International Journal of Exercise Metabolism and Sports Nutrition
Accepted version, 21/07/2018

Title of Article: Dietary Anthocyanins: A Review of the Exercise Performance Effects and Related Physiological Responses

Running Title: Exercise Performance Responses to Anthocyanins

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Abstract

Foods and supplements high in anthocyanins are gaining popularity within sports nutrition. Anthocyanins are pigments within berries and other colourful fruits and vegetables. They have anti-oxidative and anti-inflammatory actions that improve recovery from exercise. Furthermore, anthocyanins can also affect vasoactive properties, including decreasing mean arterial blood pressure and increasing vasodilation during exercise. *In vitro* observations have shown anthocyanin- and metabolite-induced activation of endothelial nitric oxide synthase and human vascular cell migration. However, effects of anthocyanins on exercise performance without a prior muscle-damaging or metabolically demanding bout of exercise is less clear. For example, exercise performance effects have been observed for blackcurrant, but are less apparent for cherry, therefore indicating that the benefits could be due to the specific source-dependent anthocyanins. The mechanisms by which anthocyanin intake can enhance exercise performance may include effects on blood flow, metabolic pathways, and peripheral muscle fatigue, or a combination of all. This narrative review focuses on the experimental evidence for anthocyanins to improve exercise performance in humans.

Keywords: polyphenols; anthocyanin metabolites; sports nutrition

INTRODUCTION

Epidemiological studies have indicated that high intake of dietary polyphenols is associated with lower risk for multiple diseases (Kuriyama et al., 2006; Ivey et al., 2017). Based on chemical structures, there are four groups of polyphenols, i.e. phenolics, flavonoids, stilbenes and lignans, with classes within the groups. Anthocyanins are a class of the flavonoids. The dietary intake of the main anthocyanins are glycosides of their respective aglycones; pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Wu et al., 2006). Anthocyanins are water-soluble and act as natural pigments causing purple, blue, red and

orange colouration to flowers, leaves, fruits and vegetables. Over 500 different anthocyanins exist, based on structural variety such as the number and position of hydroxyl and methoxyl groups, the specific type and number of bonded sugars, the aliphatic, or aromatic carboxylates bonded to the sugar and the bond position (Speciale et al., 2014).

Observational studies indicate a causal link between anthocyanin intake and decreased disease risk, including cardiovascular disease (Cassidy et al., 2016), type-2 diabetes (Muraki et al., 2013) and ageing associated cognitive decline (Letenneur et al., 2007). For many years, benefits were attributed to the anthocyanins scavenging free radicals by B ring hydroxyl groups and conjugated double bonds. However, anthocyanins also affect signalling pathways (Qin et al., 2012), particularly the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Cimino et al., 2013; Yan et al., 2017). Nrf2 is a transcription factor regulating gene expression of antioxidant proteins. Oh et al., (2017) observed upregulation of Nrf2 in mice and increased endurance exercise performance, and similar responses may occur in humans. However, such observations have not been made in human studies. Furthermore, effects on blood flow (Matsumoto et al., 2005), blood vessel diameter during exercise (Cook et al., 2017) and endothelial nitric oxide synthase (Xu et al., 2004b) by anthocyanins may all provide mechanisms for improved exercise performance.

In a recent systematic review and meta-analysis, polyphenol supplementation for at least 7-days or more increased exercise performance by 1.90% (95% CI 0.40-3.39), with the analysis including studies using quercetin, anthocyanins, epigallocatechin gallate, epicatechin and *trans*-resveratrol (Somerville et al., 2017). This narrative review, however, will focus on the effect of anthocyanin intake by humans on exercise performance. Berry fruits such as blackcurrant, blueberries and raspberries and drupes such as cherry contain high concentrations of anthocyanins, but each with a specific make-up of anthocyanins. For example, the main anthocyanin in blackcurrant is delphinidin-3-rutinoside, whereas in cherry

it is cyanidin-3-glucosylrutinoside (Rothwell et al., 2013). In humans, foods containing primarily delphinidin improved metabolic and cardiovascular disease risk biomarkers (Stull et al., 2010; Zhu et al., 2011), whereas cyanidin did not provide the same protective benefits (Curtis et al., 2009; Wright et al., 2013). In addition, delphinidin has a higher potency of activity towards the superoxide radical than cyanidin (Rahman et al., 2006). Therefore, different berries with specific anthocyanin contents may provide different physiological effects, indicating that not all berries may improve exercise performance. This narrative literature review will focus on studies in humans examining the effects of anthocyanin intake on exercise performance and not focus on a specific fruit.

EXERICSE PERFORMANCE

The first study to observe that anthocyanins could be beneficial for exercise performance was published by Willems et al. (2015). Within the study, thirteen trained triathletes (8 males) were supplemented for 7-days with 6 g·day^{-1} of New Zealand blackcurrant (NZBC) powder (~139 mg anthocyanin·day^{-1}) dissolved in 140 mL of water before completing an incremental cycle ergometer test. NZBC powder caused a downward shift of the lactate curve during incremental intensity cycling with lower plasma lactate at 40, 50, 60 and 70% of maximum power. In addition, the intensity at 1 mmol·L^{-1} lactate rise was 4% higher (placebo: 184±52 vs. blackcurrant: 192±52 W) and the intensity at 4 mmol·L^{-1} was 6% higher (placebo: 223±57 vs. blackcurrant: 236±60 W). There was no difference in heart rate and oxygen uptake at the pre-defined reference points (i.e. 1 and 4 mmol·L^{-1}), and diastolic, systolic, mean arterial pressure, heart rate, stroke volume, cardiac output and total peripheral resistance during the incremental exercise. Maximum oxygen uptake ($\dot{V}O_{2max}$) (placebo: 49.1±6.2 vs. blackcurrant 49.7±6.1 mL·kg^{-1}·min^{-1}) and maximum power output at $\dot{V}O_{2max}$ (placebo: 305±68 vs. blackcurrant 307±62 W) were also not different. These results show no effect of blackcurrant on the oxygen cost of the exercise, $\dot{V}O_{2max}$ or maximum power ability,

however, lactate observations suggest that exercise performance could be enhanced by blackcurrant anthocyanins.

