c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? **YES**  **NO**
- 7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? **YES**  **NO**
- 
8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*  
If the above condition(s) is/are present, answer questions 8a-8c If **NO**  go to question 9
- 8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? **YES**  **NO**   
(Answer **NO** if you are not currently taking medications or other treatments)
- 8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting? **YES**  **NO**
- 8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)? **YES**  **NO**
- 
9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*  
If the above condition(s) is/are present, answer questions 9a-9c If **NO**  go to question 10
- 9a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? **YES**  **NO**   
(Answer **NO** if you are not currently taking medications or other treatments)
- 9b. Do you have any impairment in walking or mobility? **YES**  **NO**
- 9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? **YES**  **NO**
- 
10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**  
If you have other medical conditions, answer questions 10a-10c If **NO**  read the Page 4 recommendations
- 10a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months? **YES**  **NO**
- 10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? **YES**  **NO**
- 10c. Do you currently live with two or more medical conditions? **YES**  **NO**
- PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: \_\_\_\_\_

**GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.**

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If you answered **NO** to all of the **FOLLOW-UP** questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the **PARTICIPANT DECLARATION** below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition: You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+** at **www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

**⚠ Delay becoming more active if:**

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and **NO** changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

## PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

*I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.*

NAME \_\_\_\_\_ DATE \_\_\_\_\_  
 SIGNATURE \_\_\_\_\_ WITNESS \_\_\_\_\_  
 SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER \_\_\_\_\_

For more information, please contact  
**www.eparmedx.com**  
**Email: eparmedx@gmail.com**

Citation for PAR-Q+  
 Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):9-23, 2011.

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## Appendix C

### Demographic Questionnaire

1. What is your gender?
  - Male
  - Female
  - Other \_\_\_\_\_
2. What is your age?
  - 21 – 25
  - 26 – 30
  - 31 – 35
3. What ethnic group do you identify with?
  - Non-Hispanic white
  - Latino or Hispanic
  - Indian
  - Middle Eastern or Arab
  - Other \_\_\_\_\_
  - Black
  - South Asian
  - Southeast Asian
  - Native American or Alaskan native
4. What is your marital status?
  - Single
  - Married
  - In a relationship
5. What is your education status?
  - Associate
  - Bachelor
  - Doctoral
  - Other \_\_\_\_\_
6. What's your mode of transportation?
  - Bike
  - Walk
  - Car or other
7. How long is your commute?  
\_\_\_\_\_ minutes
8. How far do you commute?  
\_\_\_\_\_ miles
9. How often do you exercise per week?
  - Never
  - Once a week
  - 2-3 days a week
  - 4-5 days a week
  - 6-7 days a week
10. How long do you work out each time?
  - 0 - 30 minutes
  - 30 – 60 minutes

- 60 – 90 minutes
- 90 – 120 minutes
- More than 2 hours

11. Do you do more upper body or lower body exercises?

12. How often do you consume vegetables in a day?

- less than 1
- 1 - 2
- 2 - 3
- More than 3

13. How often do you consume fruits in a day?

- less than 1
- 1 - 2
- 2 - 3
- More than 3

## Appendix D

### Compliance sheet

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Comments
	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no	
Did you take your supplements on time?															
Did you consume anything on the high antioxidant list?															
Did you abstain from the exercise on the list?															
Did you abstain from chewing gum?															
Did you abstain from mouthwash?															
Did you abstain from caffeine such as tea/coffee?															



## Appendix E

### Intervention Questionnaire

Subject ID:

1. How many cups of liquid, including water, did you drink today?
2. How many hours of sleep did you have the night before?
3. What is your stress level today on a scale of 1 to 5, with 1 being relax and 5 being stressful?
4. Did you consume any food 3 hours prior to arrival?
5. Are you currently on your menstrual cycle?  
 Yes  
 No
6. If yes, what is your pain level today from a range of 1 to 5, with 5 being extremely painful?
7. Have you experienced any positive or negative side effect during this week of supplementation?

## **Appendix F**

### Modified Wingate Protocol on Monark 928E testing bike

Resistance equivalent to 10% of participant's body weight is added

1. Begin with 3-minute warm-up
2. 3-second warm up sprint
3. Pedal at a comfortable pace for one minute
4. Take 2-minute break on the bike without pedaling
5. Cycle 10 seconds
6. 30-second sprint test
7. End with a 2-minute cool down

## Appendix E

### RPE (Borg) Scale

RPE Scale	Equivalent % HR <sub>max</sub>	Exercise Intensity % VO <sub>2max</sub>
6		
7 Very, very light		
8		
9 Very light		
10		
11 Fairly light	52-66	31-50
12		
13 Somewhat hard	61-85	51-75
14		
15 Hard	86-91	76-85
16	92	85
17 Very hard		
18		
19 Very, very hard		