WILD BLUEBERRIES INCREASE FAT OXIDATION RATE DURING MODERATE INTENSITY EXERCISE

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ABSTRACT

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Consumption of fruits high in anthocyanins, such as wild blueberries (WB)s, have been shown to influence lipolytic enzymes and increase fatty acid (FA) oxidation during rest. Purpose: Examine the effect of a WB drink on FA oxidation during moderate intensity exercise. Methods: Eleven aerobically trained males (26 ± 7.5 years, 74.9 ± 7.54 kg, 10.5 ± 3.2% body fat) completed an incremental cycling test to determine VO_{2peak} (55.1 ± 7.5 ml/kg/min) followed by a washout diet avoiding foods high in anthocyanins. After two weeks, subjects completed a control cycling protocol at 65% of VO_{2peak} for 40 minutes. Next, subjects consumed 375 g anthocyanins from WB powder for two weeks, and repeated the exercise protocol. Results: The WB trials increased FA oxidation by 19.7 (P = 0.049), 43.2 (P = 0.010), 31.1% (P = 0.012) at 20, 30 and 40 minutes, respectively. Carbohydrate oxidation rates were significantly lower by 10.1 (P = 0.024), 19.2 (P = 0.014) and 14.8% (P = 0.045) at 20, 30 and 40 minutes respectively, in the WB trials. Lactate was significantly lower at 20 (P = 0.005), 30 (P = 0.005) and 40 (P = 0.013) minutes in WB trial. Conclusion: Fourteen-day intake of WBs increased FA oxidation while decreasing carbohydrate reliance and lactate production during submaximal intensity exercise.

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INTRODUCTION

During exercise adipose tissue, plasma triglycerides (TG)s and intramuscular TGs supply fatty acids (FA) used for the production of energy (Horowitz, 2003; Horowitz & Klein, 2000). A large percentage of free FAs reattach to glycerol and form new TGs at rest, however, during low and moderate intensity exercise the rate of appearance of free FAs within the blood increases two to six-fold compared to rest (Achten & Jeukendrup, 2004; Horowitz, Mora-Rodriguez, Byerley & Coyle, 1997; Klein, Coyle & Wolfe, 1994; Romijn, Coyle, Sidossis, Gastaldelli, Horowitz, Endert & Wolfe, 1993; Wolfe, Klein, Carraro & Weber, 1990). TGs are utilized as fuel to decrease the rate of endogenous carbohydrate use during exercise (Burke et al., 2002) as potential energy storage in the form of TGs is 40x greater than glycogen storages (Horowitz, 2003). As the exercise intensity continues to increase, there is a shift in substrate utilization as the concentration of free FAs in the plasma drops as the body begins to depend more heavily on carbohydrate for energy (Brooks, 1998; Horowitz, 2003; Jeukendrup, 2006; Loon, Greenhaff, Constantin-Teodosiu, Saris & Wagenmakers, 2001; Romijn, Coyle, Sidossis, Gastaldelli, Horowitz, Endert & Wolfe, 1993; Spriet, 2014; Venables, Achten & Jeukendrup, 2004). Fat oxidation cannot meet the energy demands of vigorous intensity exercise which requires a greater reliance on glycogen sources (Brooks, 1998; Horowitz, 2003; Jeukendrup, 2006; Martin & Klein, 1998; Romijn et al., 1993; Spriet, 2014; Loon et al., 2001; Venables et al., 2005). However, reliance on glycogen during lower intensity activities causes depletion of glycogen which causes fatigue and decreased exercise

capacity (Burke et al., 2017; Hargreaves, Hawley & Jeukendrup, 2004). Therefore, maximizing fat mobilization during low and moderate intensity exercise prevents glycogen storage depletion (Burke et al., 2017; Hargreaves et al., 2004). Research has focused on finding interventions that help the preservation of glycogen for later, more intense bouts in exercise or competition.

Endurance trained individuals have demonstrated increased rates of lipolysis and greater capacity to oxidize FAs compared to untrained individuals at the same relative intensity (Coggan, Raguso, Gastaldelli, Sidossis & Yeckel, 2000; Glisezinski et al., 2001). Endurance trained individuals present lower respiratory exchange ratio (RER) values and a greater rate of FA oxidation during rest and exercise than their untrained counterparts (Klein et al., 1994). Mechanisms in which endurance trained individuals display greater ability to depend on fat as an energy source include an increase in oxidative enzymes and mitochondria size which enhance oxidative efficiency (Holloszy & Coyle, 1984; Jansson & Kaijser, 1987), an increase in amount and oxidation rate of intramuscular triglycerides (Hurley, Nemeth, Martin, Hagberg, Dalsky & Holloszy, 1986) and an increased uptake of FAs by skeletal muscle (Kiens, Essen-Gustavsson, Christensen & Saltin, 1993; Klein et al., 1994). The physiological adaptations that increase FA oxidation occurring from endurance training increase exercise performance and the addition of dietary interventions may maximize this response.