Cook et al. (2015) examined the effects of blackcurrant anthocyanins on exercise performance in a 16.1 km cycling ergometer time-trial. Following 7-days intake of blackcurrant extract capsules (105 mg anthocyanin·day^{-1}) in 14 trained male cyclists, the study observed a 2.4% faster 16.1 km time (P=0.027) with blackcurrant (placebo 1722±131 vs blackcurrant 1678±108 s). There have been similar observations of increased exercise performance following 7-days intake of blackcurrant extract in different exercise models. For example, Perkins et al. (2015) observed an increase of 10.6% (P=0.023) in total running distance during an incremental intermittent high-intensity running protocol to exhaustion on a treadmill (placebo 3871±622 vs. blackcurrant 4282±833 m) and Murphy et al. (2017) observed an increased performance of 0.82% (P=0.034) for a repeated 4 km cycling time-trial (placebo 771±60 vs. blackcurrant 764±56 s), but sample size could have been a limitation to allow firm conclusions for each of the two separate 4 km tests. Increased resistance to fatigue during exercise has also been shown following intake of blackcurrant extract with less slowing of maximal sprint running in the last 15-minute block of the Loughborough Intermittent Shuttle Test (Willems et al., 2016). The positive effects of blackcurrant extract on exercise performance have been identified in trained cyclists (Cook et al., 2015; Murphy et al., 2017; Willems et al., 2015), active but untrained males (Perkins et al., 2015; Willems et al., 2016) and trained youth footballers (Godwin et al., 2017). The effects in elite athletes are unknown and need to be examined in future research, especially in those with a relatively low anthocyanin intake. It has been recently shown that baseline antioxidant status can be a determinant in the effectiveness of supplementing with antioxidants. For example, individuals with a low baseline status of vitamin C (Paschalis et al., 2016) and glutathione (Paschalis et al., 2018) improved their $\dot{V}O_{2max}$ following supplementation with vitamin C and N-

acetylcysteine, respectively, however those with higher baseline levels did not respond. In addition, all these performance studies except Willems et al. (2015) used men, therefore studies in women are needed to confirm no differences between the sexes. A recent study by Strauss et al. (2018) replicated for females findings by Cook et al. (2017) in males of increased fat oxidation by New Zealand blackcurrant during 120-minutes cycling at 65% $\dot{V}O_{2max}$, therefore increased exercise performance from blackcurrant anthocyanins in females is likely.

The effects of other high anthocyanin content fruits on exercise performance are less clear though. For example, cherry has received considerable interest for its effects on recovery (Bell et al., 2014; Bell et al., 2016; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010), however there is limited evidence on its potential to increase exercise performance without a mechanically damaging or metabolically fatiguing protocol (Table 1).

The first study to examine the effect of cherry anthocyanins on exercise performance was by Clifford et al. (2013). The study compared 120 mg Pycnogenol® (citrus bioflavonoids), 200 mg CherryActive® and placebo (200 mg maltodextrin) on 20 km cycle ergometer time-trial performance in nine moderately trained triathletes and cyclists. Participants were supplemented for 2-days before and on the day of the time-trial, with results showing no difference between the conditions (Pycnogenol®: 1990.07 ± 93.18 vs. CherryActive®: 2008.56 ± 97.50 vs. placebo: 2030.30 ± 124.73 s). However, the *P*-value of 0.117 suggests it was approaching a trend for a performance effect and the sample size of nine subjects may therefore indicate that the study was underpowered to allow a firm conclusion. In a study by Howatson et al. (2010) to examine the effects of cherry juice on recovery following marathon running, a secondary measure identified if there was any influence on marathon running performance (i.e. finish times) in recreational marathon runners. Supplementing two groups matched for their predicted finish time with a short intake (5-days) before the marathon with

cherry juice (~40 mg·day^{-1} anthocyanins) or placebo allowed comparison between the groups on their marathon performance. The study observed no difference in marathon finishing time for the two conditions, however, the difference between the actual and predicted finish time was smaller for the cherry group (predicted: 3:41:00±0:26:01 vs. actual: 3:48:04±0:48:48 h:min:s) than the placebo group (predicted: 3:56:40±0:40:37 vs. actual: 4:15:48±1:01:22 h:min:s), although this was not significantly different. However, sample size may have been an issue in Howatson et al. (2010) to not showing a significant beneficial effect for the cherry juice. In a similar study, Levers et al. (2016) used endurance trained runners and split them in two matched groups on predicted race pace (from results of previous year). The study supplemented in a double-blind design with a powdered form of tart cherry skins (66 mg·day^{-1} anthocyanins) or placebo for 7-days prior and on the day of a half marathon, in turn, allowing comparison on exercise performance in the race. Half-marathon finish time was 13% faster (P=0.001) in the cherry group (cherry: 103±9.28 vs. placebo: 118±9.72 minutes). However, within the studies by Howatson et al. (2010) and Levers et al. (2016), there was no cross-over condition, therefore, it is difficult to determine if exercise performance was improved by cherry in those studies.

What is more, in the studies by Howatson et al. (2010) and Levers et al. (2016), the exercise durations were long, and therefore the exercise intensities are likely lower than those of the blackcurrant studies with short duration exercise periods of Cook et al. (2015), Murphy et al. (2017), Perkins et al. (2015) and Willems et al. (2016). Anthocyanins have been shown to increase vasodilation and cardiac output (Cook et al., 2017) and peripheral blood flow in the forearms (Matsumoto et al., 2005). Alterations in blood flow may benefit exercise performance where the intensity results in an imbalance of perfusion to support metabolism and causes a decrease in intramuscular oxygen partial pressure (Bylund-Fellenius et al., 1981) and acidic conditions (Costill et al., 1983) such as those during high intensity exercise.

Recently, Keane et al. (2018) was the first study to examine the effects of an acute intake of cherry on exercise performance. They observed 60 mL Montmorency cherry juice containing ~60 mg anthocyanins to have no effect on cycling time-to-exhaustion during severe intensity exercise (CherryActive®: 772±32 vs. placebo: 733±32 s, *P*=0.323), however in a 60-s all-out sprint following the time-to-exhaustion, cherry increased peak power by 9.5% (CherryActive®: 363±42 vs placebo: 330±26 W, *P*=0.034) and total work by 10% (CherryActive®: 21±3 vs. 19±3 kJ, *P*=0.021).

The studies by Cook et al. (2015), Godwin et al. (2017), Murphy et al. (2017), Perkins et al. (2015) and Willems et al. (2016) observed blackcurrant extract taken for 6-days before and on the morning of the seventh-day, 2-hours before performance testing, with Keane et al. (2018) supplementing cherry acutely 90-minutes before. This raises questions if the performance benefits are entirely affected by intake of last dose, or a result of the previous 6-days intake. Furthermore, the plasma metabolites by anthocyanin intake are likely key to the observed performance and physiological responses. For example, following a 500 mg intake of cyanidin-3-glucoside, Czank et al. (2013) observed a peak concentration of 0.14±0.05 µmol/L and area under the curve in 48-hours of 0.31±0.13 µmol·h/L for cyanidin-3-glucoside, while the metabolite hippuric acid had a peak concentration of 1.96±1.39 µmol/L and area under the curve in 48-hours of 46.42±30.31 µmol·h/L. Therefore, bio-accumulation of metabolites including phase II conjugates (Czank et al., 2013) by anthocyanin intake over 7-days is possible and may have been required to cause the exercise performance benefits. In an animal study by Kirakosyan et al. (2015), three weeks of cherry feeding resulted in diverse tissue distribution of anthocyanins. To the author's knowledge, no studies have examined if bio-accumulation of metabolites and diverse tissue distribution of anthocyanins in human occurs following multiple days intake of anthocyanins. However, Kalt et al. (2014) observed that following an intake of 250 mL of blueberry juice, metabolites of anthocyanins are still

present in urine 5-days following no further intake of anthocyanins. Most interestingly, metabolites from the anthocyanin pelargonidin were present, which were not in the blueberry juice, which may indicate dihydroxylation and demethylation of anthocyanin by xenobiotic and colonic bacteria (Kalt et al., 2014).

