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Title of Article: New Zealand Blackcurrant Extract Improves Cycling Performance and Fat Oxidation in Cyclists

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ABSTRACT

PURPOSE: Blackcurrant intake increases peripheral blood flow in humans, potentially by anthocyanin-induced vasodilation which may affect substrate delivery and exercise performance. We examined the effects of New Zealand blackcurrant (NZBC) extract on substrate oxidation, cycling time-trial performance and plasma lactate responses following the time-trial in trained cyclists.

METHODS: Using a randomized, double-blind, crossover design, fourteen healthy men (age: 38 ± 13 years, height: 178 ± 4 cm, body mass: 77 ± 9 kg, $\dot{V}O_{2max}$: 53 ± 6 ml·kg^{-1}·min^{-1}, mean ± SD) ingested NZBC extract (300 mg·day^{-1} CurraNZ™ containing 105 mg anthocyanin) or placebo (PL, 300 mg microcrystalline cellulose M102) for 7-days (washout 14-days). On day 7, participants performed 30 min of cycling (3x10 min at 45, 55 and 65% $\dot{V}O_{2max}$), followed by a 16.1 km time-trial with lactate sampling during a 20-minute passive recovery.

RESULTS: NZBC extract increased fat oxidation at 65% $\dot{V}O_{2max}$ by 27% ($P < 0.05$) and improved 16.1 km time-trial performance by 2.4% (NZBC: 1678 ± 108 s, PL: 1722 ± 131 s, $P < 0.05$). Plasma lactate was higher with NZBC extract immediately following the time-trial (NZBC: 7.06 ± 1.73 mmol·L^{-1}, PL: 5.92 ± 1.58 mmol·L^{-1} $P < 0.01$).

CONCLUSIONS: Seven days intake of New Zealand blackcurrant extract improves 16.1 km cycling time-trial performance and increases fat oxidation during moderate intensity cycling.

Keywords time-trial; substrate oxidation; lactate; recovery; anthocyanin; indirect calorimetry; New Zealand blackcurrant; sports nutrition

Abbreviations:

CHox	Carbohydrate oxidation
FATox	Fat oxidation
NZBC	New Zealand Blackcurrant
PL	Placebo
$\dot{V}O_{2max}$	Maximal oxygen uptake
WR_{max}	Maximum work rate

INTRODUCTION

Blackcurrant (*Ribes nigrum*) is a food source rich in polyphenols, including the anthocyanins delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-glucoside, in addition to some flavanols and flavonols in smaller quantities. Anthocyanins are a flavonoid group that has been associated with benefits for human health through anti-inflammatory effects (Zhu et al. 2013) and anti-oxidant activity (De la Cruz et al. 2013). These effects are of interest to counteract the production of reactive oxygen species during exhaustive exercise (Viña et al. 2000), which is the primary cause of excise-induced disturbance in the oxidation-reduction status (i.e. redox balance) of skeletal muscle (Powers et al. 2004). In addition, blackcurrant intake has also been reported to increase peripheral blood flow by 22% during typing work in humans (Matsumoto et al. 2005), and retina blood flow in patients with normal tension glaucoma (Ohguro et al. 2007) potentially by anthocyanin-induced vasorelaxation and vasodilation as shown in thoracic aortic rings in male Wistar rats (Ziberna et al. 2013). This may be mediated by the ability of anthocyanins to increase nitric oxide by endothelial cells and also a reduced breakdown of nitric oxide by free radicals (Martin et al. 2002; Nagi et al. 2002).

The evidence that blackcurrant can improve blood flow and reduce oxidative stress may represent a potential ergogenic affect upon exercise performance in an event with a large aerobic component such as a 16.1 km time-trial as restricted blood flow is considered an important limiting factor in muscle oxygenation during high intensity exercise (Basset and Howley 2000). However, the effects of short duration (7-days) blackcurrant intake on endurance exercise performance have not been examined. Following blackcurrant supplementation (300 $mg \cdot day^{-1}$ anthocyanin) alongside a short-duration (i.e. 3 weeks) high-intensity training programme in 23 female runners, Braakhuis et al. (2014) reported a possible peak running speed improvement of 1.9 ± 2.5% during an incremental running test of the fastest runners (i.e. runners faster by 1 SD of mean speed on an incremental running test) in the study cohort. However, Skarpańska-Stejnborn et al. (2006) reported no change in best effort 2000m rowing ergometer performance in rowers taking blackcurrant (250 mg blackcurrant powder, 3 times daily) for 6 weeks in a training camp. Both of these studies supplemented athletes over a training period with physiological assessment before and after training with different daily doses and supplementation periods. The dose- and time-dependent responses of blackcurrant on physiological responses are unknown. In addition, no studies have addressed the potential ergogenic properties of short-term (7 days) blackcurrant supplementation on a performance-based test that simulates competition in a trained population without a training period. As anthocyanins reach maximum serum

