Beneficial physiological effects with blackcurrant intake in endurance athletes

Authors: Mark Elisabeth Theodorus Willems, Stephen David Myers, Mandy Lucinda Gault, Matthew David Cook

Affliliation: University of Chichester
Department of Sport & Exercise Sciences
College Lane
Chichester, PO19 6PE
United Kingdom

Running head: Blackcurrant and exercise-mediated effects

Corresponding author: Professor Mark Willems
Phone: +44 (0)1243 816468
Email: m.willems@chi.ac.uk

ABSTRACT

Blackcurrant contains anthocyanins, known to influence vasorelaxation and peripheral blood flow. We examined the effects of 7 days intake of Sujon New Zealand blackcurrant powder (6g/day) on the lactate curve, maximum oxygen uptake, and cardiovascular responses at rest and during cycling. Thirteen trained triathletes with >3 yrs experience (8 men, age: 38±8 yrs, body

mass: 71±9 kg, BF%: 19±5%, mean±SD) performed two incremental cycling protocols with recording of physiological and cardiovascular responses (Portapres® Model 2). Cardiovascular function was also measured in rest. Experimental design was double-blind, placebo-controlled, randomized and cross-over (wash-out 4 wks). Data was analysed with two-tailed t-tests and 2-way ANOVA and significance accepted at $p<0.05$. Plasma lactate was lower at 40%, 50%, 60% and 70% of maximum power by 27%, 22%, 17% and 13%. Intensity at 4 $mmol \cdot L^{-1}$ OBLA was 6% higher with blackcurrant without effect on heart rate and oxygen uptake. Maximum values of oxygen uptake, heart rate and power were not affected by blackcurrant, but obtained with 14% lower lactate. In rest, blackcurrant increased stroke volume and cardiac output by 25% and 26%, and decreased total peripheral resistance by 16%, with no changes in blood pressure and heart rate. Cardiovascular responses during exercise at 40%, 50%, 60%, 70% and 80% intensity were not affected. Sujon New Zealand blackcurrant powder affects lactate production and/or clearance during exercise. Sujon New Zealand blackcurrant powder affects physiological and cardiovascular responses in rest and during exercise that may have implications for exercise performance.

Key words: Plasma lactate; cardiovascular function, New Zealand blackcurrant

INTRODUCTION

Blackcurrant is a rich source for anthocyanin, a flavonoid that has attracted attention recently for human health benefits such as reduced risks for myocardial infarction (Cassidy et al., 2013) and type 2 diabetes (Wedick et al., 2012), inhibition of the proliferation of cancer cells

(Bishayee et al., 2010), anti-inflammatory effects (Zhu et al., 2013), and anti-oxidant activity (De la Cruz et al., 2013). Ergogenic aids with anti-inflammatory and anti-oxidant activity counteract potentially negative responses to exercise. For example, catechins, shiitake, resveratrol, quercetin, montmorency cherries, that are known ergogenic aids with anti-inflammatory and anti-oxidant properties, have been examined primarily for their effectiveness on exercise recovery (Haramizu et al., 2011; McAnulty et al., 2013; Zembron-Lacny et al., 2013). In addition, anthocyanin-containing fruits and berries, including blackcurrant, were examined for post-exercise effects on immune and functional responses (montmorency cherry juice: Bowtell et al., 2011; tart cherry juice: Connolly et al., 2006; blackcurrant extract: Lyall et al., 2009; blueberry: McLeay et al., 2012). It is not known whether the effects are caused by intake of a total amount of anthocyanins or amount of specific anthocyanins. However, the effectiveness of such ergogenic aids on the physiological responses during exercise has not been addressed.

Blackcurrant has an effect on human cardiovascular responses during activity. Peripheral blood flow was increased by 22% during typing work in humans (Matsumoto et al., 2005), potentially by anthocyanin-induced vasorelaxation and vasodilation as shown in thoracic aortic rings in male Wistar rats (Ziberna et al., 2013). In intact rat aortic rings (i.e. with functional endothelium), anthocyanins induced relaxation by 37%, possibly by involvement of the purinergic pathway producing nitric oxide (Mendes et al., 2003). Edirisinghe et al (2011) provided evidence using human umbilical vein endothelial cells that blackcurrant concentrate activated eNOS via the Akt/PI3 kinase pathway. In addition, Nakamura et al (2002) observed that blackcurrant concentrate enhanced the synthesis of nitric oxide and induced dose-dependent relaxation up to 80%. In this study, removal of the endothelium blunted the effect of the anthocyanins, supporting endothelial function to be affected. Caution is required, however, to

generalize from in vitro exposure of arteries with blackcurrant to an in-vivo condition in humans. However, dietary intake of anthocyanin intake was associated with lower arterial stiffness and central blood pressure in women (Jennings et al., 2012). All together, these studies provide the evidence that may support an effect of anthocyanin on cardiovascular responses in rest and during exercise.

An anthocyanin effect on cardiovascular responses during exercise may influence the delivery of substrates and removal of metabolic products of skeletal muscles. It is a classic observation, for example, that during exercise of incremental intensity, blood lactate accumulates in an exponential fashion as a consequence of production and removal mechanisms. An increase in peripheral blood flow by anthocyanin may benefit the removal mechanisms of lactate as uptake may be enhanced by liver, heart, kidney and skeletal muscles. As such, intracellular lactate oxidation (Gladden, 2008) and lactate conversion into glucose may be enhanced and could potentially lead to glycogen sparing (Emhoff et al., 2013). Increases in peripheral blood flow may also affect the oxygen consumed during exercise and potentially reduce effects of peripheral fatigue mechanisms and beneficial for exercise performance. The effects of blackcurrant on the lactate responses during incremental exercise and maximum oxygen uptake have not been examined.