The consumption of a diet high in carbohydrate has been linked to increased muscle glycogen and liver glycogen stores, improved performance in endurance events, and increased rate of carbohydrate use during exercise (Zajac, Poprzecki, Maszczyk,

Czuba, Michalczyk & Zydek, 2014). Therefore, the consumption of a high carbohydrate diet especially before, during and after exercise has been reinforced as the supreme paradigm for optimizing performance and recovery (Achten & Jeukendrup, 2004; Beelan, Burke, Gibala & Loon, 2010). Recently, the nutritional guidelines for athletes have shifted away from this universal approach to meet the needs of individual activities (Burke, Hawley, Wong & Jeukendrup, 2011; Burke, Kiens & Ivy, 2004). Researchers have found that the previous high carbohydrate approach is unsuitable due to the anticipated demands of each individual activity should determine the quantity and timing of carbohydrates, high intake of carbohydrates is favorable for performance in competition and sessions of high intensity, but is not necessary for less intense exercise, and the amount and timing of carbohydrate intake should be individualized to their specific period of training or competition (Burke et al., 2004; Burke et al., 2011). Different dietary methods have been employed to determine the optimal feeding for endurance athletes including periodized carbohydrate intake which has had promising effects on exercise performance (Burke et al., 2017). Recently, researchers have investigated the effects of a high fat diet on parameters of exercise performance.

Zajac et al. (2014) examined the concept of high fat diets on male off-road cyclists were fed a diet consisting of 70% fat and 15% carbohydrate intake for four weeks (Zajac et al., 2014). Results included a two-fold increase in plasma FA concentration and a decrease in body mass and body fat, however their ability to exercise at a high intensity was also decreased (Zajac et al., 2014). A similar study on elite race walkers found a ketogenic diet (75-80% fat) impaired exercise economy and did not provide any

improvements that could be transferred to future competition (Burke et al., 2017). Intake of a high fat, low carbohydrate generates metabolic adaptions to increase rates of FA oxidation during exercise however, there is a lack of evidence to support an enhanced performance after following a high fat diet (Burke & Kiens, 2006; Burke et al., 2002; Burke, 2015; Burke et al., 2017). Overall, dietary methods aimed at increasing fat oxidation rates to maintain glycogen stores appear to work by generating metabolic adaptations but also result in decreases in endurance performance (Burke et al., 2017). Therefore, there is a need for an intervention that can increase FA reliance without hindering endurance exercise capacity.

The addition of wild blueberries (WB) to the diet may have potential benefits to exercise performance. Health benefits associated with blueberries are the result of the bioactive properties of their phytochemicals (Shi, Loftus, McAinch & Su, 2017). Phytochemicals are a classification of plant derived, biochemical compounds including polyphenols (Kim & Park, 2016; Rupasinghe, Sekhon-Loodu, Mantso & Panayiotidis, 2016; Tucci, 2010) with flavonoids as a class of polyphenols. Within flavonoids there are subclasses including anthocyanins (Cook, Myers, Blacker & Willems, 2015; Pojer, Mattivi, Johnson & Stockley, 2013) which are responsible for giving foods red, blue and purple colors (Kim & Park, 2016) and found abundantly in WB (Hosseinian & Beta, 2007). When compared to other anthocyanin containing berries, WB displayed significantly higher (558.33 mg/ 100 g) levels of anthocyanins than raspberries (365.21 mg/100 g) and chokeberries (177.39 mg/100 g) (Hosseinian & Beta, 2007). Evidence indicates a link between high intake of dietary anthocyanins and lower risk of

cardiovascular disease, myocardial infarction and ischemic damage (Ahmet et al., 2009; Cassidy, Mukamal, Liu, Franz, Eliassen & Rimm, 2013; McCollough, Peterson, Patel, Jacques, Shah & Dwyer, 2012). Additional benefits of anthocyanin consumption include improvements to blood pressure (Basu et al., 2010; McAnulty et al., 2014; Mykkanen, Huotari, Herzig, Dunlop, Mykkanen & Kirjavainen, 2014; Shaughnessy, Boswall, Scanlan, Gottschall-Pass & Sweeny, 2009), endothelial function (Kalea, Clark, Schuschke & Klimis-Zacas, 2009; Kalea, Clark, Schuschke, Kristo & Klimis-Zacas, 2010; Kristo, Kalea, Schuschke & Klimis-Zacas, 2010; Stull et al., 2015), dyslipidemia (Basu et a., 2010; Prior, Wu, Gu, Hager, Hager, Wilkes & Howard, 2009), oxidative stress (Cassidy, Rogers, Peterson, Dwyer, Lin & Jacques, 2015; Zepeda, Aguayo, Fuentealba, Figueroa, Acevedo, Salgado, Calaf & Faria, 2012), inflammatory status (Cassidy et al., 2015; Lau, Bielinski & Joseph, 2007; Mykkanen, Huotari, Herzig, Dunlop, Mykkanen & Kirjavainen, 2014; Vendrame, Daughtry, Kristo, Riso & Klimis-Zacas, 2013) and blood glucose control (DeFuria, Bennett, Strissel, Perfield II, Milbury, Greenberg & Obin, 2009; Jacques, Cassidy, Rogers, Peterson, Meigs & Dwyer, 2013; Stull, 2010).