concentrations in 1.81 ± 0.16 h following ingestion, and metabolites remain in the blood stream for at least 48 hours (Czank et al. 2013), a potential build-up of metabolites from a short-term intake (i.e. 7-days) and the subsequent physiological responses such as, altered nitric oxide availability and increased peripheral blood flow, may alter exercise performance. It should be noted however, that the acute and chronic responses of anthocyanin intake on exercise performance are not known, but the chronic exposure as used in the above training studies may result in different physiological responses during the training period which may alter the training adaptations, than the physiological responses that result from 7-days exposure which may improve performance in high intensity exercise with a large aerobic component.

As lactate redistribution following exercise occurs via blood flow (Gladden 2004), an improved peripheral blood flow induced by anthocyanin related vasodilation may benefit lactate removal through greater uptake by liver, heart, kidney and skeletal muscles. Nutritional interventions that improve blood lactate responses after high intensity exercise are therefore of interest to athletes to promote faster recovery, slow lactate accumulation and potentially influence the performance of subsequent high intensity exercise.

Experimental studies have also indicated that consumption of some of the anthocyanins within blackcurrant in C57BL/6 mice can inhibit body mass gain, positively alter insulin responses, attenuate lipid accumulation and decrease leptin secretion (Benn et al. 2014) and also enhance adipokine secretions in rat adipocytes (Tsuda et al. 2004). These physiological responses may alter fat oxidation during low and moderate intensity exercise where fat oxidation rates are highest (Achten et al. 2002).

Therefore, the objectives of the present study addressed whether there are effects of short-term (7-days) NZBC extract on performance, metabolic and physiological responses. The first objective was to examine the effect of New Zealand blackcurrant (NZBC) extract on substrate oxidation at three different exercise intensities. The second objective was to examine the effects of NZBC extract on 16.1 km (10-mile) cycling time-trial performance. The third objective was to examine the lactate responses following the 16.1 km time-trial. It was hypothesized that NZBC extract would enhance endurance performance, increase fat oxidation and alter lactate responses during passive post-exercise recovery.

METHODS

Participants

Fourteen healthy men volunteered and provided written informed consent to participate in the study with participant characteristics presented in Table 1. Participants were recruited from local cycling and triathlon clubs with a history of sport participation of greater than 3 years and were not involved in a structured training programme at the time of the study, but typically performed cycling exercise of 8 to 10 hours a week. All participants had a personal best time for a 16.1 km cycling time-trial of less than 30 minutes. Participants were screened for intake of other dietary supplements before commencing participation with only one participant required to undergo a wash out period of 14 days for taking beetroot supplements. The study was approved by the University of Chichester Research Ethics Committee with protocols and procedures conformed to the 2013 Declaration of Helsinki. Participants did not receive payment for participation.

Experimental Design

Each participant visited the laboratory for 4 morning sessions (<2 hours difference). In preparation for all testing sessions, participants were instructed to abstain from strenuous exercise for 48 hours prior, alcohol intake for 24 hours prior and caffeine-containing products on the day of testing. All exercise was performed with the participant's own cycling shoes and pedals attached to the SRM ergometer (SRM ergometer, SRM International, Germany). Saddle height and setback, handle bar reach and drop were personalized in the first visit and replicated for all additional visits. On the first visit, participants stature (Seca 213, Seca, Birmingham, UK), body mass (Kern ITB, Kern, Germany) and body fat (Tanita BC418 Segmental Body Composition analyzer, Tanita, Illinois, USA) were measured. Subsequently, participants completed an intermittent incremental-intensity cycling test until a blood plasma lactate $\geq$ 4 mmol·L^{-1} was obtained. This was followed by a familiarization of the 16.1 km time-trial. In the second visit, participants completed a maximal incremental cycling test to volitional exhaustion to allow measurement of maximal oxygen uptake ($\dot{V}O_{2max}$) and maximum work rate (WR_{max}; the last completed work rate, plus the fraction of time spent in the final non-completed work rate multiplied by the work rate), followed by a rest period and a second 16.1 km time-trial for familiarization.