Lactate threshold or absolute lactate values during incremental exercise and maximum oxygen uptake are recognized indicators of the ability for endurance performance [for a review see (Bentley et al., 2007)]. An increased peripheral blood flow may affect lactate clearance and oxygen delivery influencing the exercise intensity at lactate indicators (e.g. onset of blood lactate accumulation (OBLA) at 4 $mmol \cdot L^{-1}$), maximum oxygen uptake and the cardiovascular responses in rest and during exercise. Therefore, the primary objective was to examine the effect

of 7 days supplementation with Sujon New Zealand blackcurrant powder on the blood plasma lactate curve and aerobic capacity of trained triathletes. Second objective was to examine whether such supplementation would affect the cardiovascular responses at rest and exercise.

METHODS

Healthy male (n=8) and female (n=5) triathletes with >3 yrs triathlon experience (age: 38±8 yrs, height: 174±5 cm, body mass: 71±9 kg, BMI: 23±2, BF%: 19±5%, mean±SD) were recruited from local triathlon clubs and volunteered without payment. Participants provided written informed consent after explanation of the experimental procedures, potential risks and benefits. Ethical approval was obtained from the University of Chichester Ethics Research Committee. In brief, participants visited for one familiarization and two experimental testing sessions. Familiarization consisted of practicing all experimental procedures and recording of baseline subject characteristics [i.e. age, height, body mass, BMI, body fat (%) (Tanita BC418 segmental body composition analyser)]. Participants attended for two experimental visits to perform cycling protocols to examine the effectiveness of 7 days of intake of Sujon New Zealand blackcurrant powder [NZBC, 6g/day (138.6 mg anthocyanins) or placebo (PBO)]. Placebo was a commercially available blackcurrant juice with British blackcurrants containing likely about 3-4 mg anthocyanins per dose (Mattila et al., 2011). We were not able to quantify the bioavailability of anthocyanins in the blood. Optimal dosing strategy of New Zealand blackcurrant powder is not known and the dose was according manufacturers guidelines. Studies on the effectiveness of berry juices applied also multiple days of intake before exercise testing (e.g. 8 days: Bowtell et al., 2011; 4 days: Connolly et al., 2006; 6 days: Howatson et al., 2010). The experimental design

was double blind, randomized, placebo-controlled, and cross-over with drinks provided in unlabelled bottles. Participants recorded their dietary intake for 48 hr before attending the first experimental visit and followed the identical dietary pattern before the second visit. Participants were instructed not to exercise and consume alcohol 24 hr before each visit, be well-rested and hydrated on arrival, and not take other supplements that add further nutritional value to the normal diet. For the experimental visits, participants visited 2-3 hr postprandial, after a light breakfast of toast and water and intake of the final supplement. All testing occurred in a temperature controlled (~ 18°C) exercise physiology laboratory. The sequence of testing during an experimental visit comprised of 20 min recording of resting cardiovascular function followed by the cycling protocol for lactate responses with recording of physiological and cardiovascular responses. Then, following a 30 min rest, the cycling protocol for maximum oxygen uptake was performed.

Experimental procedures

Incremental cycling protocols

The incremental cycling protocol for lactate responses consisted of 4 min stages with 2 min recovery, start power 50 W with 30 W increments [adapted from (González-Haro et al., 2007)], with termination by obtaining a plasma lactate response close to or over 4.0 $mmol \cdot L^{-1}$. The maximum oxygen uptake protocol had a start power 50 W for 4 min with 30 $W \cdot min^{-1}$ increments [adapted from (Bailey et al., 2009)] with termination by voluntary exhaustion. Cycling protocols were performed at self-selected pedal cadence (70-90 rpm) on an ergometer (SRM ergometer, SRM International, Germany). In the last minute of each stage during the cycling protocol for lactate, expired air was collected using the Douglas bag technique and heart

rate (Polar Vantage NV, Polar Electro Oy, Kempele, Finland) recorded. Expired air was analysed with three-point calibrated Servomex gas analysers (Servoflex MiniMP, 5200 Multipurpose) and volume measured (Harvard dry gas meter). Gas volumes were calculated using Haldane transformation and standardisation to STPD conditions with consideration of fractions of oxygen and carbon dioxide of inspired air. Blood samples for analysis of plasma lactate were taken using finger-prick method immediately after each stage and 3 min after voluntary exhaustion during the maximum oxygen uptake test (2300 STAT Plus™ analyser, YSI Life Sciences, Yellow Springs, USA). In at least the last 4 minutes of the maximum oxygen uptake protocol, expired gases were collected in 45 seconds samples with 30 seconds samples in the last minute using Douglas bags (Plysu Protection Systems Limited, Milton Keynes, UK). A blood sample was taken 3 minutes after the end of the test.

Cardiovascular measurements

Cardiovascular responses were recorded using a beat-to-beat blood pressure monitoring system (Portapres® Model 2, Finapres Medical Systems BV, Amsterdam, The Netherlands) in rest and during the incremental cycling protocol for lactate responses. The Portapres® Model 2 is a beat-to-beat finger pressure analyser that allows the non-invasive continuous measurement of hemodynamic parameters. The Portapres has shown reliability for the relative changes in cardiac output (Sugawara et al., 2003). Clear outliers in recorded data were removed. The finger cuff was positioned around the same finger of the hand of the left arm. For the resting condition, participants were in a supine position for 20 min. Cardiovascular measures in rest were averaged over 10 consecutive beats, with the lowest systolic blood pressure (BP) and associated measures analysed. For the exercise condition, cardiovascular measures were averaged for the last minute

of each stage in which participants held the left arm in front of their chest to enable recording of the signal with less noise. The following parameters were derived: stroke volume, cardiac output, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and total peripheral resistance (Beatscope 1.1a., Finapres Medical Systems BV, Amsterdam, The Netherlands).

Supplementation protocol

Participants were provided with a 4 day supply of Sujon New Zealand blackcurrant powder (24 gram in 571 mL of water) followed by 3 day supply (18 gram in 429 mL of water), dissolved in opaque bottles. Sujon New Zealand blackcurrant powder contains 138.6 mg anthocyanin, 49 mg Vit C and 5.2 gram of carbohydrates per 6 gram serving. Per serving, total phenolic content was 271.6 mg. Placebo was a commercially available low-calorie blackcurrant juice drink (Ribena Blackcurrant), containing 32 mg Vit C and ~1.6 gram of carbohydrates per 250 ml serving. Participants were instructed to take the supplement or placebo with breakfast with the last intake about 2 hrs before an experimental visit. Participants were provided with a marked plastic cup to ensure equal daily intake over a 7-day period. Wash-out period was 4 weeks (Jin et al., 2011).

