Eur J Appl Physiol (2017) 117:1207–1216
DOI 10.1007/s00421-017-3607-z

ORIGINAL ARTICLE

Dose effects of New Zealand blackcurrant on substrate oxidation and physiological responses during prolonged cycling

Matthew David Cook[1,2] · Stephen David Myers[1] · Mandy Lucinda Gault[1] · Victoria Charlotte Edwards[1] · Mark Elisabeth Theodorus Willems[1]

Received: 13 October 2016 / Accepted: 3 April 2017 / Published online: 8 April 2017

Abstract
Purpose It has been previously shown that New Zealand blackcurrant (NZBC) extract increased fat oxidation during short duration cycling. The present study examined the effect of different doses of NZBC extract on substrate oxidation and physiological responses during prolonged cycling.
Methods Using a randomized counterbalanced Latin-square design, 15 endurance-trained male cyclists (age: 38 ± 12 years, height: 187 ± 5 cm, body mass: 76 ± 10 kg, $\dot{V}O_{2max}$: 56 ± 8 mL kg^{-1} min^{-1}, and mean ± SD) completed four separate 120-min cycling bouts at 65% $\dot{V}O_{2max}$ after ingesting no dose, or one of three doses (300, 600, or 900 mg day^{-1}) of NZBC extract (CurraNZ™) for 7 days.
Results A dose effect ($P < 0.05$) was observed for average fat oxidation (0, 300, 600, and 900 mg day^{-1} values of 0.63 ± 0.21, 0.70 ± 0.17, 0.73 ± 0.19, and 0.73 ± 0.14 g min^{-1}) and carbohydrate oxidation (0, 300, 600, and 900 mg day^{-1} values of 1.78 ± 0.51, 1.65 ± 0.48, 1.57 ± 0.44, and 1.56 ± 0.50 g min^{-1}). The individual percentage change of mean fat oxidation was 21.5 and 24.1% for 600 and 900 mg day^{-1} NZBC extract, respectively, compared to no dose. Heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, plasma lactate, and glucose were not affected.
Conclusion Seven-day intake of New Zealand blackcurrant extract demonstrated a dose-dependent effect on increasing fat oxidation during 120-min cycling at 65% $\dot{V}O_{2max}$ in endurance-trained male cyclists.

Keywords Substrate oxidation · New Zealand blackcurrant · Anthocyanins · Polyphenols · Sports nutrition · Cycling

Abbreviations

ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
FAT/CD36	Fatty acid translocase/cluster of differentiation 36
FMD	Flow-mediated dilation
GTE	Green tea extract
NZBC	New Zealand blackcurrant
RER	Respiratory exchange ratio
$\dot{V}O_2$	Oxygen consumption
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_{2max}$	Maximum oxygen uptake
WR_{max}	Maximum work rate

Introduction

Among berries, blackcurrant (*Ribes nigrum*) has one of the highest concentrations of the polyphenol, anthocyanin, and typically contains delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside. Anthocyanins are flavonoids that have been associated with health benefits acting through inflammatory or antioxidant activity (Pojer et al. 2013). Increased peripheral blood flow during typing activity was shown with blackcurrant

Communicated by Anni Vanhatalo.

✉ Mark Elisabeth Theodorus Willems
m.willems@chi.ac.uk

[1] Department of Sport and Exercise Sciences, University of Chichester, College Lane, Chichester PO19 6PE, UK

[2] Institute of Sport and Exercise Science, University of Worcester, Henwick Grove, Worcester WR2 6AJ, UK

intake (Matsumoto et al. 2005), potentially through anthocyanin-induced vasodilation and vasorelaxation (Ziberna et al. 2013).

Studies on New Zealand blackcurrant (NZBC) during exercise have observed that 7-day intake (~105 mg day^{-1} of anthocyanins) had no effect on rating of perceived exertion during repeated high-intensity treadmill running sprints (Perkins et al. 2015) or maximum oxygen uptake ($\dot{V}O_{2max}$) during cycling (Willems et al. 2015). However, an increased 16.1 km cycling time trial (Cook et al. 2015) and intermittent running performance (Perkins et al. 2015), a greater absolute lactate decrease following exercise (Cook et al. 2015; Perkins et al. 2015) and an increase in lactate threshold (Willems et al. 2015), were observed. In addition, a 27% higher fat oxidation rate was observed at 65% of $\dot{V}O_{2max}$ during 10-min cycling, with no changes in heart rate, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and plasma lactate at 45, 55, and 65% of $\dot{V}O_{2max}$ (Cook et al. 2015). However, the metabolic and physiological responses during prolonged exercise (i.e., greater than 60 min) with intake of NZBC are not known. The physiological responses influencing fat oxidation during prolonged exercise are different to those during short duration exercise. For example, fatty acid translocase/cluster of differentiation 36 (FAT/CD36) on the mitochondrial membrane is increased at 120 min after exercise, but not after 30 min (Holloway et al. 2006). In addition, prolonged cycling exercise causes a gradual decrease in insulin concentration, a gradual increase in plasma free fatty acid and glycerol concentration (Jeukendrup et al. 1999), and a lowering of intramuscular glycogen stores (Vøllestad and Blom 1985). Therefore, the substrate oxidation and the physiological responses may be different with NZBC during prolonged exercise of 120 min, to those reported during shorter duration steady state exercise; however, this has not been examined.