In animal trials, anthocyanins have been found to positively affect fat metabolism regulation (Overall, Bonney, Wilson, Beerman, Grace, Esposito, Lila & Komarnytsky, 2017; Prior et al., 2009) and stimulation of lipolysis leading to increased FA oxidation rates (Feillet-Coudray et al., 2009; Shimoda, Tanaka, Kikuchi, Fukuda, Ito, Hatano & Yoshida, 2009). The research on anthocyanin effects on physiological measures for FA oxidation during endurance exercise in humans is limited to the use of New Zealand

blackcurrant (NZBC) supplementation. Recent studies have found that 105 mg of anthocyanin intake for seven-days resulted in 27% increased rate of FA oxidation in male trained cyclists at 65% of VO_{2peak} (Cook, Myers, Blacker & Willems, 2015). Further research with female trained cyclists consuming 210 mg of anthocyanins for seven days resulted in a 27% increase in fat oxidation and a significant increase in non-esterified fatty acids (Strauss, Willems & Shepard, 2018). Therefore, the purpose of this study was to determine the effects of 14-day intake of WB powder on the FA oxidation rate in healthy males during a submaximal cycling bout. Comparing WB consumption to a control condition, the objectives included to determine 1) the rate of FA oxidation during submaximal exercise using respiratory exchange ratio and fat and carbohydrate oxidation rate calculations and 2) the response of plasma lactate during submaximal exercise. It was hypothesized that 14-intake of WB would increase FA oxidation rates during 40 minutes of submaximal intensity exercise.

METHODS

Subjects

Eleven healthy, aerobically trained males volunteered (Table 1) to participate in the study. A total of 13 participants completed the study, however two individuals were excluded in this analysis du to sickness or changes in lifestyle (physical activity and drug use) during the WB condition. Participation required the following inclusion criteria: 18 years old or above, non-tobacco users, waist circumference <102 cm, free of any cardiovascular, metabolic, respiratory and orthopedic conditions, not taking blood pressure and/or cholesterol lowering medications and engaging in moderate intensity cardiovascular exercise at least three days a week for 30 minutes for the past two years. Prior to participation and throughout the duration of the study, all subjects consumed a mixed diet. Recruitment for subjects took place on the Humboldt State University campus and in the surrounding community. All those recruited for possible participation had their intake of all polyphenic compounds screened. This determined the difficulty of adherence to avoiding foods containing anthocyanins throughout the study.

Table 1. Descriptive statistics of subjects

Variable	Initial Session	WB Drink
Age	26.55 ± 7.95	26.55 ± 7.95
Weight (kg)	74.74 ± 8.22	74.91 ± 8.53
Height (cm)	180.16 ± 3.42	180.16 ± 3.42
Body Fat (%)	10.15 ± 3.37	10.15 ± 3.27
Waist Circumference (cm)	79.41 ± 6.36	78.68 ± 5.81
VO_{2peak} (ml/kg/min)	54.43 ± 7.99	N/A

All data presented as means ± standard deviations

Experimental Design

The design was a non-randomized, free living trial and subjects came into the Human Performance Lab at Humboldt State University on three separate occasions (Figure 1). The initial session consisted of reading and signing an informed consent and completion of a health questionnaire, anthropometric measures, VO_{2peak} test and a familiarization trial at moderate intensity. An incremental stage protocol until volitional fatigue on a Velotron cycle ergometer (RacerMate, Seattle, WA) was used to determine VO_{2peak}. The protocol included a 3-minute warm up at zero watts followed by 25 watt increments every minute. Following the initial test, participants performed a familiarization trial at 65% of their VO_{2peak} for 15 minutes. Upon leaving the lab,

participants were given instructions (Appendix A) on which foods to avoid for the following two-week washout. Participants were instructed to keep an exercise log (Appendix B) for the entire two-week washout period and to log food intake (Appendix C) for the three days before their second session. Fluid intake was standardized for each subject during the two days prior to the next session.

Two weeks after the initial session in the lab, participants returned at 7 am after a 12-hour fast following a mixed night before meal. Other instructions included no strenuous exercise 48 hours prior, no alcohol within 24 hours of the session, no caffeine, aspirin or NSAIDs the morning of the session and to avoid breakfast. The control exercise trial consisted of a 15-minute warm up of cycling as the intensity was slowly increased to 65% of VO_{2peak}. Immediately following, the subjects cycled at 65% of VO_{2peak} for 40 minutes. After this session, participants followed the same instructions (food and physical activity logs, fluid intake) given prior to the second session. In addition, subjects were instructed to consume the WB drink twice a day for the following two weeks. The third and final session occurred two weeks after the second session and approximately four to five weeks after recruitment. Following the previously mentioned instructions for the second session, participants returned to the lab and repeated the protocol from the second visit. All methods were approved by the Institutional Review Board on April 4th, 2018.