It is likely that the efficacy of anthocyanin supplementation on exercise performance without a prior fatiguing bout will depend on several inter-related factors. Firstly, the subjects training status, age, health, habitual anthocyanin consumption and expression of genes. Secondly, the dose and duration of intake, the specific anthocyanins consumed and/ or the food source of anthocyanins consumed. Lastly, the intensity, duration and type of exercise. An acute intake may influence cardiovascular alterations, such as vasodilation (Cook et al., 2017) and increased peripheral blood flow (Matsumoto et al., 2005), but longer intake durations may be required to result in changes in cellular signalling [see below for discussion].

TRAINING RESPONSES

While benefits to exercise performance following a short duration of intake of anthocyanins have been observed (Table 2) (Cook et al., 2015; Godwin et al., 2017; Keane et al 2018; Murphy et al., 2017; Perkins et al., 2015; Willems et al., 2016) the effects of regular intake on training adaptations are an important consideration. Blunting of training adaptations has been observed following a high intake of antioxidants, and it is possible that the blunting requires an intake threshold. For example, vitamin C intake of 200 $mg \cdot day^{-1}$ can be justified for health reasons, though an intake of >1000 $mg \cdot day^{-1}$ appears to blunt training adaptations by limiting mitochondrial biogenesis and possibly altering vascular function (Braakhuis et al., 2012). It is not known if anthocyanins can have the same effects, however, cyanidin-3-glucoside have been shown to increase gene expression for sirtuin 1 and proliferator-activated receptor gamma coactivator-1α (PGC1-α) in myotubes (Matsukawa et al., 2015). PGC1-α activation is required for mitochondrial biogenesis in skeletal muscle (Islam et al., 2018). Anthocyanins

also increased activation of AMP-activated protein kinase and expression of PGC1-α in mice hepatocytes with non-alcoholic steatohepatitis (Tang et al., 2015). Extrapolation of these findings to human muscle during a period of physical training could provide positive benefits of regular anthocyanin intake on training adaptations. However, this is speculative, therefore further research on combined effects of anthocyanin intake and physical training on biological adaptations is required.

Braakhuis et al. (2014) also examined the influence of blackcurrant on 5 km road running time-trial and an incremental treadmill test to exhaustion following supplementation during a training period in female runners. Using a randomised, three-condition, cross-over placebo-controlled design, twenty-three trained female runners consumed 250 mL of fruit drink concentrate mixed with blackcurrant juice powder twice daily providing 300 $mg \cdot day^{-1}$ of anthocyanins and vitamin C mixed with fruit juice or placebo. Intake was for 24-days while undergoing high intensity running training controlled for estimation of the training impulse with a washout of 26 days between conditions. There were no effects on 5 km time-trial performance, however during the incremental test to exhaustion, they reported with inferential statistics (with 90% confidence limits) a possible improvement of 1.9±2.5% for the fastest runners by 1 SD and 2.3±3.6% for the runners faster by 2 SD (i.e. runners faster by 1 and 2 SD of mean speed on an incremental running test, respectively) for the blackcurrant condition. Interestingly, the average runners in the cohort had no change in performance following the training and supplementation period and were possibly slower. In addition, Godwin et al. (2017) observed also the beneficial effect of blackcurrant on sprint performance in more highly trained football players.

Controlling a 6-week training period of treadmill and cycle exercise (3 times a week, 60-90 min) so that participants started at 60-65% of maximum heart rate and progressed to 75-85% of maximum heart rate by the end of the training period, Yarahmadi et al. (2014) used a

double-blind randomised design to supplement with 100 mg·day^{-1} anthocyanin capsules (food source and individual anthocyanins not stated) or placebo across the training period in active (>3 years history of athletic training) males and females. Following the training period, the anthocyanin group increased their $\dot{V}O_{2max}$ (anthocyanin pre: 48.65±4.73, post: 52.62±5.04 mL·kg^{-1}·min^{-1}), whereas the placebo group did not (placebo pre: 49.88±5.23, post: 49.61±5.33 mL·kg^{-1}·min^{-1}). However, as $\dot{V}O_{2max}$ is only an indicator of endurance exercise performance potential, it is not possible to state that endurance performance can improve from training while supplementing with anthocyanins.

To the author's knowledge, these are the only studies to have used anthocyanin supplementation during training. As there was no detriment in performance in either study following intake of blackcurrant anthocyanins compared to placebo, it is likely that for these doses and conditions, there is no suppression of training responses despite the anti-oxidative properties of anthocyanins. However, further research is recommended on high doses and prolonged intakes of anthocyanins during controlled training periods to identify if negative responses can occur.

RECOVERY RESPONSES

Many studies examined the effects of anthocyanins on markers of oxidative stress (e.g. thiobarbituric acid reactive substances, total antioxidant status, lipid hydroperoxides and protein carbonyls) and inflammation (e.g. interleukin 6, tumour necrosis factor α, C-reactive protein) following muscle-damaging and metabolically demanding exercise (Bell et al., 2014; Bell et al., 2015; Bell et al., 2016 ; Howatson et al., 2010). Identifying marker responses during recovery is outside the scope of this review, however, we will address effects of anthocyanins on the functional recovery from muscle-damaging and/or fatiguing exercise.

To the best of our knowledge, Connolly et al. (2006) was the first study to observe that anthocyanins could be beneficial for recovery. Using a single blind crossover design, male

students were supplemented with Montmorency cherry juice mixed with apple juice, each intake containing at least 40 mg anthocyanins for 9-days (4-days pre, day of and 4-days post exercise), or the placebo of black cherry soft drink mixed with water. In the 96 hours following a muscle damaging protocol of the elbow flexors, Montmorency cherries showed significant attenuation ($P<0.0001$) in the decline of isometric strength compared to placebo (4% vs. 22%, respectively). Similarly, Bowtell et al. (2011) demonstrated for well-trained males in a double-blind crossover design that 10-days of Montmorency cherry containing ~234 $mg \cdot day^{-1}$ anthocyanin (7-days pre, on the day and 2 days post exercise) resulted in a more rapid recovery ($P=0.04$) of isokinetic knee extensor force than the placebo (i.e. fruit concentrate) following an eccentric-induced muscle damage protocol of 100 knee extension at 80% of one-repetition maximum. McLeay et al. (2012) also observed that anthocyanins from a New Zealand Blueberry smoothie (~97 mg anthocyanin per smoothie), taken 5 and 10 hours prior to a bout of eccentric isokinetic contractions of the knee extensors and 12 and 36 hours post bout, were associated with a faster rate of recovery of isometric peak torque 36 hours post bout in physically active females ($P=0.047$). While these three studies have observed ergogenic effects on the recovery of muscle function following eccentric exercise-induced muscle damage, the results should be taken with caution. The studies used crossover designs so that the participants completed the experimental exercise protocol on two visits. This was achieved by exercising the contralateral limb in the second visit. In addition, repeated eccentric exercise is known for the repeated bout effect, whereby a second bout of eccentric exercise experiences a protective effect on damage and muscle function (McHugh et al., 1999). Protective effects may happen as well to the contralateral limb not previously exposed to eccentric exercise (Howatson and van Someren, 2007; Starbuck and Eston, 2012). Therefore, matched groups or sufficient time between sessions would be a better study design

to examine these responses. However, the crossover design ensures that the digestion and bioavailability of anthocyanins will likely remain constant between visits.