Statistical analysis.

The mathematical relationship between cycling power and lactate for each individual was determined with a third degree polynomial using lactate analysis software (Newell et al., 2007) [R^2 values of 0.9868±0.0174 (placebo) and 0.9871±0.0198 (Sujon New Zealand blackcurrant powder)] and calculated at 30%, 40%, 50%, 60%, 70% and 80% of maximal cycling power obtained with the maximum oxygen uptake cycling test. Intensity, oxygen uptake and heart rate were calculated at a lactate rise of 1 $mmol \cdot L^{-1}$ and a lactate value of 4 $mmol \cdot L^{-1}$ (OBLA, onset of

blood plasma lactate accumulation) using the lactate analysis software. Cardiovascular responses during exercise were taken for the stage closest to 40%, 50%, 60%, 70% and 80% of maximum oxygen uptake resulting in intensity for placebo of 41±5%, 51±4%, 60±6%, 70±3%, and 79±4% and for the Sujon New Zealand blackcurrant powder condition 41±3%, 48±2%, 57±3%, 67±6%, and 80±4%. Data for 3 participants for the cardiovascular responses during exercise was excluded due to the inability of recording of a clean signal. A sample size of 9 would allow detection of a 20% difference in cardiac output at rest (power: 0.80: α-level: 0.05). Resting cardiovascular data passed normality check with D'Agostino and Pearson omnibus normality test (Prism v5.04, Graphpad Software Inc.) to allow paired t-tests. Lactate and cardiovascular responses during exercise were analysed with 2-way ANOVA and post hoc t-tests to examine condition effects at each intensity. Significance was accepted at $p<0.05$.

RESULTS

Lactate responses

The intensity at 1 $mmol \cdot L^{-1}$ lactate rise was 4% higher with Sujon New Zealand blackcurrant powder (PBO: 184±52, NZBC: 192±52 W, range -2 to 14%, 8 participants showed an increase and 1 no change) ($p=0.02$). In both conditions at a 1 $mmol \cdot L^{-1}$ lactate rise, there were no differences in heart rate (PBO: 141±16, NZBC: 141±14 $b \cdot min^{-1}$, $p=0.83$) or oxygen uptake (PBO: 2.54±0.66, NZBC: 2.49±0.63 $L \cdot min^{-1}$, $p=0.41$). The intensity at 4 $mmol \cdot L^{-1}$ OBLA was 6% higher with Sujon New Zealand blackcurrant powder (PBO: 223±57, NZBC: 236±60 W, range -5 to 22%, 11 participants showed an increase and 1 no change) ($p=0.007$) (Figure 1). In both conditions at 4 $mmol \cdot L^{-1}$ OBLA, there were no differences in heart rate (PBO: 159±7, NZBC: 164±10 $b \cdot min^{-1}$, $p=0.13$) or oxygen uptake (PBO: 2.91±0.73, NZBC: 2.96±0.71 $L \cdot min^{-1}$,

p=0.31). Plasma lactate was lower at 40% (PBO: 1.24±0.52, NZBC: 0.91±0.46 mmol·L^{-1}, p=0.001), 50% (PBO: 1.58±0.78, NZBC: 1.23±0.64 mmol·L^{-1}, p=0.002), 60% (PBO: 2.29±0.96, NZBC: 1.91±0.87 mmol·L^{-1}, p=0.001) and 70% (PBO: 3.52±1.10, NZBC: 3.08±1.21 mmol·L^{-1}, p=0.004) of maximum power, decreases of 27%, 22%, 17% and 13%, respectively (Figure 2).

Maximum oxygen uptake

There was no effect on maximum values of oxygen uptake (PBO: 49.1±6.2, NZBC: 49.7±6.1 mL·kg^{-1}·min^{-1}, p=0.16), power (PBO: 305±68, NZBC: 307±62 W, p=0.66) or heart rate (PBO: 172±10, NZBC: 172±11 b·min^{-1}, p=0.68). However, maximum oxygen uptake with Sujon New Zealand blackcurrant powder was obtained with 14% lower lactate values (measured 3-min after exhaustion; PBO: 7.85±1.69, NZBC: 6.79±1.51 mmol·L^{-1}, range -27 to 48%, 10 participants showed a decrease and 1 no change) (p=0.02) (Figure 3).

Cardiovascular function in rest

There were no differences in systolic BP (PBO: 121±23, NZBC: 120±23 mmHg, p=0.92), diastolic BP (PBO: 69±16, NZBC: 63±14 mmHg, p=0.12), mean arterial BP (PBO: 86±18, NZBC: 82±18 mmHg, p=0.33), and heart rate (PBO: 58±9, NZBC: 59±10 beats·min^{-1}, p=0.95). Stroke volume (PBO: 82±23, NZBC: 99±25 mL, p=0.006) and cardiac output (PBO: 4.8±1.6, NZBC: 5.8±1.7 L·min^{-1}, p=0.015, Figure 4A) were increased by 25% and 26%, respectively. There was a 16% lower total peripheral resistance (PBO: 20.2±8.9, NZBC: 15.2±5.3 mmHg·L^{-1}·min^{-1}, p=0.05) (Figure 4B). The changes in resting cardiovascular function were observed in 10 participants.

231 *Cardiovascular responses during exercise*

232 At each intensity, there were no differences in diastolic BP (p=0.56), systolic BP (p=0.76), mean
233 arterial blood pressure (p=0.54), heart rate (p=0.78), stroke volume (p=0.88), cardiac output
234 (p=0.97) and total peripheral resistance (p=0.58) between placebo and New Zealand blackcurrant
235 at each intensity (Table 1).