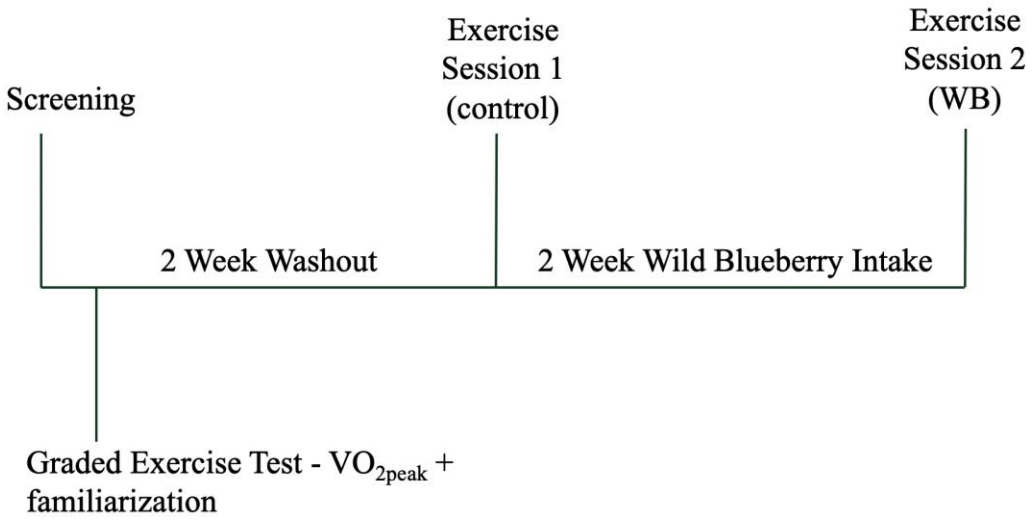

Figure 1. Experimental design

Intervention

Freeze-dried WB powder was donated by The Wild Blueberry Association in Maine containing berries from the 2017 crop. The process of freeze drying the berry results in the least reduction in polyphenol content (Wojdylo, Figiel & Oszmianski, 2009). Subjects were provided with a jar and blender ball and instructed to mix 12.5 grams of the powder in 125 mL of water twice a day for two weeks. The 25 grams of total daily intervention intake is equivalent to one cup raw fruit providing 375 mg of anthocyanins. Further instructions included to avoid food intake, especially dairy products, 30 minutes prior or post WB consumption. Subjects were also asked to consume the WB drink in the morning and evening with a minimum of 8 hours apart.

Lastly, it was recommended that subjects consume the WB drink around the same time in the morning and evening throughout the entire two weeks.

Measures

Body composition was measured using the seven-site skinfold technique (Beta Technology, Santa Cruz, CA). Physical activity was recorded using daily logs kept by each participant. Subjects were instructed to record all physical activity description, duration and intensity. The primary measures considered in this study include fat and carbohydrate oxidation rates. Secondary measures include lactate, heart rate (HR), respiratory exchange ratio (RER), ventilation (V_e), power output (W) and rate of perceived exertion (RPE). All measures were taken at baseline and at 10-minute intervals throughout the 40 minutes of cycling at 65% of VO_{2peak}. Plasma lactate was taken via finger puncture using the Lactate Plus Meter (Nova Biomedical, Waltham, MA). RER and V_e were measured using the TrueOne 2400 metabolic cart (PARVO Medics, Sandy, UT). Rates of whole-body fat and carbohydrate oxidation were determined using the following mathematical equations with the assumption that protein contribution is minor (Jeukendrup & Wallis, 2005).

$$\textit{Fat oxidation (g/min)} = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2$$

$$\textit{Carbohydrate oxidation (g/min)} = 4.210 \times \dot{V}CO_2 - 2.962 \times \dot{V}O_2$$

Statistical Analysis

Statistical analyses were completed using SPSS 20.0 (SPSS, Chicago, USA). The subjects' descriptive data is presented in mean ± standards deviations. Differences between the dependent variables before, during and after the 40 minutes of cycling were analyzed using a condition (WB vs. control) by time-point (0, 10, 20, 30, 40 minutes) one-way analysis of variance (ANOVA) repeated measures. Due to significance between conditions, a paired sample T-test was used to determine which time point varied. Differences in physical activity and food intake between the two weeks before each condition were analyzed using paired t-tests. All data is reported in mean ± standard deviations and significance is set at alpha level of $P \leq 0.05$.

RESULTS

Anthropometrics, Dietary Intake and Physical Activity

No differences in body mass, waist circumference or body fat (Table 1) were found pre-post intervention. Subject's average total aerobic and anaerobic physical activity are shown for the two-week period following both the control and WB conditions in Table 2. There was no significant difference between aerobic or anaerobic physical activity minutes between the control and WB conditions.

Table 2. Dietary intake and physical activity comparison between conditions

Variable	Control	WB	P value
Aerobic Activity (mins)	313.3 ± 324.0	325.7 ± 251.4	0.753
Anaerobic Activity (mins)	110.5 ± 142.5	146.1 ± 160.8	0.292

All data presented as means ± standard deviations

Physiological Data and Cycling Parameters

During both control and WB drink sessions, there were no differences in V_e, heart rate, power output, cadence or RPE (Table 3) detected, however, lactate values were significantly lower at minutes 20 (WB: 2.6 ± 1.0, C: 3.0 ± 1.1, $P = 0.005$), 30 (WB: 2.2 ± 0.9, C: 2.9 ± 1.0, $P = 0.005$) and 40 (WB: 1.9 ± 0.8, C: 2.5 ± 0.9, $P = 0.013$) following WB consumption (Figure 2).