Prior to visits 3 and 4, participants consumed 1 capsule of concentrated NZBC extract (300 mg active cassis containing 105mg of anthocyanins, i.e. 35-50% delphinidin-3-rutinoside, 5-20% delphinidin-3-glucoside, 30-45% cyanidin-3-rutinoside, 3-10% cyanidin-3-glucoside) (CurraNZ^{TM}, Health Currancy Ltd, Surrey, UK) or an identical looking placebo capsule (300mg microcrystalline cellulose M102) every morning with breakfast for 7 days. The

NZBC capsules were independently analysed for contents, which confirmed ingredients present and that ingredients such as caffeine were absent. On the morning of the final day of supplementation, participants reported to the laboratory at the same time of day, approximately 2 hours postprandial of a standard breakfast (i.e. one slice of buttered toast or bread) and their last supplement capsule. On arrival, participants rested for 10 minutes before their blood pressure was taken four times using an automated cuff (OMRON 705 IT, Medisave, Weymouth, UK) with the last three measurements averaged for quantification of blood pressure. Subsequently, a finger prick blood sample was taken to record resting blood plasma lactate and glucose (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs, USA). After the resting sample was provided, participants performed a continuous 30 min cycling protocol, consisting of three 10 min stages at 45, 55 and 65% $\dot{V}O_{2max}$ with expired gas samples collected and analysed. Following a 15-minute rest, participants performed a 16.1 km best effort time-trial on the SRM ergometer. The two experimental conditions (NZBC and placebo) were performed in a randomized, double-blind, cross-over design with a 14-day washout period. An anthocyanin intake three times higher than our study for one month reported return to baseline of biochemical parameters and biomarkers of antioxidant status after 15 days washout (Alvarez-Suarez et al. 2014). Six participants received NZBC extract as first condition. All exercise tests were conducted in a temperature-controlled laboratory at 18ºC.

Physical Activity and Dietary Standardization

Participants were instructed to keep their weekly exercise schedule as consistent as possible. Each participant recorded their dietary intake on a written food diary for the 48 hours prior to the first of the experimental condition visits (visit 3). Participants were instructed to replicate this diet for the 48 hours prior to the second experimental condition visit (visit 4) using their previous food diary as a guide, while recording on a new diary their dietary intake for that visit. Food diaries were analysed using Nutritics (Nutritics LTD, Dublin, Ireland) for carbohydrate, fat and protein intake and total energy intake (kJ). There were no differences in absolute or relative to per kilogram of body mass for carbohydrate, fat protein and total energy intake ($P > 0.05$) between the experimental visits (Table 2). Analysis of diaries demonstrated a 100% reported adherence to dietary instructions. Participants reported 100% compliance to the supplementation protocol.

Incremental cycling test

The intermittent incremental cycling test in visit 1 was performed to establish the relationship between oxygen uptake and submaximal power outputs. The protocol began at 50 W for 4 minutes with subsequent stages increasing

by 30 W every 4 minutes. Between each exercise stage, participants rested on the ergometer without pedalling for 2 minutes, in which time, a capillary blood sample was taken from the finger and plasma lactate concentration analyzed. The test was terminated when participants blood plasma lactate reached a value ≥ 4 mmol·L^{-1}. Expired gas samples were collected using the Douglas bag technique (Cranlea & Co. Bourneville, Birmingham, UK) in the last minute of each exercise stage.

Maximal Rate of Oxygen Uptake

Maximal oxygen uptake ($\dot{V}O_{2max}$) was calculated following an incremental exercise test. The test began at 50 W for 4 minutes, and subsequent work rate increased by 30 W every minute until volitional exhaustion. The participants were asked to maintain a pedalling cadence between 70 and 90 rev·min^{-1}. A visual display in front of the participants was used to maintain this cadence. Expired gas samples were collected using the Douglas bag technique and separate gas samples were collected for a minimum of 3-minutes of before participants reached volitional exhaustion. The last collection bag was only analyzed when collection time and expired volume was greater than 30 sec and 65 L, respectively. Expired and inspired fractions of oxygen and carbon dioxide were determined with a gas analyzer (Series 1400, Servomex, Crowborough, UK), calibrated using known gases (Linde Gas UK Ltd., West Bromwich, UK), and expired volumes measured using a dry gas meter (Harvard Apparatus Ltd., Edenbridge, UK). A finger prick capillary blood sample was taken four minutes after the end of the test and analysed for plasma lactate concentration. All participants attained at least two of the following $\dot{V}O_{2max}$ criteria; 1) plateau in $\dot{V}O_2$ of < 2.1 ml·kg^{-1}·min^{-1} between the last two gas collections, 2) blood plasma lactate > 8 mmol·L^{-1}, 3) respiratory exchange ratio ≥ 1.15 (Howley and Bassett 1995).

Submaximal Cycling Intensities

The power to oxygen uptake (as a percentage of $\dot{V}O_{2max}$) relationship during the intermittent incremental exercise, performed during visit 1, was used to establish power at 45, 55 and 65% of participants $\dot{V}O_{2max}$. Participants cycled at each intensity for 10 minutes with a finger prick blood plasma sample measured 5 minutes into each stage (i.e. at 5, 15, 25 minutes of the protocol) with duplicate measurements averaged to provide blood plasma lactate and glucose. Two, one-minute gas sample were collected between minutes 7-9 of each stage, and analyzed. Data collection of one subject was stopped due to technical problems with the SRM ergometer during this part of the session.