Evidence that polyphenols may increase fat oxidation is also provided by studies using green tea extract (GTE), which is rich in the polyphenol catechins. Venables et al. (2008) observed that a 24-h GTE ingestion (366 mg day^{-1}) increased fat oxidation rate by 17% (placebo: 0.35 ± 0.03 vs. GTE: 0.41 ± 0.03 g min^{-1}) during 30 min of cycling at 60% $\dot{V}O_{2max}$ in young healthy men (26 ± 2 years). A similar effect was observed when dosing chronically for 3 months with catechins (218 mg day^{-1}) in healthy men (range 26–42 years), and a 24% higher fat oxidation rate was observed (control: 3956 ± 1205 vs. catechin: 5217 ± 904 kcal day^{-1}) during 30 min of treadmill walking at 5 km h^{-1} compared to a control of no catechins (Ota et al. 2005). However, a lower dose of GTE containing 160 mg day^{-1} catechins (of which 70 mg day^{-1} was epigallocatechin gallate) for 3 weeks did not effect fat oxidation during 120-min cycling at 50% of maximum work rate in endurance-trained men (Eichenberger et al. 2009). Taken together, these studies suggest that increases in fat oxidation during exercise from catechin polyphenols within GTE are dose-dependent.

Following anthocyanin intake, vascular function has also demonstrated dose-dependent responses. For example, in healthy individuals, Rodriguez-Mateos et al. (2013) reported a dose-dependent increase in flow-mediated dilation (FMD) up to 310 mg anthocyanin and then a plateau above this dose, with additional intake causing no further increases. Previous studies on the effectiveness of New Zealand blackcurrant during exercise (Cook et al. 2015; Perkins et al. 2015; Willems et al. 2015) have not examined if the physiological responses are dose dependent. However, based upon previous responses to polyphenol intake, dose-dependent changes on physiological responses during exercise may occur. Therefore, this study aimed to examine if dose-dependent changes in physiological responses occur following New Zealand blackcurrant taken for 7 days during prolonged cycling in trained cyclists.

Methods

Participants

Fifteen endurance-trained men volunteered for the study without payment and provided written informed consent to participate. They were recruited from local cycling clubs with a history of participation of greater than 3 years and were not engaged in a structured training program for the duration of the study but typically performed cycling exercise 6–10 h a week. Participants were screened for intake of other dietary supplements before commencing participation with all not taking any nutritional supplements. Participant characteristics are presented in Table 1. The study was approved by the University of Chichester Research Ethics Committee with protocols and procedures conforming to the 2013 Declaration of Helsinki.

Experimental design

Participants visited the laboratory on five occasions at the same time of day (see Fig. 1 for the timeline of experimental sessions and testing). Prior to all visits, participants were instructed to abstain from vigorous exercise for 48 h, alcohol for 24 h, and caffeine-containing products on the day of testing. On the first visit, participants were measured for position on the electronically controlled cycle ergometer (SRM ergometer, SRM International, Jülich, Germany) with saddle height and setback, handlebar height, and drop replicated for all visits. The ergometer was fitted with the

Table 1 Participant characteristics

Age (years)	38 ± 12
Height (cm)	178 ± 5
Body mass (kg)	76 ± 10
%BF	13.4 ± 2.4
$\dot{V}O_{2max}$ (mL kg^{-1} min^{-1})	56.7 ± 7.9
$\dot{V}O_{2max}$ (L min^{-1})	4.3 ± 0.5
RER_{max}	1.13 ± 0.06
$Lactate_{peak}$ (mmol L^{-1})	7.4 ± 1.4
Heart $rate_{max}$ (beats min^{-1})	184 ± 10
WR_{max} (W)	378 ± 55
Power (lactate 4 mmol L^{-1}) (W)	271 ± 41
Anthocyanin intake (mg day^{-1})	67 ± 47

Maximum values were collected during the incremental maximal cycling test to volitional exhaustion. Data reported as mean ± SD from 15 participants

$\dot{V}O_{2max}$ 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, $Lactate_{peak}$ peak lactate value achieved 4 min after the end of the test, $Heart\ Rate_{max}$ maximum heart rate, WR_{max} maximum work rate, *%BF* percentage body fat, *Anthocyanin intake* daily intake of anthocyanin calculated from a food frequency questionnaire

participant's saddle and pedals, with participants also using their own cycling shoes.