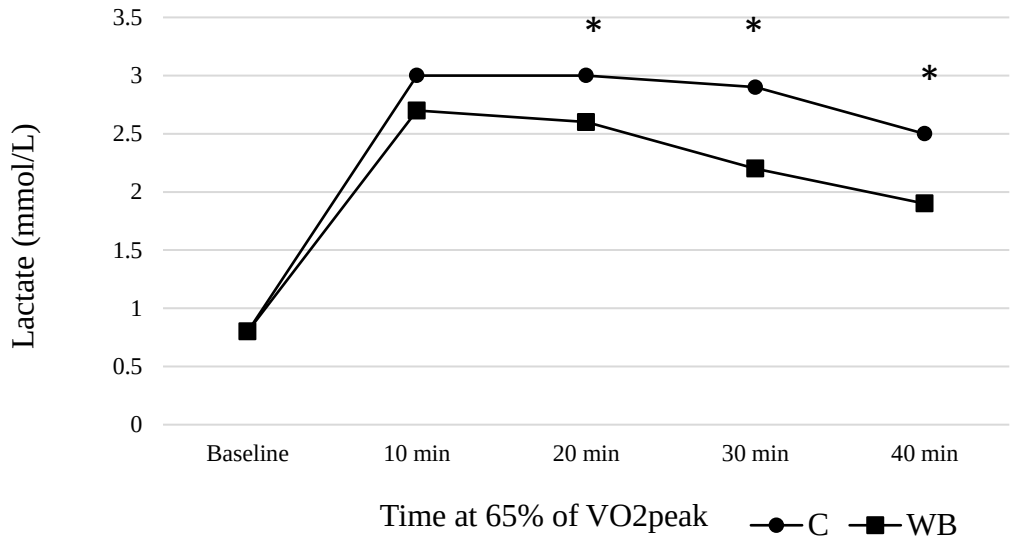

Figure 2. Lactate responses between conditions

Note: * depicts significant difference between conditions ($P \leq 0.05$)

Metabolic Data and Substrate Oxidation

There was no significant difference in RER over time during between the control and WB conditions (Table 3). At 65% of VO_{2peak}, fat oxidation rates were 19.7, 43.2 and 31.1% higher following WB condition at 20 minutes ($P = 0.049$), 30 minutes ($P = 0.010$) and 40 minutes ($P = 0.012$) (Figure 3) respectively. These values were matched by significantly lower carbohydrate oxidation rates of 10.1 ($P = 0.024$), 19.2 ($P = 0.014$) and 14.8% ($P = 0.045$) at 20, 30 and 40 minutes respectively, in the WB trials (Figure 3).

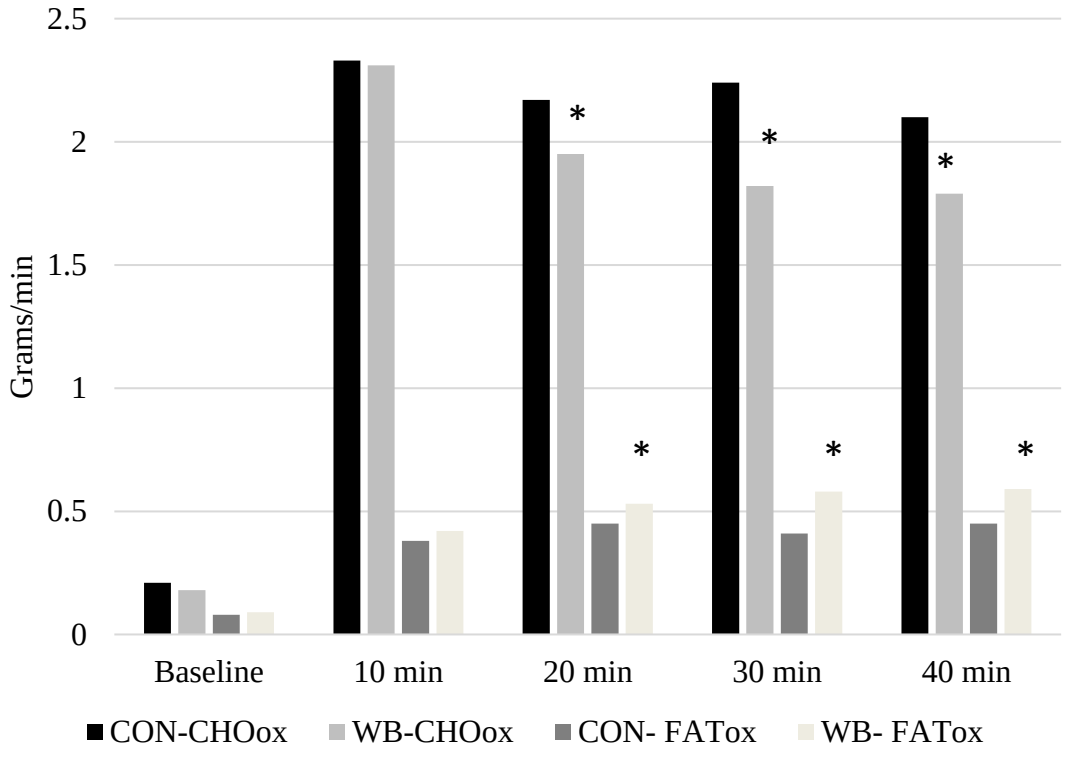

Figure 3. CHO and FAT oxidation rates between conditions

Note: * depicts significant difference between conditions ($P \leq 0.05$)