Rates of whole-body carbohydrate and fat oxidation (i.e. CHox and FATox, respectively) were calculated based on the following equations by Jeukendrup and Wallis (2005) for low (45% $\dot{V}O_{2max}$) and moderate intensity exercise (55 and 65% $\dot{V}O_{2max}$) with the assumption that protein oxidation during exercise was negligible:

Fat Oxidation=1.695*$\dot{V}O_2$-1.701*$\dot{V}CO_2$

Low intensity (45% $\dot{V}O_{2max}$), Carbohydrate oxidation=4.344*$\dot{V}CO_2$-3.061*$\dot{V}O_2$

Moderate intensity (55 and 65% $\dot{V}O_{2max}$), Carbohydrate oxidation=4.210*$\dot{V}CO_2$-2.962*$\dot{V}O_2$

16.1 km Cycling Time-Trial

Participants completed 16.1 km time-trials on the SRM ergometer. As per manufactures instructions, the large flywheel was attached to the ergometer to simulate kinetic energy as would be experienced during road cycling. Participants could freely choose the cycling gear and cadence. The software program recorded power output, pedal cadence, time and distance. Water was provided *ad libitum*. Participants received no temporal, verbal or physiological feedback during the time-trial and were only aware of the distance they had covered. In order not to interfere with the performance-based setting, no expired gas samples or blood samples were taken during the time-trial. Immediately following the time-trial, participants rested passively and a blood sample for plasma lactate was taken, with subsequent samples then taken every minute for the first 5 minutes, and then taken every 5 minutes for a total of 15 minutes. Samples were analysed in duplicate and averaged.

Statistical Analysis

All statistical analyses were completed using SPSS 20.0 (SPSS, Chicago, IL). Data normality assumptions were assessed using Kolmogorov-Smirnov test. Paired samples t-tests used were to compare physiological responses and 48 hours dietary intake between the supplement and placebo conditions. A *priori* power analysis showed a sample size of 14 would allow detection of a 2-3% difference in 16.1 km time-trial performance with a high statistical power ($1 - \beta = 0.80$: $0.05 = \alpha$ level). To determine the time-trial effect size, Cohen's *d* and subsequent power were calculated (Cohen 1988). Differences between plasma lactate following the time-trial were analysed using a condition (control vs. NZBC) by time-point (0, 1, 2, 3, 4, 5, 10, 15, and 20 min post time-trial) repeated measures analysis of variance (ANOVA) with post-hoc t-tests. Mauchley's Test of Sphericity was conducted to test for homogeneity of data and where violations were present Greenhouse-Geiser adjustments were made. All data are reported as mean ± SD and significance was set at alpha level of $P \leq 0.05$.

RESULTS

Blood Pressure, Lactate and Glucose in Rest

Resting systolic blood pressure (NZBC: 124 ± 7, PL: 123 ± 6 mmHg, P = 0.556), diastolic blood pressure (NZBC: 79 ± 5, PL = 78 ± 5 mmHg, P = 0.190), blood plasma lactate (NZBC: 1.15 ± 0.25, PL: 1.02 ± 0.24 mmol·L$^{-1,}$ P = 0.23) and glucose (NZBC: 4.57 ± 0.45, PL: 4.52 ± 0.44 mmol·L^{-1}, P = 0.77) were not different between conditions after 7-days of supplementation.

Steady State Exercise, Energy Expenditure and Substrate Oxidation

Across the three intensities, there were no differences between treatments in $\dot{V}O_2$, $\dot{V}CO_2$, heart rate, cycling economy, absolute power, blood plasma lactate, blood glucose or energy expenditure indicating that the participants experienced similar relative exercise intensities and physiological responses between treatments (Table 3). However, there were trends with NZBC for whole-body FATox rates to be 15 and 13% higher at 45% (P = 0.077) and 55% $\dot{V}O_{2max}$ (P = 0.102), but these were not matched by a significantly lower CHox rate (P > 0.05). At 65% $\dot{V}O_{2max}$, FATox was 27% higher following NZBC supplementation (P = 0.044), in line with a strong trend for lower CHox (P = 0.06). Correspondingly, the RER had a trend to be lower at 45% $\dot{V}O_{2max}$ (P = 0.066) and 55% $\dot{V}O_{2max}$ (P = 0.120). At 65% $\dot{V}O_{2max}$ RER was lower (P = 0.043) (Table 3).