236

237 **DISCUSSION**

238

239 The present study provides evidence for an effect on the physiological responses during
240 exercise and resting cardiovascular function by short duration (1 week) intake of an anthocyanin-
241 containing supplement. Short-term supplementation with Sujon New Zealand blackcurrant
242 powder shifted the lactate curve during exercise, allowed maximum oxygen uptake with lower
243 lactate, increased resting cardiac output and stroke volume and decreased resting total peripheral
244 resistance. No effects were observed for cardiovascular responses during exercise. In addition,
245 no effects were observed for functional capacity (i.e. power at maximum oxygen uptake), thus
246 the practical implications of the supplement intervention are still unclear.

247 As far as we know, the substantial combined downward and rightward shift of the lactate
248 curve in the present study with Sujon New Zealand blackcurrant powder has not been reported
249 by other supplement intake. It is well known that the downward and rightward shift of the lactate
250 curve results from physiological and metabolic adaptations by endurance training (Faude et al.,
251 2009) and high-intensity training (Evertsen et al., 2001); in our study, the right-ward shift of the
252 lactate curve resulted that OBLA was obtained at a 6% higher cycling intensity. A delay in
253 OBLA may have beneficial implications for endurance athletes as it would allow prolonged high

intensity exercise during competition and endurance exercise training. In addition, a delay in OBLA could be predictive for an enhancement of cycling endurance performance. However, supplementation with Sujon New Zealand blackcurrant powder did not replicate other common endurance training adaptations in physiological responses because heart rate and oxygen uptake at OBLA, for example, were not affected. Other supplements have been reported to be able to shift the lactate curve right-ward. For example, recreationally active men delayed OBLA after 28 days of beta-alanine supplementation but with increased absolute heart rate as a percentage of maximum values (Jordan et al., 2010). In endurance-trained cyclists, OBLA with beta-hydroxy betamethylbutarate and leucine was delayed by 9.1% and 2.1%, respectively (Vukovich & Dreifort, 2001). Neither of these studies reported a downward shift of the lactate curve. Therefore, the mechanisms causing delayed OBLA in previous studies by supplementation (Jordan et al., 2010) or training (Evertsen et al., 2001) are possibly explained by different physiological mechanisms that delayed the OBLA and blood lactate accumulation in the present study. Blood lactate accumulation results from an imbalance between lactate appearance and removal mechanisms (Brook, 1985). Our data provides evidence for the following acute physiological adaptations with short-term anthocyanin supplementation. First, short-term supplementation with anthocyanin seems to alter the balance of lactate appearance and removal mechanisms to blood lactate accumulation. Second, it seems that the alteration of the balance of factors contributing to blood lactate accumulation is/are intensity-dependent with exercise at relatively low intensity to be more affected. The lactate appearance may have been altered by an anthocyanin effect on substrate oxidation such that there was potentially an increased contribution of fat oxidation at relatively low intensities. Future work should address whether substrate oxidation is altered with blackcurrant supplementation. The removal mechanisms may

have been enhanced by an increase in peripheral blood flow. An increase in peripheral blood flow was reported by Matsumoto et al (2005), in typing work, a physical activity performed at relatively low intensity. We were not able to quantify the contribution of lactate appearance and removal mechanism towards reduced lactate at low and moderate exercise intensities. However, increased peripheral blood flow by anthocyanin vasodilating effects may have contributed to increased lactate clearance.

Maximum oxygen uptake was not affected by blackcurrant intake but obtained with lower lactate values. Although the protocols for obtaining the lactate curve and maximum oxygen uptake were different in stage duration, the lower lactate value may indicate reduced contribution of anaerobic glycolysis needed to reach exhaustion or increased clearance immediately following exercise and being at rest.

At rest (but without preceding exercise), total peripheral resistance was reduced with blackcurrant supplementation. As far as we know, there is no evidence that anthocyanins or their metabolites can reduce sympathetic drive, but if this had happened, resting heart rate would have been affected and that was not the case. In humans, it was observed that orally provided anthocyanins (320 mg containing 17 different anthocyanins from bilberry and blackcurrant) induced an increase in brachial flow-mediated dilation of about 10% 2hr after intake in hypercholesterolemic individuals (Zhu et al., 2011). The dose in our study was 138.6 mg of total anthocyanins in Sujon New Zealand blackcurrant powder. In addition, Zhu et al (2011) provided the anthocyanin in capsule form bypassing possible anthocyanin-dependent degradation in mouth saliva (Kamonpatana et al., 2012; Kamonpatana et al., 2014) by powder intake in the present study, although potential effects are small due to the time needed for degradation by saliva. It is possible that in our study, blackcurrant anthocyanins (or its metabolites) increased nitric oxide

production (Xu et al., 2004; Ziberna et al., 2013) leading to a decrease in total peripheral resistance. However, the reciprocal changes of reduced total peripheral resistance and increased cardiac output (by increased stroke volume), but no changes in resting heart rate and blood pressure suggests probably more complex hemodynamic mechanisms. For example, increased resting cardiac output could normally result from an increase in resting heart rate but this was not the case.

There are some limitations of the present study. First, our understanding of the mechanisms of the effects by oral intake of Sujon New Zealand blackcurrant powder requires information on the bioavailability of anthocyanins and metabolites. In addition, work is required to examine the mechanisms of physiological effects by anthocyanins and metabolites in *in-vivo* exercise models. Second, we did not consider sex of the participants, and differences in substrate oxidation, for example, may affect the observed lactate responses. Third, we do not know whether the observed effects were due to the duration of the intake. Fourth, maximum oxygen uptake values are likely indicative of moderately trained individuals and effectiveness in more trained populations is recommended.

In summary, effects of Sujon New Zealand blackcurrant powder for the endurance athletes during exercise on the lactate curve and resting cardiovascular function may initiate a new direction in applied sports nutrition research. There is scope to focus on the physiological, metabolic and performance effects of New Zealand blackcurrant in different exercise modalities. Intake of Sujon New Zealand blackcurrant powder was associated with 1) a downward and rightward shift of the lactate curve during cycling over a wide range of exercise intensities, 2) lower plasma lactate at aerobic capacity suggesting increased lactate clearance or altered

substrate oxidation, 3) improved cardiovascular function at rest. These findings may have implications for training practice, aerobic performance and recovery of endurance athletes.