Using matched groups, by assigning participants to experimental conditions (i.e. cherry juice or placebo) based on predicted marathon finish time, Howatson et al. (2010) showed that in the 48-hours post-marathon, the recovery of maximal voluntary isometric force of the knee extensors was improved (P<0.024) with the total intake of cherry juice 5 days before, on the day of the marathon and 48-hours post-marathon. However, decrements in maximal voluntary isometric force immediately following the marathon were similar (cherry: 24.3% vs. placebo: 26.9%) indicating that cherry anthocyanins (80 mg·day^{-1}) do not affect muscle damage and fatigue sustained from the marathon.

Bell et al. (2014) examined in trained cyclists the responses of Montmorency cherry extract on multiple bouts of 109 minutes stochastic road cycling simulation with intake 4-days pre-cycling and 3 consecutive days of time-trials with supplementation. While a decrease in total work performed was observed across the three time-trial days, there was no difference between Montmorency cherry extract or placebo on total work performed indicating no effect on repeated cycling performance over several days. A further study by Bell et al. (2015) examined the recovery responses 72 hours following one bout of the same 109 minutes stochastic road cycling simulation and demonstrated that maximal voluntary isometric contraction of the knee extensors was significantly attenuated with Montmorency cherry versus placebo, with between-group differences of 10%, 12% and 21% at 24, 48 and 72 hours post bout, respectively. This was the first study to highlight that anthocyanins were associated with maintenance of muscle function following exercise that did not include damage by eccentric contractions such as those of Bowtell et al. (2011), Connolly et al. (2006), Howatson et al. (2010) and McLeay et al. (2012). Bell et al. (2016) examined further the attenuation of muscle function following another exercise simulation, this time adapting the

Loughborough Intermittent Shuttle Test to standardise the distance covered by all participants (the original protocol requires running to exhaustion). Maximal voluntary isometric force of the knee extensors was maintained with Montmorency cherry at 24, 48 and 72 hours following the exercise, with a peak difference between placebo and cherry of 19% occurring 48-hours post bout. Performance in other functional measurements such as the counter movement jump, 5-0-5 agility test and 20-metre sprint time, were also all attenuated with cherry within the recovery period. Taken together, these studies indicate that supplementation with anthocyanins from cherry protect against declines in muscle function following strenuous activity and has positive effects on subsequent functional recovery after damaging or fatiguing exercise.

MECHANISMS

The mechanisms for increased exercise performance by anthocyanin intake have not been fully elucidated, however may result from alterations in blood flow. For example, Matsumoto et al. (2005) observed in a maximal voluntary contraction (MVC) of the trapezius muscle following 30-minutes of typing a change in total haemoglobin (measured by near-infrared spectroscopy) within the muscle of 106.0±12.8% of the pre-value following blackcurrant concentrate capsules, however following placebo it decreased to 53.2±21.6%. Furthermore, Cook et al. (2017) observed during a 120-second isometric contraction of the knee extensors at 30% of MVC that the femoral artery diameter of the exercising limb was between 6.9 and 8.2% larger following 7-day intake of New Zealand blackcurrant extract. This was also coupled with a decrease in activation of the *vastus medialis muscle*, a decrease in total peripheral resistance and an increase in cardiac output during the contraction (Cook et al., 2017).

In the studies of Cook et al. (2015, 2017), it was reported how many of the participants had a change in exercise performance and femoral artery diameter following blackcurrant extract

intake, respectively. In both studies ~80% of the participants responded to the New Zealand blackcurrant intake and it could be possible that changes in exercise performance and blood flow alterations by anthocyanins are associated with genotype. For example, George et al. (2012) examined the effect of a high flavonoid fruit and vegetable drink on vasodilation within the forearm (measured by Laser Doppler with iontophoresis) 180-minutes following intake. Vasodilation in the forearm following consumption was different for those with different expressions of the endothelial nitric oxide synthase (eNOS) gene Glu298Asp, whereby there was higher endothelium-dependent vasodilation in response in GG individuals compared to GT. Interestingly, plasma nitrate and nitrite increased from baseline following intake for both genotypes, however there was no difference between the genotypes in the amount. The metabolite hippuric acid also increased for both genotypes, indicating that the polyphenols within the flavonoid drink were absorbed and metabolised, however, at 300-minutes post intake for those with GG expression, there was approximately a 120% increase from baseline, whereas for the GT expression this increase was approximately 500% from baseline. The cause for this large difference is unknown, however may result from the gut microflora which are responsible for converting polyphenols to phenolic acids.

If an alteration in blood flow is a causal factor for improved exercise performance, then increasing availability of nitric oxide could be the mechanism. *In vitro* cyanidin-3-glucoside has been shown to up-regulate eNOS (Xu et al., 2004a, Xu et al., 2004b; Sorrenti et al., 2007). This was observed using cultured endothelial cells at concentrations from 0.001 to 250 µM. However, Czank et al. (2013) and de Ferras et al. (2014) have confirmed a physiological range of anthocyanin metabolites in humans of 0.1 – 10 µM. Using doses of 0.1, 1 and 10 µM, Edwards et al. (2015) observed differential activity between the whole-body anthocyanin and metabolites, with cyanidin-3-glucoside up-regulating eNOS on every dose, but the metabolites protocatechuic acid and vanillic acid having no influence at any dose within a

human vascular cell model. However, the metabolites can maintain vascular homeostasis by increasing nitric oxide bioactivity through mechanisms involving NADPH inhibition or inducing cytoprotective enzyme haem oxygenase-1, an enzyme that catalyzes the degradation (Edwards et al., 2015). Interestingly, Keane et al. (2016a) observed no *in vivo* increase in plasma nitrate and nitrite (i.e. proxy markers of eNOS activity) following intake of Montmorency cherry concentrate, yet Keane et al. (2016b) observed a combination of the anthocyanin metabolites protocatechuic acid and vanillic acid to increase human vascular smooth muscle cell migration *in vitro*. Therefore, the metabolites of the anthocyanins are likely key to the vascular effects observed. So far only a few metabolites have been examined for their effects, yet it is worth noting that Czank et al. (2013) observed 24 metabolites from the anthocyanin cyanidin-3-glucoside in human serum. As a result, this indicates many metabolites, alone and in combination, have to be examined for cardiovascular bioactivity rather than the few which have currently been studied.