Table 3. Dependent variable comparisons between conditions at 65% of VO_{2peak}

Time	Condition	HR	Power Output	Cadence	RPE	V_e
Baseline	C	69 ± 10				10.9 ± 1.9
	WB	67 ± 7				10.0 ± 2.1
10 min	C	155 ± 17	191 ± 24	82 ± 6	5 ± 1	67.7 ± 7.5
	WB	155 ± 13	190 ± 26	77 ± 25	5 ± 1	68.7 ± 8.9
20 min	C	144 ± 44	185 ± 25	83 ± 6	6 ± 2	68.5 ± 7.2
	WB	157 ± 15	183 ± 27	83 ± 10	6 ± 2	66.9 ± 9.6
30 min	C	162 ± 17	179 ± 25	83 ± 7	7 ± 2	69. 7 ± 9.1
	WB	160 ± 14	181 ± 26	83 ± 10	7 ± 2	66.7 ± 9.6
40 min	C	165 ± 17	177 ± 24	85 ± 9	7 ± 3	69.1 ± 9.5
	WB	160 ± 16	177 ± 26	83 ± 9	7 ± 2	66.5 ± 8.0

All data presented as means ± standard deviations

HR (bpm), Power Output (watts), Cadence (rpm), RPE (1-10 scale), V_e (L/min)

* $P < 0.05$

DISCUSSION

The use of anthocyanin containing foods to increase FA oxidation during exercise has, to date, focused on the effects of NZBCs, making this is the first study to investigate the effects of WBs on submaximal exercise parameters. The effects of WBs are important due to the greater ability to find this food in the U.S compared to NZBC. Consumption of WBs for 14 days resulted in the novel findings of 1) increased FA oxidation and corresponding decreased carbohydrate oxidation values and 2) decreased plasma lactate values during 40 minutes of moderate cycling in recreational aerobically trained males.

The results indicate a 19.7, 43.2 and 31.1% increase in FA oxidation at 20, 30 minutes and 40 minutes, respectively, following WB consumption. These results are more substantial than both findings of 27% increase in FA oxidation rates in recreational trained male and female cyclists during cycling at 65% of VO_{2peak} following supplementation of whole NZBC extract (Cook et al., 2015; Strauss et al., 2017). Similar to the current study, subjects in both NZBC studies experienced a corresponding trend in lower carbohydrate contributions during the exercise bout following anthocyanin supplementation (Cook et al., 2015; Strauss et al., 2018). In addition, Strauss et al. (2018), detected an increase in non-esterified FAs and glycerol concentrations at rest in the NZBC condition, although no difference between the placebo and NZBC conditions were found during exercise. Discrepancies between the current study and findings in the NZBC studies in fat oxidation rates following anthocyanin intake may be explained by differences in methodology, exercise protocols and supplementation dose.

Both sets of subjects in the NZBC studies exercised two hours postprandial following a standardized breakfast consisting of porridge with semi-skimmed milk, orange juice and a cereal bar (Strauss et al., 2018) or one slice of buttered toast or bread (Cook et al., 2015) while the current study required subjects to exercise following an overnight 12-hour fast. Research has shown that exercising in a fasted state at a low to moderate intensity (< 65% of VO_{2max}) results in a significant increase (3.09 g) of FA oxidation (Viera, Costa, Macedo, Coconcelli & Kruel, 2016) without supplementation. Furthermore, the intensity and total time spent exercising at a specific intensity between the between the current study and previous work is another difference in methodology.

The current study had participants cycle at 65% of VO_{2peak} for 40 minutes while Cook et al., (2015) had participants cycle for only 10 minutes at three different intensities (45, 55 and 65% of VO_{2peak}). Although, a shorter amount of time spent exercising may not be a cause in different FA oxidation rates between WB and NZBC. Strauss et al. (2017) required participants to exercise for 120 minutes at 65% of VO_{2peak} and found similar increases in FA oxidation rates as the shorter trials (Cook et al., 2015). Lastly, both Cook et al. (2015) and Strauss et al. (2017) supplemented with a lower daily dose of anthocyanins, amounting to 210 mg for 7 days compared to the current study's WB supplementation of 375 mg for 14 days. A higher dosage and longer anthocyanin supplementation period may be most accountable for the differences in fat oxidation in the current study compared to past findings. Or the entire matrix of the WB compared to the NZBC could be accountable for the differences in fat oxidation.

The exact mechanism behind anthocyanin consumption effecting fat oxidation is still unknown. It has been proposed that anthocyanins directly influence lipolytic enzymes to increase FA mobilization and oxidation at rest (Jayaprakasam et al., 2006; Prior et al., 2010). Specifically, it is thought that anthocyanins may upregulate adenosine monophosphate protein kinase (AMPK) activity which reduces the inhibition of FA transport into the mitochondria through the impedance of acetyl-CoA carboxylase (ACC) (Rupasinghe et al., 2016). ACC is responsible for the synthesis of manonyl-CoA which inhibits FA uptake in the mitochondria and down regulates carnitine palmitoyl transferase (CPT-1) (Rupasinghe et al., 2016). CPT-1 is responsible for the transportation of acetyl-CoA derived from fatty acids into the inner membrane of the mitochondria (Pekala et al., 2011).