Acknowledgement

Funding for this study was provided by the University of Chichester, Health Currancy Ltd (United Kingdom) and Gibb Holdings (Nelson) Limited (New Zealand).

References

Bailey, S.J., Winyard, P., Vanhatalo, A. Blackwell, J.R., Dimenna, F.J., Wilkerson, D.P., Tarr, J., Benjamin, N., & Jones A.M. (2009). Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology (1985), 107*, 1144-1155. doi: 10.1152/japplphysiol.00722.2009.

Bentley, D.J., Newell, J., & Bishop, D. (2007). Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. *Sports Medicine, 37,* 575-586. doi: 10.2165/00007256-200737070-00002.

Bishayee, A., Háznagy-Radnai, E., Mbimba, T., Sipos, P., Morazzoni, P., Darvesh, A.S., Bhatia, D., & Hohmann, J. (2010). Anthocyanin-rich black currant extract suppresses the growth of human hepatocellular carcinoma cells. *Natural Product Communications, 5*, 1613-1618. doi: 21121259.

Bowtell, J.L., Sumners, D.P., Dyer, A., Fox, P., & Mileva, K.N. (2011). Montmorency cherry juice reduces muscle damage caused by intensive strength exercise. *Medicine & Science in Sports & Exercise, 43*, 1544-1551. doi: 10.1249/MSS.0b013e31820e5adc.

Brooks, G.A. (1985). Anaerobic threshold: review of the concept and directions for future research. *Medicine & Science in Sports & Exercise*, *17*, 22-34.

Cassidy, A., Mukamal, K.J., Liu, L., Franz, M., Eliassen, A.H., & Rimm, E.B. (2013). High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. *Circulation, 127*, 188-196. doi: 10.1161/CIRCULATIONAHA.112.122408.

Connolly, D.A., McHugh, M.P., Padilla-Zakour, O.I., Carlson, L., & Sayers, S.P. (2006). Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *British Journal of Sports Medicine, 40*, 679-683. doi: 10.1136/bjsm.2005.025429.

De la Cruz, A.A., Hilbert, G., Mengin, V. Rivière, C., Ollat, N., Vitrac, C., Bordenave, L., Decroocq, S., Delaunay, J.C., Mérillon, J.M., Monti, J.P., Gomès, E., & Richard, T. (2013). Anthocyanin phytochemical profiles and anti-oxidant activities of Vitis candicans and Vitis doaniana. *Phytochemical Analysis*, *24*, 446-452. doi: 10.1002/pca.2447.

Edirisinghe, I., Banaszewski, K., Cappozzo, J., McCarthy, D., & Burton-Freeman, B.M. (2011). Effect of black currant anthocyanins on the activation of endothelial nitric oxide synthase (eNOS) in vitro in human endothelial cells. *Journal of Agricultural and Food Chemistry, 59*, 8616-8624. doi: 10.1021/jf201116y.

Emhoff, C.A., Messonnier, L.A., Horning, M.A., Fattor, J.A., Carlson, T.J., & Brooks, G.A. (2013). Direct and indirect lactate oxidation in trained and untrained men. *Journal of Applied Physiology (1985), 115*, 829-838. doi: 10.1152/japplphysiol.00538.2013.

366 Evertsen, F., Medbø, J.I., & Bonen, A. (2001). Effect of training intensity on muscle lactate
367 transporters and lactate threshold of cross-country skiers. *Acta Physiologica Scandinavica, 173,*
368 195-205. doi: 10.1046/j.1365-201X.2001.00871.x
369 Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate threshold concepts: how valid are
370 they? *Sports Medicine, 39,* 469-490. doi: 10.2165/00007256-200939060-00003.
371 Gladden, L.B. (2008). 200th anniversary of lactate research in muscle. *Exercise and Sport*
372 *Sciences Reviews, 36,* 109-115. doi: 10.1097/JES.0b013e31817c0038.
373 González-Haro, C., Galilea, P.A., Drobnic, F., & Escanero, J.F. (2007). Validation of a field test
374 to determine the maximal aerobic power in triathletes and endurance cyclists. *British Journal of*
375 *Sports Medicine, 41,* 174-179. doi: 10.1136/bjsm.2006.031310.
376 Haramizu, S., Ota, N., Hase, T., & Murase, T. (2011). Catechins attenuate eccentric exercise-
377 induced inflammation and loss of force production in muscle in senescence-accelerated mice.
378 *Journal of Applied Physiology (1985), 111,* 1654-1663. doi: 10.1152/japplphysiol.01434.2010.
379 Howatson, G., McHugh, M.P., Hill, J.A., Brouner, J., Jewell, A.P., van Someren, K.A., Shave,
380 R.E., & Howatson, S.A. (2010). Influence of tart cherry juice on indices of recovery following
381 marathon running. *Scandinavian Journal of Medicine & Science in Sports, 20,* 843-852. doi:
382 10.1111/j.1600-0838.2009.01005.x.
383 Jennings, A., Welch, A.A., Fairweather-Tait, S.J., Kay, C., Minihane, A.M., Chowienczyk, P.,
384 Jiang, B., Cecelja, M., Spector, T., Macgregor, A., & Cassidy, A. (2012). Higher anthocyanin
385 intake is associated with lower arterial stiffness and central blood pressure in women. *The*
386 *American Journal of Clinical Nutrition, 96,* 781-788. doi: 10.3945/ajcn.112.042036.
387 Jin, Y., Alimbetov, D., George, T., Gordon, M.H., & Lovegrove, J.A. A randomised trial to

investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects. (2011). *European Journal of Clinical Nutrition, 65,* 849-856. doi: 10.1038/ejcn.2011.55.

Jordan, T., Lukaszuk, J., Misic, M., & Umoren, J. (2010). Effect of beta-alanine supplementation on the onset of blood lactate accumulation (OBLA) during treadmill running: Pre/post 2 treatment experimental design. *Journal of the International Society of Sports Nutrition, 7,* 20. doi: 10.1186/1550-2783-7-20.