LIMITATIONS AND FUTURE CONSIDERATIONS

The anthocyanin content of berry fruits is heavily influenced by growing conditions. For example, ultraviolet light exposure (i.e. sun light) is one of the biggest predictors of concentrations of anthocyanins (Guo et al., 2008). Therefore, to get a similar intake of anthocyanin from berries grown in different countries needs different amounts to be consumed. In addition, the cultivar of the berry is influential in the anthocyanin concentration (Moyer et al., 2002). The ripeness can also effect the anthocyanin content, with riper fruit containing higher levels than partially ripe fruit. For example, Gonçalves et al. (2004) observed in partially ripe cherries the anthocyanin concentration to be very low (5 to 23 mg/100 fresh weight), yet the ripe fruits have substantially higher concentrations (19 to 96 mg/100 fresh weight). Another factor to consider is that berry fruits are also seasonal, implying that intake from food sources is likely harder during winter. The doses of

anthocyanins used within the studies discussed in this review indicate that to get a similar intake from foods would result in a large portion. For example, Cook et al. (2015) supplemented with 105 mg New Zealand blackcurrant capsules for 7-days, which is equivalent to ~80 blackcurrants per day, while Howatson et al. (2010) supplemented with ~455 mL (16 fl oz) cherry juice containing 80 mg anthocyanins and equivalent to 120 cherries per day. For the sports nutritionist, manufactured products provide an ideal and convenient source of dietary anthocyanins for supplementation to improve performance. These can include powders, drinks and encapsulated powders, which with a known dose of anthocyanins in the products provide a reliable intake that can be used throughout the year, despite seasonal supply issues.

To the author's knowledge, there have been no studies that have compared the exercise performance effects of different berries with specific anthocyanin profiles. Future studies should also ensure to be conducted with appropriate sample sizes. It is possible that some published berry studies lacked sufficient power to allow firm conclusions on berry effects. However, it is known that anthocyanins, anthocyanin metabolites and other polyphenols can act synergistically (Shanmuganayagam et al., 2002; Dai et al., 2009), therefore appropriate dietary controls would be needed to determine these factors. Some studies have addressed this by using wash-out diets void of all anthocyanins (Bell et al., 2014; 2015; 2016). The use of wash-out diets allows the study design to control for these potential interactions, however it is problematic for ecological validity. In addition, by removing polyphenols from the diet, it is possible that the potential for change is reduced or even increased (Paschalis et al. 2018) and it could be argued that the ergogenic effects are only of interest when they can be observed imposed on top of normal dietary intake, for example in the design used by Levers et al. (2016).

CONCLUSION

The use of anthocyanin containing products indicate that exercise performance benefits may be fruit and/or berry specific. Performance benefits have been observed following blackcurrant ingestion, whereas performance improvements following intake of other anthocyanin containing fruits have not been demonstrated to the same extent. This may be due to the individual and specific anthocyanin make ups within the fruits and future work is needed to confirm this. Future work is also needed to identify if suppression of training responses occurs, however current evidence indicates no detriment to performance when anthocyanins are taken during training. The mechanisms for improved exercise performance may result from increases in blood flow, while training adaptations may be influenced by alterations in cellular signalling and faster recovery through antioxidative and anti-inflammatory pathways.

Acknowledgement

The manuscript preparation was undertaken by MC and METW. Both authors approved the final version of the paper.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Bell, P.G., Stevenson, E., Davison, G.W., & Howatson, G. (2016). The Effects of Montmorency Tart Cherry Concentrate Supplementation on Recovery Following Prolonged, Intermittent Exercise. *Nutrients, 8*(7), E441. doi*:* 10.3390/nu8070441

Bell, P.G., Walshe, I.H., Davison, G.W., Stevenson, E., & Howatson, G. (2014). Montmorency Cherries Reduce The Oxidative Stress and Inflammatory Responses to Repeated Days High-Intensity Stochastic Cycling. *Nutrients*, *6*(2), 829–843. doi.org/10.3390/nu6020829

449 Bell, P.G., Walshe, I.H., Davison, G.W., Stevenson, E.J., & Howatson, G. (2015). Recovery
450 facilitation with Montmorency cherries following high-intensity, metabolically challenging
451 exercise. *Applied Physiology Nutrition and Metabolism, 40*(4), 414–423. doi: 10.1139/apnm-
452 2014-0244
453 Bowtell, J.L., Sumners, D.P., Dyer, A., Fox, P., & Mileva, K.N. (2011). Montmorency cherry
454 juice reduces muscle damage caused by intensive strength exercise. *Medicine and Science in*
455 *Sports and Exercise, 43*(8), 1544–1551. doi: 10.1249/MSS.0b013e31820e5adc
456 Braakhuis, A.J. (2012). Effect of vitamin C supplements on physical performance. *Current*
457 *Sports Medicine Reports, 11*(4), 180–184. doi: 10.1249/JSR.0b013e31825e19cd
458 Braakhuis, A.J., Hopkins, W.G., & Lowe, T.E. (2014). Effects of dietary antioxidants on
459 training and performance in female runners. *European Journal of Sport Science, 14*(2), 160–
460 168. doi:10.1080/17461391.2013.785597
461 Bylund-Fellenius, A.C., Walker, P.M., Elander, A., Holm, S., Holm, J., & Scherstén, T.
462 (1981). Energy metabolism in relation to oxygen partial pressure in human skeletal muscle
463 during exercise. *Biochemical Journal, 200*(2), 247–255.
464 Cassidy, A., Bertoia, M., Chiuve, S., Flint, A., Forman, J., & Rimm, E.B. (2016). Habitual
465 intake of anthocyanins and flavanones and risk of cardiovascular disease in men. *American*
466 *Journal of Clinical Nutrition, 104*(3), 587–594. doi :10.3945/ajcn.116.133132
467 Cimino, F., Speciale, A., Anwar, S., Canali, R., Ricciardi, E., Virgili, F., . . . Saija, A. (2013).
468 Anthocyanins protect human endothelial cells from mild hyperoxia damage through
469 modulation of Nrf2 pathway. *Genes and Nutrition, 8*(4), 391–399. doi: 10.1007/s12263-012-
470 0324-4
471 Clifford, T., Scott, A., & Mitchell, N. (2013). The influence of different sources of
472 polyphenols on submaximal cycling and time trial performance. *Journal of Athletic*
473 *Enhancement, 2(6),* S10.

474 Connolly, D.A., McHugh, M.P., Padilla-Zakour, O.I., Carlson, L., & Sayers, S.P. (2006).
475 Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *British*
476 *Journal of Sports Medicine, 40*(8), 679–683. doi: 10.1136/bjsm.2005.025429
477 Cook, M.D., Myers, S.D., Blacker, S.D., & Willems, M.E.T. (2015). New Zealand
478 blackcurrant extract improves cycling performance and fat oxidation in cyclists. *European*
479 *Journal of Applied Physiology, 115*(11), 2357–2365. doi: 10.1007/s00421-015-3215-8
480 Cook, M.D., Myers, S.D., Gault, M.L., Edwards, V.C., & Willems, M.E.T. (2017). Dose
481 effects of New Zealand blackcurrant on substrate oxidation and physiological responses
482 during prolonged cycling. *European Journal of Applied Physiology, 177*(6), 1207–1216. doi:
483 10.1007/s00421-017-3607-z
484 Cook, M.D., Myers, S.D., Gault, M.L., & Willems, M.E.T. (2017). Blackcurrant Alters
485 Physiological Responses and Femoral Artery Diameter during Sustained Isometric
486 Contraction. *Nutrients, 9*(6), E556. doi: 10.3390/nu9060556
487 Costill, D.L., Barnett, A., Sharp, R., Fink, W.J., & Katz, A. (1983). Leg muscle pH following
488 sprint running. *Medicine and Science in Sports and Exercise*, *15*(4): 325–329.
489 Crum, E.M., Che Muhamed, A.M., Barnes, M., & Stannard, S.R. (2017). The effect of acute
490 pomegranate extract supplementation on oxygen uptake in highly-trained cyclists during
491 high-intensity exercise in a high altitude environment. *Journal of the International Society of*
492 *Sports Nutrition, 14*(1), 14. doi: 10.1186/s12970-017-0172-0
493 Curtis, P.J., Kroon, P.A., Hollands, W.J., Walls, R., Jenkins, G., Kay, C.D., & Cassidy, A.
494 (2009). Cardiovascular disease risk biomarkers and liver and kidney function are not altered
495 in postmenopausal women after ingesting an elderberry extract rich in anthocyanins for 12
496 weeks. *Journal of Nutrition, 139*(12), 2266–2271. doi:10.3945/jn.109.113126
497 Czank, C., Cassidy, A., Zhang, Q., Morrison, D.J., Preston, T., Kroon, P.A., . . . Kay, C.D
498 (2013). Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a