During the first visit, participants' stature (Seca 213, Seca, Birmingham, UK) and body mass (Kern ITB, Kern, Germany) were measured. Subsequently, participants completed an incremental-intensity cycling test until a blood plasma lactate ≥4 mmol L^{-1} (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs, USA) was reached. After a 15-min break, participants then completed a maximal cycling test to volitional exhaustion for calculation of $\dot{V}O_{2max}$ 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 increment).

Participants were assigned, in a randomized, counterbalanced Latin-square design, to three NZBC dose conditions (1, 2, or 3 capsules a day) for 7 days and a no dose condition. Optimal dosing strategy of NZBC is not known; however, multiple days of intake have been used previously before exercise testing [e.g., 5 days before (Bell et al. 2015)]. Each 300 mg NZBC capsule contained 105 mg of anthocyanins, consisting of 35–50% delphinidin-3-rutinoside, 5–20% delphinidin-3-glucoside, 30–45% cyanidin-3-rutinoside, and 3–10% cyanidin-3-glucoside (CurraNZ™, Health Currancy Ltd, Surrey, UK). The NZBC capsules were independently analysed and confirmed the ingredients present with no presence of caffeine. Participants were instructed to take the capsules, with breakfast (one-a-day), 12-h apart (two-a-day), and evenly spaced through the day (three-a-day). On the final day of supplementation, participants reported to the laboratory, 2-h post-prandial of standard breakfast (i.e., one slice of buttered bread or toast) and all the capsules required for that condition. Participants then completed a 120-min cycling protocol at a power calculated to elicit ~65% $\dot{V}O_{2max}$, with expired gas samples collected and lactate measured every 15 min. Thirteen participants performed the 120-min cycle at an intensity below lactate threshold. Participants were allowed to drink plain water ad libitum, with all exercise tests conducted in a temperature-controlled laboratory at 18 °C with a fan in front of participants to limit unwanted heat storage. Between dosing conditions, there was a 14-day washout period. An anthocyanin intake similar to

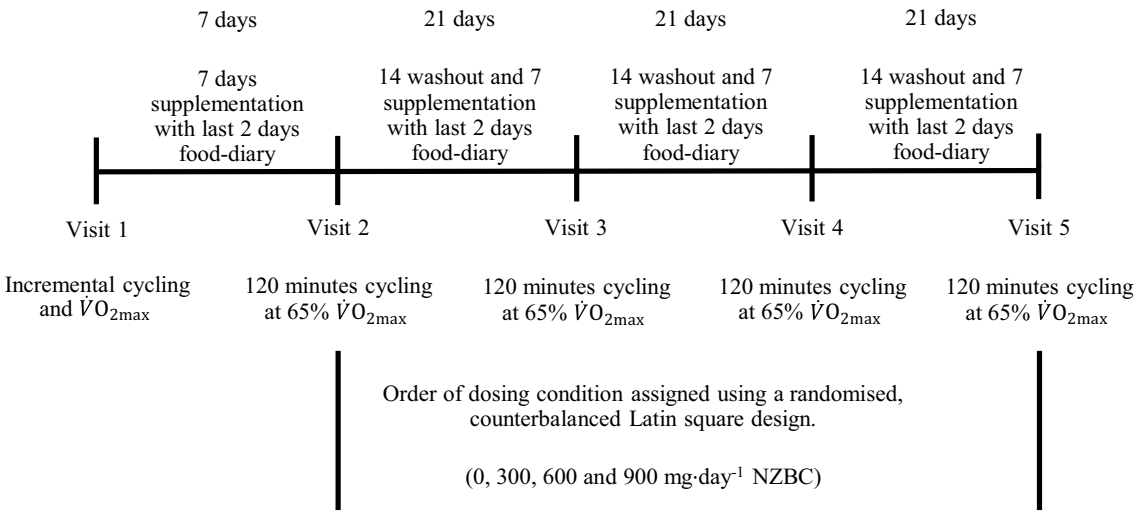

Fig. 1 Experimental design and timeline of the five visits

that of our highest dose for 1 month showed a return to baseline of biochemical and biomarkers of antioxidant status after 15-day washout (Alvarez-Suarez et al. 2014).

Anthocyanin consumption, physical activity, and dietary standardization

Participants completed a food frequency questionnaire, which detailed the amount and frequency of anthocyanin containing foods eaten complied from the Phenol Explorer database (Neveu et al. 2010). Intake of anthocyanin was calculated as the sum of the consumption frequency of each anthocyanin containing food multiplied by the content of the anthocyanin content for the portion size (Table 1).