Kamonpatana, K., Failla, M.L., Kumar, P.S., & Giusti, M.M. (2014). Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa. *Journal of Agricultural and Food Chemistry, 62,* 6903-6910. doi: 10.1021/jf405180k.

Kamonpatana, K., Giusti, M.M., Chitchumroonchokchai, C., MorenoCruz, M., Riedl, K.M., Kumar, P., & Failla, M.L. (2012). Susceptibility of anthocyanins to ex vivo degradation in human saliva. *Food Chemistry, 135,* 738-747. doi: 10.1016/j.foodchem.2012.04.110.

Lyall, K.A., Hurst, S.M., Cooney, J., Jensen, D., Lo, K., Hurst, R.D., & Stevenson, L.M. (2009). Short-term blackcurrant extract consumption modulates exercise-induced oxidative stress and lipopolysaccharide-stimulated inflammatory responses. *American Journal of Physiology Regul Integr Comp Physiol, 297,* R70-81. doi: 10.1152/ajpregu.90740.2008.

Mattila, P.H., Hellström, J., McDougall, G., Dobson, G., Pihlava, J.M., Tiirikka, T., Stewart, D., & Karjalainen, R. (2011). Polyphenol and vitamin C contents in European commercial blackcurrant juice products. *Food Chemistry, 127,* 1216-1223. doi: 10.1016/j.foodchem.2011.01.129.

Matsumoto, H., Takenami, E., Iwasaki-Kurashige, K., Osada, T., Katsumura, T., & Hamaoka, T. (2005). Effects of blackcurrant anthocyanin intake on peripheral muscle circulation during typing

411 work in humans. *European Journal Applied Physiology*, *94*, 36-45. doi: 10.1007/s00421-004-
412 1279-y.
413 McAnulty, L.S., Miller, L.E., Hosick, P.A., Utter, A.C., Quindry, J.C., & McAnulty, S.R. (2013).
414 Effect of resveratrol and quercetin supplementation on redox status and inflammation after
415 exercise. *Applied Physiology, Nutrition, and Metabolism*, *38*, 760-765. doi: 10.1139/apnm-2012-
416 0455.
417 McLeay, Y., Barnes, M.J., Mundel, T., Hurst, S.M., Hurst, R.D., Stannard, S.R. (2012). Effect of
418 New Zealand blueberry consumption on recovery from eccentric exercise-induced muscle
419 damage. *Journal of the International Society of Sports Nutrition*, *9*, 19. doi: 10.1186/1550-2783-
420 9-19.
421 Mendes, A., Desgranges, C., Chèze, C., Vercauteren, J., & Freslon, J.L. (2003). Vasorelaxant
422 effects of grape polyphenols in rat isolated aorta. Possible involvement of a purinergic pathway.
423 *Fundamental & Clinical Pharmacology*, *17*, 673-681. DOI: 10.1046/j.1472-8206.2003.00198.x.
424 Nakamura, Y., Matsumoto, H., & Todoki, K. (2002). Endothelium-dependent vasorelaxation
425 induced by black currant concentrate in rat thoracic aorta. *The Japanese Journal of*
426 *Pharmacology*, *89*, 29-35. doi: 10.1254/jjp.89.29.
427 Newell, J., Higgins, D., Madden, N., Cruickshank, J., Einbeck, J., McMillan, K., & McDonald,
428 R. (2007). Software for calculating blood lactate endurance markers. *Journal of Sports Sciences*,
429 *25*, 1403-1409. doi: 10.1080/02640410601128922.
430 Sugawara, J., Tanabe, T., Miyachi, M., Yamamoto, K., Takahashi, K., Iemitsu, M., Otsuki, T.,
431 Homma, S., Maeda, S., Ajisaka, R., & Matsuda, M. (2003). Non-invasive assessment of cardiac
432 output during exercise in healthy young humans: comparison between Modelflow method and

433 Doppler echocardiography method. *Acta Physiologica Scandinavica, 179*, 361-366. doi:
434 10.1046/j.0001-6772.2003.01211.x
435 Vukovich, M.D., & Dreifort, G.D. (2001). Effect of beta-hydroxy beta-methylbutyrate on the
436 onset of blood lactate accumulation and V(O)(2) peak in endurance-trained cyclists. *The Journal*
437 *of Strength and Conditioning Research, 15*, 491-497.
438 Wedick, N.M., Pan, A., Cassidy, A., Rimm, E.B., Sampson, L., Rosner, B., Willett, W., Hu,
439 F.B., Sun, Q., & van Dam, R.M. (2012). Dietary flavonoid intakes and risk of type 2 diabetes in
440 US men and women. *American Journal of Clinical Nutrition*, *95*, 925-933. doi:
441 10.3945/ajcn.111.028894.
442 Xu, J.W., Ikeda, K., & Yamori Y. (2004). Cyanidin-3-glucoside regulates phosphorylation of
443 endothelial nitric oxide synthase. *FEBS Letters*, *574*, 176-180. doi:
444 http://dx.doi.org/10.1016/j.febslet.2004.08.027
445 Zembron-Lacny, A., Gajewski, M., Naczk, M., & Siatkowski, I. (2013). Effect of shiitake
446 (Lentinus edodes) extract on antioxidant and inflammatory response to prolonged eccentric
447 exercise. *Journal of Physiology and Pharmacology, 64*, 249-254.
448 Zhu, Y., Ling, W., Guo, H., Song, F., Ye, Q., Zou, T., Li, D., Zhang, Y., Li, G., Xiao, Y., Liu,
449 F., Li, Z., Shi, Z., & Yang, Y. (2013). Anti-inflammatory effect of purified dietary anthocyanin
450 in adults with hypercholesterolemia: a randomized controlled trial. *Nutrition, Metabolism and*
451 *Cardiovascular Diseases*, *23*, 843-849. doi: 10.1016/j.numecd.2012.06.005.
452 Zhu, Y., Xia, M., Yang, Y., Liu, F., Li, Z., Hao, Y., Mi, M., Jin, T., & Ling, W. (2011). Purified
453 anthocyanin supplementation improves endothelial function via NO-cGMP activation in
454 hypercholesterolemic individuals. *Clinical Chemistry*, *57*, 1524-1533. doi:
455 10.1373/clinchem.2011.167361.