(13)C-tracer study. *American Journal of Clinical Nutrition, 97*(5), 995–1003. doi: 10.3945/ajcn.112.049247

Dai, J., Gupte, A., Gates, L., & Mumper, R.J. (2009). A comprehensive study of anthocyanin-containing extracts from selected blackberry cultivars: extraction methods, stability, anticancer properties and mechanisms. *Food and Chemical Toxicology, 47*(4), 837–847. doi: 10.1016/j.fct.2009.01.016

de Ferrars, R.M., Czank, C., Zhang, Q., Botting, N.P., Kroon, P.A., Cassidy, A., & Kay, C.D. (2014). The pharmacokinetics of anthocyanins and their metabolites in humans. *British Journal of Pharmacology, 171(13)*, 3268–3282. doi:10.1111/bph.12676

Edwards, M., Czank, C., Woodward, G.M., Cassidy, A., & Kay, C.D. (2015). Phenolic metabolites of anthocyanins modulate mechanisms of endothelial function. *Journal of Agricultural and Food Chemistry Research, 63*(9), 2423–2431. doi: 10.1021/jf5041993

George, T.W., Waroonphan, S., Niwat, C., Gordon, M.H., & Lovegrove, J.A. (2012). The Glu298Asp single nucleotide polymorphism in the endothelial nitric oxide synthase gene differentially affects the vascular response to acute consumption of fruit and vegetable puree based drinks. *Molecular Nutrition & Food Research, 56*(7), 1014–1024. doi: 10.1002/mnfr.201100689

Godwin, C., Cook, M.D., & Willems, M.E.T. (2017). Effect of New Zealand Blackcurrant Extract on Performance during the Running Based Anaerobic Sprint Test in Trained Youth and Recreationally Active Male Football Players. *Sports, 5(3),* 69. doi: org/10.3390/sports5030069

Gonçalves, B., Landbo, A.K., Knudsen, D., Silva, A.P., Moutinho-Pereira, J., Rosa, E., & Meyer, A.S. (2004). Effect of Ripeness and Postharvest Storage on the Phenolic Profiles of Cherries (*Prunus avium L.*). *Journal of Agricultural and Food Chemistry, 52*(3), 523–530. doi: 10.1021/jf030595s

524 Guo, J., Han, W., & Wang, M (2008). Ultraviolet and environmental stresses involved in the
525 induction and regulation of anthocyanin biosynthesis: A review. *African Journal of*
526 *Biotechnology, 7*(25), 6
527 Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., Jewell, A. P., van Someren, K. A., . . .
528 Howatson S.A. (2010). Influence of tart cherry juice on indices of recovery following
529 marathon running. *Scandinavian Journal of Medicine & Science in Sports, 20*(6), 843–852.
530 doi:10.1111/j.1600-0838.2009.01005.x
531 Howatson, G., & van Someren, K.A. (2007). Evidence of a contralateral repeated bout effect
532 after maximal eccentric contractions. *European Journal of Applied Physiology*, *101*(2), 207–
533 214. doi:10.1007/s00421-007-0489-5
534 Islam, H., Edgett, B.A., & Gurd, B.J. (2018). Coordination of mitochondrial biogenesis by
535 PGC-1α in human skeletal muscle: a re-evaluation. *Metabolism*, *79*, 42–51. doi:
536 10.1016/j.metabol.2017.11.001
537 Ivey, K.L., Jensen, M.K., Hodgson, J.M., Eliassen, A.H., Cassidy, A., & Rimm, E.B. (2017).
538 Association of flavonoid-rich foods and flavonoids with risk of all-cause mortality. *British*
539 *Journal of Nutrition, 117*(10), 1470–1477. doi: 10.1017/S0007114517001325
540 Kalt, W., Liu, Y., McDonald, J.E., Vinqvist-Tymchuk, M.R., & Fillmore, S.A. (2014).
541 Anthocyanin metabolites are abundant and persistent in human urine. *Journal of Agricultural*
542 *and Food Chemistry Research, 62*(18), 3926–3934. doi: 10.1021/jf500107j
543 Keane, K.M., Bailey S.J., Vanhatalo, A., Jones, A.M., & Howatson, G. (2018). Effects of
544 montmorency tart cherry (L. Prunus Cerasus) consumption on nitric oxide biomarkers and
545 exercise performance. *Scandinavia Journal of Medicine & Science in Sports, 28*(7), 1746–
546 1756. doi: 10.1111/sms.13088
547 Keane, K.M., Bell, P.G., Lodge, J.K., Constantinou, C.L., Jenkinson, S.E., Bass, R., &
548 Howatson, G. (2016a). Phytochemical uptake following human consumption of

549 Montmorency tart cherry (L. Prunus cerasus) and influence of phenolic acids on vascular
550 smooth muscle cells in vitro. *European Journal of Nutrition, 55*(4), 1695–1705. doi:
551 10.1007/s00394-015-0988-9
552 Keane, K.M., George, T.W., Constantinou, C.L., Brown, M.A., Clifford, T., & Howatson, G.
553 (2016b). Effects of Montmorency tart cherry (Prunus Cerasus L.) consumption on vascular
554 function in men with early hypertension. *American Journal of Clinical Nutrition, 103*(6),
555 1531–1539. doi: 10.3945/ajcn.115.123869
556 Kirakosyan, A., Seymour, E.M., Wolforth, J., McNish, R., Kaufman, P.B., & Bolling, S.F.
557 (2015). Tissue bioavailability of anthocyanins from whole tart cherry in healthy rats. *Food*
558 *Chemistry, 171,* 26–31. doi:10.1016/j.foodchem.2014.08.114
559 Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., . . . Tsuji, I.
560 (2006). Green tea consumption and mortality due to cardiovascular disease, cancer, and all
561 causes in Japan: the Ohsaki study. *J Am Med Assoc, 296*(10), 1255–1265. doi:
562 10.1001/jama.296.10.1255
563 Letenneur, L., Proust-Lima, C., Le Gouge, A., Dartigues, J.F., & Barberger-Gateau, P.
564 (2007). Flavonoid intake and cognitive decline over a 10-year period. *American Journal of*
565 *Epidemiology, 165*(125), 1364–1371. doi 10.1093/aje/kwm036
566 Levers, K., Dalton, R., Galvan, E., O'Connor, A., Goodenough, C., Simbo, S., . . . Kreider,
567 R.B. (2016). Effects of powdered Montmorency tart cherry supplementation on acute
568 endurance exercise performance in aerobically trained individuals. *Journal of the*
569 *International Society of Sports Nutrition, 13*, 22. doi:10.1186/s12970-016-0133-z
570 Matsukawa, T., Inaguma, T., Han, J., Villareal, M.O., & Isoda, H. (2015). Cyanidin-3-
571 glucoside derived from black soybeans ameliorate type 2 diabetes through the induction of
572 differentiation of preadipocytes into smaller and insulin-sensitive adipocytes. *Journal of*
573 *Nutritional Biochemistry, 26*(8), 860–867. doi: 10.1016/j.jnutbio.2015.03.006