16.1 km Cycling Time-Trial Performance and Lactate Responses

NZBC reduced 16.1 km completion time (NZ: 1678 ± 108, PL: 1722 ± 131 sec, P = 0.027), with a group mean reduction of 2.4±3.7% (range -2.7%-8.7%) and 11 participants showing a decrease (Fig. 1). This was coupled with a trend for higher power across the time-trial (NZBC: 259 ± 29, PL: 250 ± 33 W, P = 0.155) with no difference in heart rate (NZBC: 157 ± 14, PL: 153 ± 15 beats·min^{-1}, P = 0.247) or cadence (NZBC: 92 ± 8, PL: 93 ± 8 rev·min^{-1}, P = 0.847) between conditions. Post hoc effect size calculations indicate a 0.7 (medium-large) effect magnitude, with the achieved statistical power for the time-trial at 0.80. Absolute lactate values following the time-trial (Fig. 2) showed significant time ($F_{(1,13)}$ = 108.815, P<0.001) and condition effects ($F_{(1,13)}$ = 7.637, P = 0.016) with between condition effects equating to 15% (P = 0.003), 10% (P = 0.032), 12% (P = 0.004), 11% (P = 0.025) and 15% (P = 0.048) at 0, 2, 3, 4 and 15 minutes post time-trial, respectively, although there was no interaction effect ($F_{(1,13)}$ = 2.447, P = 0.191).

DISCUSSION

This is the first study to observe that 7 days capsule intake of NZBC extract by trained endurance athletes enhanced time-trial cycling performance by 2.4%. Intake of NZBC extract also increased whole-body FATox by 27% at moderate intensity exercise (~65% $\dot{V}O_{2max}$), which was coupled with a strong trend for lower whole-body CHox (P = 0.06). A strong trend for higher whole-body FATox was also observed at low intensity exercise (~45% $\dot{V}O_{2max}$, P = 0.077).

Effects of NZBC extract on cycling time-trial performance

Paton and Hopkins (2006) proposed that the "smallest worthwhile change" for road time-trial cyclists is around 0.6%. Our finding of a 2.4% increase in time-trial performance is considerably greater than this value and comparable to other studies using supplements high in polyphenols, such as the 2.7% improvement in 16.1 km time-trial following acute (~2.5 hours before time-trial) beetroot intake in male cyclists with similar $\dot{V}O_{2max}$ values (Lansley et al. 2011) and the 3.1% improvement in a 30 km time-trial with quercetin in elite cyclists (MacRae and Mefferd 2006). Our finding of a 2.4% increase in time-trial performance represents a significant practical advantage to athletes undertaking endurance exercise training because the performance increase occurred without alteration of training or diet before the time-trial and likely results from the trend for a higher power output across the time-trial (P = 0.15). In addition, all participants conformed to dietary restrictions and between experimental visits; there was no difference in postprandial status as confirmed with resting glucose samples. The magnitude of the practical effect of NZBC supplementation on 16.1 km performance can also be represented by using effect size statistic (Cohen 1988) and the calculated effect size for the present study of 0.7 indicates a moderate-large effect of NZBC extract upon cycling time-trial performance. Participants did not report any change in frequency or type of their cycling participation and reported to be participating in cycling exercise 8-10 hours a week during the 7-day supplementation periods. In addition to using a randomised design, it is therefore unlikely the improvement in performance is attributable to a chronic training effect of undertaking 7-days supplementation and exercise and therefore represents a performance improvement achievable from a short duration (i.e. 7-days) intake. However, with absence of markers of phytochemical status in this study, that may be associated with the performance effect, we do not know whether a shorter intake of NZBC results in similar performance improvements.

A mechanism by which blackcurrant supplementation improves performance may involve improved endothelial function. Anthocyanin-induced endothelium-dependant vasorelaxation of rat thoracic aorta is mediated by increased production of endothelial-derived vasodilation factor nitric oxide (Nakamura et al. 2002). Delphinidin, a non-glycoside anthocyanin, can also relax blood vessels by increasing nitric oxide through increased Ca^{2+} concentrations in endothelial cells (Martin et al. 2002). Production of peroxynitrate from nitric oxide has also been shown to be inhibited by polyphenols (Nagi et al. 2002). Blackcurrant containing a large amount of delphinidin and other anthocyanins, therefore has the potential to increase peripheral blood flow by the combined action of increased nitric oxide by endothelial cells and a reduced breakdown by nitric oxide free radicals. Indeed, an increase in peripheral blood flow in typing work, a physical activity performed at a relatively very low intensity, following blackcurrant intake has been reported (Matsumoto et al. 2005). Given the importance of nitric oxide in control of skeletal muscle blood flow (Boushel et al. 2002) and potentially on skeletal muscle contractile efficiency (Bailey et al. 2010), it is possible that such responses confer the performance benefits observed in the present study. To elucidate such mechanisms, future studies should examine the availability of nitric oxide and blood flow measures following NZBC intake before, during and following exercise.