Ziberna, L., Lunder, M., Tramer, F., Drevenšek, G., & Passamonti, S. (2013). The endothelial plasma membrane transporter bilitranslocase mediates rat aortic vasodilation induced by anthocyanins. *Nutrition, Metabolism and Cardiovascular Diseases*, *23*, 68-74. doi: 10.1016/j.numecd.2011.02.005.

Figure legends

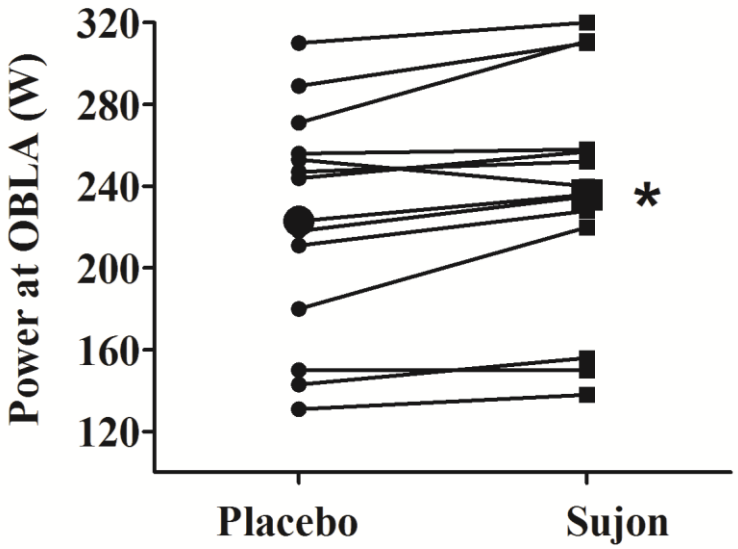
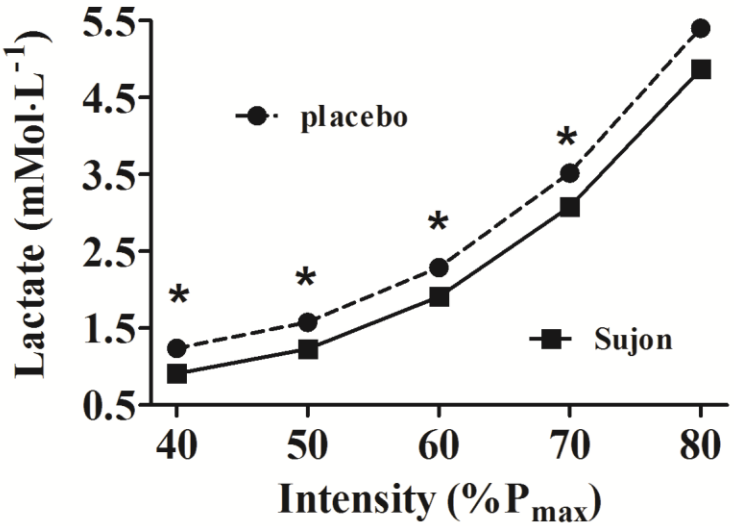


Fig.1. Power values of participants at a lactate value of 4 mmol·L^{-1} (OBLA) for placebo and after 7 days of Sujon New Zealand blackcurrant powder intake. Large symbols indicate mean values. *, difference between placebo and Sujon New Zealand blackcurrant powder.

494

495



496

497 Fig. 2. Relationship between cycling intensity and lactate. Intensity was expressed as a

498 percentage of maximum power (%P_{max}). *, difference between placebo and Sujon New Zealand

499 blackcurrant powder. Values are mean±SD.