574 Matsumoto, H., Takenami, E., Iwasaki-Kurashige, K., Osada, T., Katsumura, T., &
575 Hamaoka, T. (2005). Effects of blackcurrant anthocyanin intake on peripheral muscle
576 circulation during typing work in humans. *European Journal of Applied Physiology, 94*(1-2),
577 36–45. doi: 10.1007/s00421-004-1279-y
578 McHugh, M.P., Connolly, D.A., Eston, R.G., & Gleim, G.W. (1999). Exercise-induced
579 muscle damage and potential mechanisms for the repeated bout effect. *Sports Medicine,*
580 *27*(3), 157–170.
581 McLeay, Y., Barnes, M.J., Mundel, T., Hurst, S.M., Hurst, R.D., & Stannard, S.R. (2012).
582 Effect of New Zealand blueberry consumption on recovery from eccentric exercise-induced
583 muscle damage. *Journal of the International Society of Sports Nutrition, 9,* 19. doi:
584 10.1186/1550-2783-9-19
585 Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., & Wrolstad, R.E. (2002). Anthocyanins,
586 phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribes.
587 *Journal of Agricultural and Food Chemistry Research, 50*(3), 519–525.
588 Muraki, I., Imamura, F., Manson, J.E., Hu, F.B., Willett, W.C., van Dam, R.M., & Sun, Q.
589 (2013). Fruit consumption and risk of type 2 diabetes: results from three prospective
590 longitudinal cohort studies. *British Medical Journal, 28,* 347, f5001. doi:
591 org/10.1136/bmj.f5001
592 Murphy, C.A., Cook, M.D., & Willems, M.E.T. (2017). Effect of New Zealand Blackcurrant
593 Extract on Repeated Time-trial Performance. *Sports, 5(2),* 25. doi: 10.3390/sports5030069
594 Oh S., Komine S., Warabi E., Akiyama K., Ishii A., Ishige K., …, Shoda J. (2017). Nuclear
595 factor (erythroid derived 2)-like 2 activation increases exercise endurance capacity via redox
596 modulation in skeletal muscles. *Scientific Reports, 7(1),* 12902. doi: 10.1038/s41598-017-
597 12926-y.

598 Paschalis, V., Theodorou, A.A., Kyparos, A., Dipla, K., Zafeiridis, A., Panayiotou, G., . . .
599 Nikolaidis, M.G. (2016). Low vitamin C values are linked with decreased physical
600 performance and increased oxidative stress: reversal by vitamin C supplementation.
601 *European Journal of Nutrition, 55*(1), 45–53. doi:10.1007/s00394-014-0821-x
602 Paschalis, V., Theodorou, A.A., Margaritelis, N.V., Kyparos, A., & Nikolaidis, M.G. (2018).
603 N-acetylcysteine supplementation increases exercise performance and reduces oxidative
604 stress only in individuals with low levels of glutathione. *Free Radical Biology & Medicine, 1*,
605 288–297. doi:10.1016/j.freeradbiomed.2017.12.007
606 Perkins, I.C., Vine, S.A., Blacker, S.D., & Willems, M.E.T. (2015). New Zealand
607 Blackcurrant Extract Improves High-intensity Intermittent Running. *International Journal of*
608 *Sports Nutrition and Exercise Metabolism, 25*(5), 487–493. doi: 10.1123/ijsnem.2015-0020
609 Qin, B., & Anderson, R.A. (2012). An extract of chokeberry attenuates weight gain and
610 modulates insulin, adipogenic and inflammatory signalling pathways in epididymal adipose
611 tissue of rats fed a fructose-rich diet. *British Journal of Nutrition, 108*(4), 581–587. doi:
612 10.1017/S000711451100599X
613 Rahman, M.M., Ichiyanagi, T., Komiyama, T., Hatano, Y., & Konishi, T. (2006). Superoxide
614 radical- and peroxynitrite-scavenging activity of anthocyanins; structure-activity relationship
615 and their synergism. *Free Radical Research, 40*(9), 993–1002. doi:
616 10.1080/10715760600815322
617 Rothwell, J.A., Pérez-Jiménez, J., Neveu, V., Medina-Ramon, A., M'Hiri, N., Garcia Lobato,
618 . . . Scalbert, A. (2013). Phenol-Explorer 3.0: a major update of the Phenol-Explorer database
619 to incorporate data on the effects of food processing on polyphenol content. *Database*
620 *(Oxford)*, bat070. doi:10.1093/database/bat070
621 Shanmuganayagam, D., Beahm, M.R., Osman, H.E., Krueger, C.G., Reed, J.D., & Folts, J.D.
622 (2002). Grape seed and grape skin extracts elicit a greater antiplatelet effect when used in

combination than when used individually in dogs and humans. *Journal of Nutrition, 132*(12), 3592–3598. doi:10.1093/jn/132.12.3592

Somerville, V., Bringans. C., & Braakhuis, A. (2017). Polyphenols and Performance: A Systematic Review and Meta-Analysis. *Sports Medicine*, *47*(8), 1589–1599. doi: 10.1007/s40279-017-0675-5

Sorrenti, V., Mazza, F., Campisi, A., Di Giacomo, C., Acquaviva, R., Vanella, L., & Galvano, F. (2007). Heme oxygenase induction by cyanidin-3-O-beta-glucoside in cultured human endothelial cells. *Molecular Nutrition & Food Research, 51*(5), 580–586. doi: 10.1002/mnfr.200600204

Speciale, A., Cimino, F., Saija, A., Canali, R., & Virgili, F. (2014). Bioavailability and molecular activities of anthocyanins as modulators of endothelial function. *Genes and Nutrition, 9*(4), 404. doi:10.1007/s12263-014-0404-8

Starbuck, C., & Eston, R.G. (2012). Exercise-induced muscle damage and the repeated bout effect: evidence for cross transfer. *European Journal of Applied Physiology, 112*(3), 1005–1013. doi:10.1007/s00421-011-2053-6

Strauss, J.A., Willems, M.E.T., & Shepherd, S.O. (2018). New Zealand blackcurrant extract enhances fat oxidation during prolonged cycling in endurance-trained females. *European Journal of Applied Physiology, 118*(6), 1265–1272. doi:10.1007/s00421-018-3858-3

Stull, A.J., Cash, K.C., Johnson, W.D., Champagne, C.M., & Cefalu, W.T. (2010). Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *Journal of Nutrition, 140*(10), 1764–1758. doi:10.3945/jn.110.125336

Tang, X., Shen, T., Jiang, X., Xia, M., Sun, X., Guo, H., & Ling, W. (2015). Purified anthocyanins from bilberry and black currant attenuate hepatic mitochondrial dysfunction and steatohepatitis in mice with methionine and choline deficiency. *Journal of Agricultural and Food Chemistry Research, 63*(2), 552–561. doi:10.1021/jf504926n

Willems, M.E.T., Cousins, L., Williams, D., & Blacker, S.D. (2016). Beneficial Effects of New Zealand Blackcurrant Extract on Maximal Sprint Speed during the Loughborough Intermittent Shuttle Test. *Sports, 4*(3), 42.