The mechanism behind other polyphenols, such as catechins in green tea, increasing FA oxidation rates is more established and it is possible that WB react in a similar physiological manner. The polyphenols found in abundance in green tea are catechins and fall under the same category of flavonoids as anthocyanins (Kim, Quon & Kim, 2014). Supplementation of green tea catechins and its effects on FA oxidation has been investigated at rest when subjects were provided with 270 mg of tea catechins to be consumed over a 24-hour period (Dulloo, Durewt, Rohrer, Giardier, Mensi, Fathi, Chantre & Vandermander, 1999). This resulted in a significantly higher amount of FA oxidation (41.5%) at rest compared to the placebo group (31.6%). The results are similar in exercise trials as Venables et al. (2008) documented a 17% increase in FA oxidation during 30 minutes of cycling at 60% of VO_{2max} following a single dose of 366 mg

catechins. A four-week decaffeinated green tea extract intervention containing 400 mg of catechins resulted in 24.9% increase in rate of FA oxidation during an hour of cycling (Roberts, Roberts, Tarpey, Weekes & Thomas, 2015) indicating that the catechins, and not just caffeine, are the cause of the increased fat oxidation rates. The proposed mechanism of green tea extract increasing rate of FA oxidation is through the inhibition of catechol-O-methyltransferase (COMT) (Dulloo et al., 1999). COMT typically degrades the catecholamines from stimulating β-adrenergic receptors and decreases lipolysis (Borchardt & Huber, 1975). β-adrenergic stimulation of adenylate cyclase causes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which activates the phosphorylation of hormone sensitive lipase (HSL) (Horowitz, 2003; Prats et al., 2006). HSL splits TGs into three free FAs and a glycerol molecule, known as lipolysis (Horowitz, 2003; Melzer, 2011; Rupasinghe et al., 2016). Once split from the three FAs, the glycerol diffuses into the blood and is ready to be transported into the mitochondria to be used as energy (Pekala et al., 2011). The result of catechins consumption is prolonged norepinephrine effect and increased FA metabolism (Dulloo et al., 1999).

In addition to increased reliance on FA stores during moderate intensity exercise, there may be other benefits from WB supplementation including increasing exercise performance. Cook et al. (2015) documented increases in post 16.1 km time trial plasma lactate values following seven-day NZBC supplementation in trained male cyclists. The current study did not include a direct performance measures however differences in lactate values between conditions may have insight on WB effects on exercise

performance. Lower plasma lactate values at 20, 30 and 40 minutes in the WB condition were found. These findings are similar to intake of NZBC powder causing a downward and rightward lactate shift with significantly lower lactate values at submaximal and maximal power outputs in trained triathletes (Willems, Myers, Gault & Cook, 2015).

Lactate removal from skeletal muscle may be impacted by increases in peripheral blood flow (Matsumoto, Takenami, Iwasaki-Kurashige, Osada, Katsumura & Hamaoka, 2005) and nitric oxide mediated vasodilation following anthocyanin supplementation (Mendes, Desgranges, Cheze, Vercauteren & Freslon, 2003; Ziberna, Lunder, Tramer, Drevensek & Passamonti, 2011). Increased blood flow during exercise may result in decreased mechanisms causing fatigue, such as preventing decreases in pH in the muscle and decreased force production, thus increased exercise performance (Willems et al., 2015). An increase in fat oxidation coupled with a lower response in lactate with WB drink may be beneficial to endurance exercise performance. Therefore, future research should investigate the effects of increase FA oxidation from WB consumption on exercise performance.

Limitations

To the best of our knowledge this is the first study to investigate the effects of WB consumption on rate of FA oxidation during moderate intensity exercise in humans. However, this study has several limitations. The majority of these limitations are due to the study being a free-living trial however, these methods are more translational in nature. First, participants were entrusted to track and interpret their own physical activity and food intake during their participation. This could potentially lead to inaccurate

tracking of serving sizes, exercise minutes and intensity. Secondly, participants were given WB powder to consume on their own time with the instructions described in the methods section, leaving room for potential error. Participants may have forgotten to take a dose at the same time every day, consumed the WB with other food or forgotten to consume a serving completely. Lastly, not all participants used cycling as a method of exercise however they were required to cycle during each session, leading to some participants having difficulty determining a preferred cadence during the 40 minutes of cycling.

CONCLUSION

The current study demonstrated that 14-day intake of WB supplying 375 mg of anthocyanins increased fa oxidation rates, decreased carbohydrate oxidation rates and lowered the lactate response during 40 minutes of cycling at 65% of vo_{2peak} in endurance trained males. These findings may have potential effects on endurance exercise performance.

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BLUEBERRY DRINK AND FAT OXIDATION PARTICIPANT INSTRUCTIONS

A. Two-week washout only	**Next Session Date:**

During the 2 weeks before next session

- Resume regular diet and avoid/limit the foods attached to the food log for the entire 2 weeks
- Resume regular exercise and track all activity for the entire 2 weeks on the physical activity log

During the 3 days prior to exercise session

- Log all food intake in food log (see attached instructions)
- Stay hydrated by consuming _____ ounces of water each day until the next session

During the 2 days prior to exercise session

- Avoid strenuous exercise (light and moderate exercise is OK)

During the 24 hours prior to exercise session

- Avoid all alcohol intake

The evening before exercise session

- Consume your final meal 12 hours before your session start time
- This meal should have a balance of carbohydrates, fats and protein
- Please email Jessie if you are unsure about these instructions