500

501

502

503

504

505

506

507

508

509

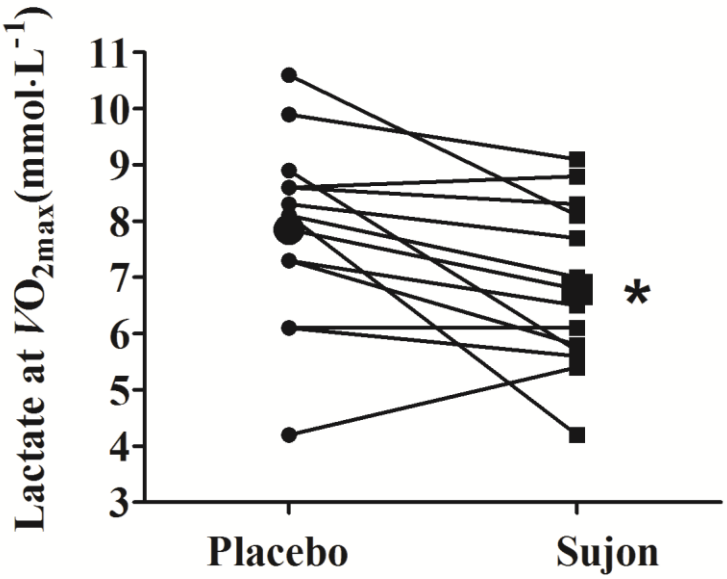
510

511

512

513

514



515

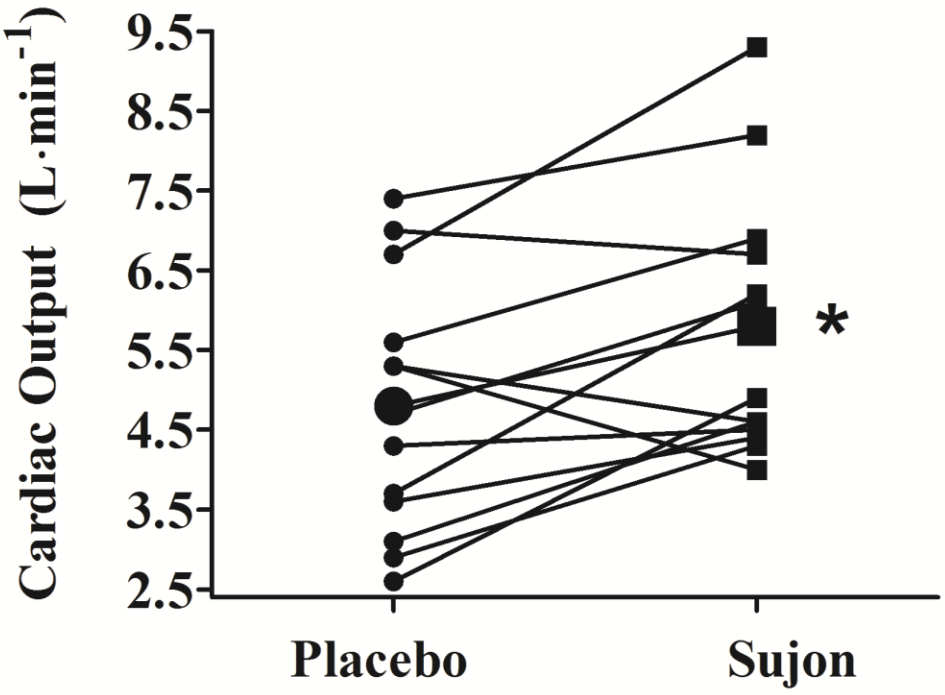
516 Fig. 3. Lactate values of participants 3 min after completion of the maximum oxygen uptake
517 protocol. Large symbols indicate mean values. Mean lactate values were lower after 7 days
518 intake of Sujon New Zealand blackcurrant powder. *, difference between placebo and Sujon
519 New Zealand blackcurrant powder.

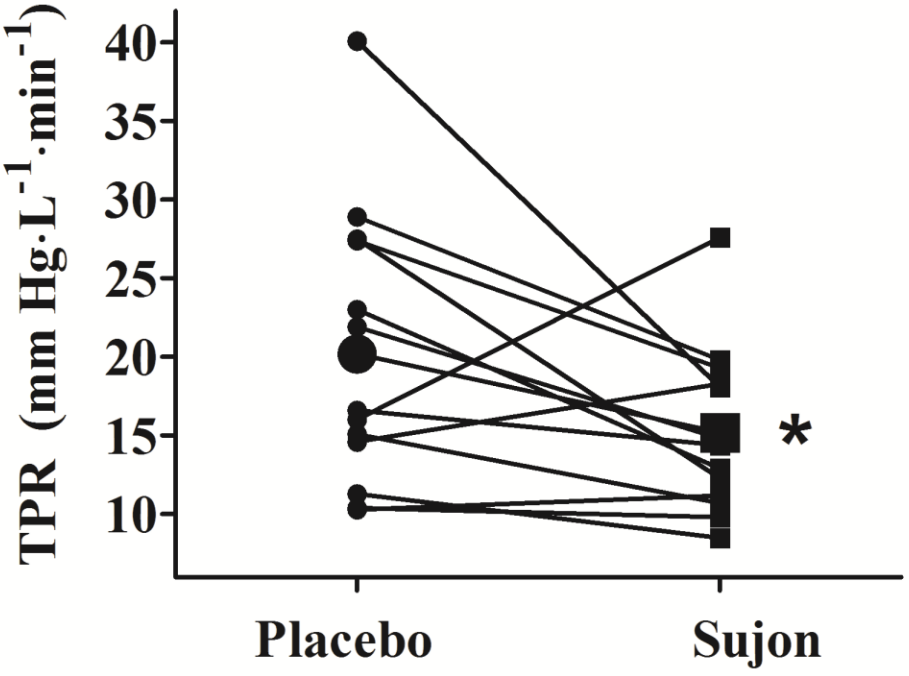
520

521

522

523





As accepted for publication, http://journals.humankinetics.com/ijsnem

Fig. 4. Resting cardiac output (A) and resting total peripheral resistance (TPR) (B) of participants. Large symbols indicate mean values. Mean values for cardiac output and TPR were higher and lower, respectively, after 7 days intake of Sujon New Zealand blackcurrant powder. *, difference between placebo and Sujon New Zealand blackcurrant powder.

533 **Table 1.** Cardiovascular responses at during cycling at 40%, 50%, 60%, 70% and 80% $\dot{V}O_{2max}$.

	40%		50%		60%		70%		80%	
	PBO	NZBC	PBO	NZBC	PBO	NZBC	PBO	NZBC	PBO	NZBC
DBP (mm Hg)	83±15	88±18	84±16	89±19	86±15	92±22	91±14	93±18	97±14	101±19
SPB (mm Hg)	185±31	195±33	188±33	192±40	196±35	203±42	202±35	201±39	215±33	218±35
MAP (mm Hg)	110±20	118±21	114±19	119±23	116±17	122±25	120±16	123±21	129±17	133±20
HR ($b \cdot min^{-1}$)	99±9	100±10	110±8	110±8	124±10	124±10	138±13	139±13	152±13	155±11
SV ($mL \cdot min^{-1}$)	101±18	95±25	96±20	92±25	94±20	93±24	91±18	93±29	88±18	87±28
CO ($L \cdot min^{-1}$)	9.9±1.9	9.6±2.4	10.6±2.4	10.1±2.7	11.7±2.7	11.5±2.9	12.6±2.5	12.9±4.2	13.3±2.6	13.6±4.4
TPR ($mmHg \cdot L^{-1} \cdot min^{-1}$)	11.8±4.5	13.5±5.9	11.7±4.9	13.1±6.3	10.7±4.2	11.8±6.2	10.1±3.1	10.7±4.7	10.3±3.3	11.3±5.5

PBO, placebo; NZ BC, Sujon New Zealand blackcurrant; DPB, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance. Data of ten subjects. There were no differences between placebo and Sujon New Zealand blackcurrant for a cardiovascular response at each intensity.Values are mean±SD.