Willems, M.ET., Myers, S.D., Gault, M.L., & Cook, M.D. (2015). Beneficial physiological effects with blackcurrant intake in endurance athletes. *International Journal of Sport Nutrition and Exercise Metabolism, 25*(4), 367–374. doi:10.1123/ijsnem.2014-0233

Wright, O.R., Netzel, G.A., & Sakzewski, A.R. (2013). A randomized, double-blind, placebo-controlled trial of the effect of dried purple carrot on body mass, lipids, blood pressure, body composition, and inflammatory markers in overweight and obese adults: the QUENCH trial. *Canadian Journal of Physiology and Pharmacology, 91*(6), 480–488. doi:10.1139/cjpp-2012-0349

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., & Prior, R.L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry Research, 54*(11), 4069–4075. doi:10.1021/jf060300l

Xu, J. W., Ikeda, K., & Yamori, Y. (2004a). Cyanidin-3-glucoside regulates phosphorylation of endothelial nitric oxide synthase. *FEBS Letters, 574*(1-3), 176–180. doi: 10.1016/j.febslet.2004.08.027

Xu, J. W., Ikeda, K., & Yamori, Y. (2004b). Upregulation of endothelial nitric oxide synthase by cyanidin-3-glucoside, a typical anthocyanin pigment. *Hypertension, 44*(2), 217–222. doi: 10.1161/01.HYP.0000135868.38343.c6

Yan, F., Chen, Y., Azat, R., & Zheng., Z. (2017). Mulberry Anthocyanin Extract Ameliorates Oxidative Damage in HepG2 Cells and Prolongs the Lifespan of Caenorhabditis elegans through MAPK and Nrf2 Pathways. *Oxidative Medicine and Cellular Longevity, 2017,* 12.doi: org/10.1155/2017/7956158

673 Yarahmadi, M., Askari, G., Kargarfard, M., Ghiasvand, R., Hoseini, M., Mohamadi, H., &
674 Asadi, A. (2014). The effect of anthocyanin supplementation on body composition, exercise
675 performance and muscle damage indices in athletes. *International Journal of Preventive*
676 *Medicine, 5*(12), 1594–1600.
677 Zhu, Y., Xia, M., Yang, Y., Liu, F., Li, Z., Hao, Y., . . . Ling, W. (2011). Purified
678 anthocyanin supplementation improves endothelial function via NO-cGMP activation in
679 hypercholesterolemic individuals. *Clinical Chemistry, 57*(11), 1524–1533. doi:
680 10.1373/clinchem.2011.167361
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684 Table 1. Summary of studies examining the effect of cherry anthocyanins on exercise performance.

Reference	Participant characteristics; design	Anthocyanin source; dose	Duration of intake	Timing of last dose before exercise	Performance protocol	Performance
Clifford et al. (2013)	9 moderately trained triathletes and cyclists, placebo controlled with counterbalanced crossover and double-blind	CherryActive®; NR	2-days	2-3 hours prior	Cycling; 20 km time-trial	No change $P = 0.117$
Levers et al. (2016)	27 endurance trained runners or triathletes (n =18 M); placebo controlled with randomised allocation after participants matched and double-blind	480 mg Montmorency tart cherry skin powder capsule; 66 mg·day^{-1}	7-days prior, day of and 2-days following	NR	Half-marathon running on closed course	Cherry had faster finish time $P = 0.001$
Keane et al. (2018)	10 trained male cyclists; placebo controlled with randomised crossover and double-blind	60 mL Montmorency cherry juice; 60 mg	1-day	90-minutes	Cycling; 60-s sprint following 6-min severe intensity cycling test	↑10% of total work during 60-s sprint, $P = 0.021$

685 NR; Not reported,
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695 Table 2. Summary of studies examining the effect of blackcurrant anthocyanins on exercise performance.

Reference	Participant characteristics; design	Anthocyanin source; dose	Duration of intake	Timing of last dose before exercise	Performance protocol	Performance
Willems et al. (2015)	14 trained triathletes (n = 8 M); placebo controlled with randomised crossover and double-blind	6 g New Zealand blackcurrant powder in 140 mL of water; 138.6 mg·day^{-1}	7-days	2 hours prior	Cycling; step protocol @ 30 W·min^{-1}	No change, $P > 0.05$
Cook et al. (2015)	14 trained male cyclists; placebo controlled with randomised crossover and double-blind	300 mg New Zealand blackcurrant extract capsule; 105 mg·day^{-1}	7-days	2 hours prior	Cycling; 16.1 km time-trial	Time to complete time-trial ↓2.4%, $P = 0.03$
Perkins et al. (2015)	13 recreationally active males; placebo controlled with randomised crossover and double-blind	300 mg New Zealand blackcurrant extract capsule; 105 mg·day^{-1}	7-days	2 hours prior	Treadmill Running; stages of 6x19 s sprints interspersed with 50% $_{v}\dot{V}O_{2max}$ for 15 s. Stage 1 started at 80% $_{v}\dot{V}O_{2max}$ and increased by 5% $_{v}\dot{V}O_{2max}$ each stage, then 2.5% $_{v}\dot{V}O_{2max}$ after 110% $_{v}\dot{V}O_{2max.}$	Running distance during sprints ↑10.8% $P = 0.02$
Murphy et al. (2017)	10 male trained cyclists; placebo controlled with crossover and double-blind	300 mg New Zealand blackcurrant capsule; 105 mg·day^{-1}	7-days	2 hours prior	Cycling; 4 km time-trial followed by 10-minutes rest and another 4 km time-trial	Total time for both time-trials ↓0.82%, P=0.034
Godwin et al. (2017)	15 recreationally active and nine trained youth players; placebo controlled with randomised crossover and double-blind	600 mg New Zealand blackcurrant extract capsules; 210 mg·day^{-1}	7-days	2 hours prior	Running Based Anaerobic Sprint Test	Reduced slowing of sprint 5, $P = 0.02$

696 NR; Not reported, $\dot{V}O_{2max}$; maximal aerobic capacity, $_{v}\dot{V}O_{2max}$; velocity at maximal aerobic capacity.