Following the time-trial, the significant affect for lactate across the 20-minute recovery period following NZBC may represent alterations in production or removal of lactate through blood flow or changes in membrane lactate transport mechanisms. However, in future studies on the effect of NZBC, measures of blood flow and lactate kinetics should be examined during and following exhaustive exercise when lactate levels are typically elevated.

Effects of NZBC on substrate oxidation

As far as we know, this is the first study to observe an improved FATox during moderate intensity cycling following NZBC extract intake and is in contrast to previous work supplementing with quercetin (MacRae and Mefferd 2006). In that study, no change in substrate oxidation was observed during a 30 km time-trial (MacRae and Mefferd 2006), however, it needs to be acknowledged that no substrate oxidation measures were obtained during the time-trial in the present study. Our increased fat oxidation at 65% $\dot{V}O_{2max}$ from 0.37 ± 0.15 in the placebo condition to 0.44 ± 0.12 $g \cdot min^{-1}$ in the NZBC condition is similar in absolute values (i.e. $g \cdot min^{-1}$) and also magnitude of change to the FATox rates observed during moderate intensity cycling following green-tea extract (Venables et al. 2008). An exact comparison of studies with different polyphenols requires caution though as the possible variation in bioavailability

and subsequent interactions with the concomitant intake of other nutrients may affect observation (for a review see Myburgh 2014). In the present study, the observed alterations in substrate utilisation occurred following a standardised absolute carbohydrate intake 2 hours before the event, with no alterations in circulating glucose or hypoglycaemia (i.e. glucose < 3 mmol·L^{-1}) present during the exercise (Table 3).

It is thought that lipolysis is not likely to limit whole-body FATox at the intensities used in the present study (Horowitz et al. 1997) and it could be that blackcurrant has additional effects on lipid metabolism. For example, chronic blackcurrant extract intake in C57BL/6J mice has been shown to elevate mRNA of genes involved with energy expenditure including peroxisome proliferator-activated receptor alpha (Benn et al. 2014) and similarly, Tsuda et al. (2005) observed that a total of 633 genes were up-regulated through treatment of rat adipocytes with cyanidin-3-glycoside, which included genes involved in in lipid metabolism and signal transduction-related genes. Therefore, the increased whole-body FATox may result from a combination of many pathways acting synergistically including up regulation of genes for proteins involved in FATox, transport of fatty acids into mitochondria, improved nitric oxide availability and increased peripheral blood flow.

Limitations

Participants were allowed to consume their normal diet 46 hours before the testing sessions (except the dietary restrictions such as caffeine on the day, alcohol the day before and the standard breakfast 2 hours before the session) and participants were instructed to use a recorded food diary from the third visit (i.e. 1st condition visit) and replicate this for the cross-over condition visit. Due to the wide availability of polyphenols within normal dietary intake, participants were not restricted in their choice of foods, therefore it cannot be ruled out that some participants may have consumed more polyphenols in the 48-hour period. We also did not measure the antioxidant status and were not able to quantify polyphenol or anthocyanin intake of participants. This therefore will not highlight if there were any intra and inter differences in phytochemical status of participants and account that activity of anthocyanins can be synergistically or antagonistically altered by other phytochemicals and vitamins found in fruits (Niki et al. 1998). In addition, it should also be recognised that a food diary collected from the first experimental condition and replicated in the second experimental condition has disadvantages such as a large variability in food intake between participants and the intake recorded the first time and then replicated may not represent an appropriate or optimal intake for that participant (Jeacocke and Burke 2010). It is also accepted that the use of a standardised breakfast of one slice of toast or bread 2 hours before the start of testing does not represent a typical pre-race condition (i.e.< 1g·kg body mass^{-1}).

However, the intake of carbohydrate before measurement of substrate utilisation required standardisation due to the affect intake of carbohydrate before exercise can have on substrate utilisation (Achten and Jeukendrup 2003).

With a 7-day NZBC supplementation representing a nutritional ergogenic aid (as in the present study), we do not know the time - and dose-dependent metabolic, physiological and performance effects of NZBC extract intake. Our daily dose of 105 mg·day^{-1} was according to manufacturers guidelines and the supplementation period in line with previous studies using berry juices also applying multiple days of intake before exercise test (e.g. Connolly et al. 2006; Howatson et al 2010; Bowtell et al. 2011). Wu et al. (2004) estimated that the average anthocyanin intake in U.S. adults as 12.5 mg·day^{-1}. Our daily dose of anthocyanin from NZBC extract capsules was approximately 8 times higher than this, but is considerably lower than other studies using polyphenol supplements such as 1000 mg·day^{-1} of quercetin (Cureton et al. 2009). In that study, the participants did not report any side-effects; however, the minimum dose and duration of NZBC extract needed to elicit ergogenic effects are unknown. Future studies should therefore examine dosing strategies of NZBC with emphasis on elucidating the optimal dose, frequency and duration of intake.