The morning of the exercise session

- Avoid food, use of NSAIDs (aspirin, ibuprofen) or caffeine
- Water is OK (not excessive amounts)
- Collect urine from the first bathroom use of the morning
- Bring completed food log, physical activity log and urine to the lab

B. Two-week washout with blueberry consumption	**Next Session Date:**

During the 2 weeks before next session

- Resume regular diet and avoid/limit the foods attached to the food log for the entire 2 weeks
- Resume regular exercise and track all activity for the entire 2 weeks on the physical activity log
- Twice a day, mix one bag of blueberry powder with ½ cup (4 ounces of water)
- Avoid all other food, especially dairy products, 30 minutes before or after drinking blueberry drink
- Blueberry drinks must be consumed at least 8 hours apart and intake should be consistent between the days (try to consume blueberry drink at the same times each day of the two weeks)

During the 3 days prior to exercise session

- Log all food intake in food log (see attached instructions)
- Stay hydrated by consuming _____ ounces of water each day until the next session

During the 2 days prior to exercise session

- Avoid strenuous exercise (light and moderate exercise is OK)

During the 24 hours prior to exercise session

- Avoid all alcohol intake

The evening before exercise session

- Consume your final meal 12 hours before your session start time
- This meal should have a balance of carbohydrates, fats and protein
- Please email Jessie if you are unsure about these instructions

The morning of the exercise session

- Avoid food, use of NSAIDs (aspirin, ibuprofen) or caffeine (water is OK)
- Collect urine from the first bathroom use of the morning
- Bring completed food log, physical activity log and urine to lab

Participant Code: _____

Physical Activity Record

Please record all workouts during the 2 week washout and the 2 week supplementation period. This includes all purposefully exercise and physical activity, as well as additional strenuous activity such as: moving, hiking, lawn care, extensive cleaning, leasure walks, play etc.

Instructions:

1. Maintain your current level of physical activity. Any activity recorded on this form should represent your usual level/intensity of exercise.
2. Please denote the day and time of activity

See back side of form for definitions of low, moderate, and high physical activity. Examples are shown in the shaded rows.

Day and Time	Physical Activity performed	Description of Activity	Intensity (*low, mod., high)	Duration
Example: 8:00am	Jogging	Jogged on trails behind campus	High plus hills	60 minutes

APPENDIX C

INSTRUCTIONS FOR RECORDING 3-DAY DIETARY RECORDS

1. Please record each food and beverage item you consume on a separate line. Be sure to include all snacks.
2. Record each item after weighing in exact amounts:
    - liquids in cups or **fluid** ounces
    - vegetables and fruits in cups, grams, or ounces
    - beans, grains, and pasta in cups **dry** or cups **cooked**
    - bread in slices, indicate what kind of bread (brand name and type)
    - meats, fish, poultry and cheeses in ounces
    - nuts in cups, ounces, or grams
    - chips or other snack type foods in cups, ounces, or grams
    - Spread (butter, cream cheese, margarine, etc.) in **tsp** or **Tbs**
3. Please specify if food is consumed raw. Also indicate if it was prepared from fresh, frozen, or canned products.
4. Indicate how the foods were **prepared**, such as fried, baked, boiled, etc.
5. If a food has a mixture of ingredients (sandwich or casserole), list the major ingredients separately in their proportions or amounts.
6. Use **brand** names whenever possible, or mention comparable brand.
7. For fruits and vegetables, please indicate if the skin was removed.
8. Indicate if **dairy** products are whole, 2%, or skim.

9. Be sure to include sauces, gravies, milk/sugar in coffee, etc.
10. Check food labels for weights, etc. Candy bars, cheeses, cookies, juices are all labeled with their weights -----Write this information down!
11. Provide any other information you feel might be helpful, such as food labels and/or recipes.
12. Record **EVERYTHING** edible that goes in your mouth.
13. **MOST IMPORTANTLY**, eat as you normally would -- please don't change your usual eating habits or modify your portion size.

Foods and Beverages to Avoid During the Study

Avoid the following:

- Blueberries (besides the ones provided daily)
- Raspberries
- Blackberries
- Cranberries
- Red Grapes
- Red Cabbage
- Strawberries
- Red Wine
- Red Currants

- Black Currants
- Egg Plant
- Chokeberry
- Plums
- Cherries
- Elderberries
- Oranges
- Radishes
- Red Onions
- Any Juice from the fruits above

Limit the following:

- Oranges: limit to 2 oranges a week
- Orange juice: limit to 16 oz per week
- Tomato sauce: limit to ½ cup, twice a week
- Tomatoes: limit to ½ cup, twice a week
- Red onions: limit excessive amounts
- Limit excessive intake of NSAIDS (aspirin, ibuprofen, and naproxen)
- Limit dietary supplements to a daily multivitamin only
- No additional vitamin C, Omega 3 fatty acids

3-DAY FOOD/BEVERAGE INTAKE RECORD

Please measure and weigh all food and beverages you eat throughout the day and write them down as you eat them. Remember to give as many details as possible, keep the food label if you think it will help describe the food better than you are able to. Providing us with recipes for homemade foods is helpful for us, too. Please list any vitamin or mineral supplements or any other supplements taken on the backside of this form and attach these labels if possible. It's best to be as descriptive as possible!

Time	Food or Beverage item	Brand/source	Type of preparation	Amount/weight