Conclusions

Short-term (7-days) intake of NZBC extract capsules is associated with an improved 16.1km time-trial cycling performance obtained with higher plasma lactate values, and an increased whole-body fat oxidation at moderate intensity exercise (~65% $\dot{V}O_{2max}$). These findings may have implications for nutritional strategies used by endurance athletes to enhance performance and alter substrate utilisation.

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FIGURE LEGENDS

Fig. 1 Exercise time of the 16.1 km time-trial. Columns show group mean ± SD. Dashed lines show the individual responses. *Completion time was reduced after NZBC extract (P<0.05).

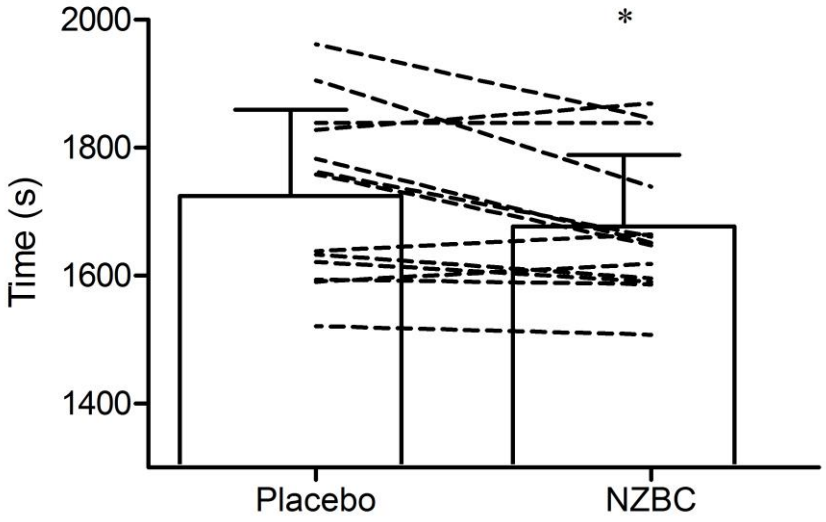

Fig. 2 Blood plasma lactate across 20-minute passive recovery following the 16.1 km time-trial after NZBC (filled circles) and placebo (open circles). Data are mean ± SD. * denotes significant difference between groups (P < 0.05).

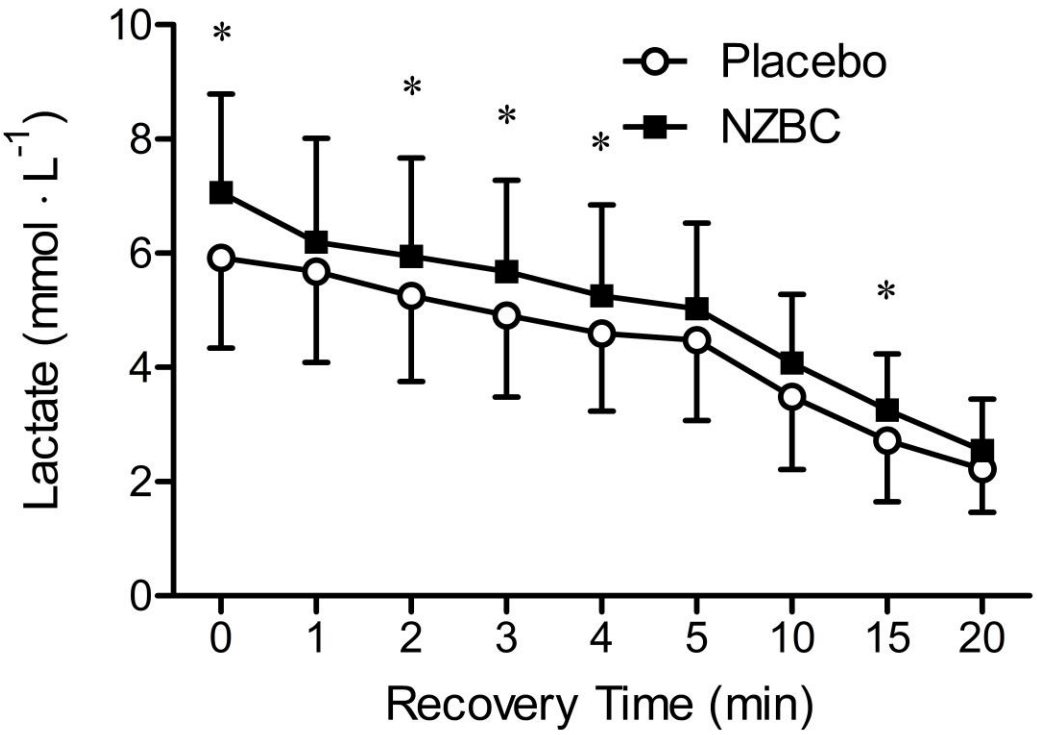

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Table 1. Participant characteristics

Age (years)	38±13
Height (cm)	178±4
Body Mass (kg)	77±9
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	53±6
$\dot{V}O_{2max}$ (L·min^{-1})	4.1±0.5
RER_{max}	1.17±0.07
Power (Lactate 4 mmol·L^{-1}) (W)	290±26
$Lactate_{max}$ (mmol·L^{-1})	7.51±0.81
Heart $Rate_{max}$ (beats·min^{-1})	182±12
WR_{max} (W)	366±36
% Body Fat	13.7±2.6

Maximum values were collected during the incremental maximal cycling test to volitional exhaustion. $\dot{V}O_{2max}$, maximum rate of oxygen uptake; RER_{max}, maximum respiratory exchange ratio; Power (Lactate 4 mmol·L^{-1}), power that elicits a plasma lactate of 4 mmol·L^{-1} measured during an intermittent incremental cycling test; $Lactate_{max}$, maximum lactate value achieved four minutes after the end of the test; Heart $Rate_{max}$, maximum heart rate; WR_{max}, maximum work rate. Data reported as mean ± SD from 14 participants.

Table 2. Absolute and relative to body mass dietary intake 48 hours before experimental visits.

	Placebo	**NZBC**
Carbohydrate (g)	474±117	460±150
(g·kg body mass^{-1})	6.3±2.4	6.3±2.9
Fats (g)	150±60	159±53
(g·kg body mass^{-1})	2.0±1.0	2.0±0.9
Protein (g)	179±48	180±42
(g·kg body mass^{-1})	2.2±0.8	2.2±0.8
Total Energy Intake (kJ)	16544±3390	16590±3818
(kJ·body mass^{-1})	204.9±77.9	206.8±86.9

Data reported as mean ± SD from 14 participants.

Table 3. Data during submaximal cycling at low (45 & 55% $\dot{V}O_{2max}$) and moderate intensities (65% $\dot{V}O_{2max}$).

	45% $\dot{V}O_{2max}$		55% $\dot{V}O_{2max}$		65% $\dot{V}O_{2max}$	
Variable	**Placebo**	**NZBC**	**Placebo**	**NZBC**	**Placebo**	**NZBC**
Power (W)	121±16	122±16	160±18	159±17	198±21	199±20
$\dot{V}O_2$ (L·min^{-1})	1.80±0.19	1.79±0.21	2.17±0.22	2.21±0.25	2.68±0.22	2.70±0.23
$\dot{V}CO_2$ (L·min^{-1})	1.62±0.21	1.60±0.22	1.97±0.23	1.99±0.26	2.43±0.28	2.42±0.26
Relative Intensity (% $\dot{V}O_{2max}$)	44±2	44±4	54±4	55±5	66±4	67±4
Cycling Economy (mL·kg^{-1}·W^{-1})	11.5±1.4	11.5±1.4	10.7±1.2	11.0±1.2	10.6±1.3	10.7±1.2
Heart rate (beats·min^{-1})	105±11	106±11	117±12	118±13	132±14	132±15
Lactate (mmol·L^{-1})	1.05±0.29	1.01±0.26	0.92±0.29	0.88±0.19	1.19±0.49	1.09±0.29
Glucose (mmol·L^{-1})	4.25±0.43	4.27±0.67	4.01±0.58	4.08±0.56	4.14±0.67	4.05±0.60
Energy Expenditure (kJ·min^{-1})	36±7	35±8	43±9	44±10	53±11	54±11
CH_{ox} (g·min^{-1})	1.6±0.39	1.52±0.40	1.85±0.43	1.80±0.43	2.36±0.54	2.23±0.48
FAT_{ox} (g·min^{-1})	0.26±0.1	0.29±0.09	0.33±0.14	0.38±0.09	0.37±0.15	0.44±0.12*
RER	0.91±0.04	0.90±0.04	0.91±0.04	0.89±0.03	0.91±0.04	0.90±0.03*

All measures were collected following 7 days supplementation with NZBC extract during steady state cycling, and 2 hours post-prandial of a standard low calorie carbohydrate breakfast (1 slice of bread and the last capsule). CHox, carbohydrate oxidation; FATox, fat oxidation; RER, respiratory exchange ratio. Data reported as mean ± SD from 13 participants. * denotes $P<0.05$ vs. placebo.