Before all experimental visits, participants were instructed to keep their weekly schedule as consistent as possible. Participants recorded their dietary intake on a written food diary 48 h prior to the first experimental dosing condition (i.e., visit 2) and were then told to replicate this for all subsequent experimental visits (i.e., visits 3, 4, and 5) using the first diary as a guide, while recording on a new diary their dietary intake for that visit. Food diaries were analysed (Nutritics LTD, Dublin, Ireland) for carbohydrate, fat and protein intake, and total energy intake (kJ). There were no differences for carbohydrate, fats, or protein intake in absolute or relative units between the experimental visits (Table 2). Analysis of the diaries indicated participants reported 100% adherence to the dietary instructions.

Incremental cycling test

The intermittent incremental cycling test performed within visit 1 was completed to establish the relationship between cycling power output and oxygen consumption. The protocol consisted of 4-min stages of work interspersed with 2-min rest where participants rested on the ergometer without pedalling. The protocol began at 50 W and increased by 30 W each stage. At the beginning of the rest stage, a capillary blood sample was taken from the finger and blood plasma lactate concentration analysed. Blood samples were not lysed and, therefore, represent plasma rather than whole blood. The test was terminated when participant's plasma lactate reached a value $\geq$4 mmol L^{-1}. In the last minute of each exercise stage, an expired gas sample was collected in 200 L plastic Douglas bags (Cranlea & Co. Bourneville, Birmingham, UK).

Maximal rate of oxygen uptake

Calculation of $\dot{V}O_{2max}$ was completed following an incremental intensity cycling test to volitional exhaustion. The test began at 50 W for 4 min and subsequently increased by 30 W each minute with participants instructed to keep pedal cadence between 70 and 90 rev min^{-1}, which was displayed on a television screen. In at least the last 3 min of the protocol, expired gases were collected in 45-s samples with 30-s samples in the last minute. Expired and inspired fractions of oxygen and carbon dioxide were determined with a gas analyser (Series 1400, Servomex, Crowborough, UK), calibrated using known gases [15.06% O_2, 5.01% CO_2, 79.93% N_2 (Linde Gas UL Ltd., West Bromwich, UK)], and expired volumes measured using a dry gas meter (6162, Harvard Apparatus Ltd., Edenbridge, UK) and expressed as standard temperature and pressure dry. A finger prick capillary blood sample was taken 4 min after the end of the test and analysed for peak plasma lactate concentration. $\dot{V}O_{2max}$ was considered to be achieved if participants attained at least three 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}; and (3) respiratory exchange ratio (RER) $\geq$1.15 (Howley et al. 1995).

Table 2 Dietary intake 48 h before each visit for each treatment condition

	0 mg day^{-1}	300 mg day^{-1}	600 mg day^{-1}	900 mg day^{-1}
Carbohydrate (g)	494 ± 91	495 ± 90	479 ± 85	490 ± 101
(g kg body $mass^{-1}$)	6.7 ± 1.8	6.7 ± 1.7	6.5 ± 1.6	6.6 ± 1.9
Fats (g)	228 ± 68	228 ± 68	230 ± 65	235 ± 73
(g kg body $mass^{-1}$)	3.1 ± 1.0	3.1 ± 0.9	3.1 ± 0.9	3.1 ± 1.0
Protein (g)	216 ± 59	221 ± 58	217 ± 56	220 ± 60
(g kg body $mass^{-1}$)	2.9 ± 0.9	3 ± 0.9	2.9 ± 0.8	3.0 ± 0.9
Total energy intake (kJ)	20,654 ± 2950	20,804 ± 3080	20,724 ± 2805	20,709 ± 2835
(kJ body $mass^{-1}$)	279 ± 63	280 ± 59	279 ± 56	278 ± 54

Intake of dietary variables for the different NZBC extract dosing conditions of 0, 300, 600, and 900 mg day^{-1}. All values were collected from 48-h food diaries before each experimental visit. Data reported as mean ± SD from $n = 15$

120-min cycling

The power to oxygen uptake relationship (as a percentage of $\dot{V}O_{2max}$) during the incremental cycling test to a plasma lactate of 4 mmol L^{-1} was used to establish the power at 65% of participant's $\dot{V}O_{2max}$. This power was a fixed load for the 120-min protocol. Participants cycled continually for 120 min, keeping a constant pedal cadence between 70 and 90 rev min^{-1} and they were allowed to consume water ad libitum. Finger prick blood sampling for lactate and glucose and one ~60 s expired air sample were collected every 15 min (i.e., at 15, 30, 45, 60, 75, 90, 105, and 120 min of the protocol). Pilot testing indicated variability of the Douglas bag technique in calculating fat oxidation of 6.3% during two 30-min bouts of cycling at 65% $\dot{V}O_{2max}$ separated by 7 days. Rates of whole body carbohydrate and fat oxidation were calculated using the following stoichiometric equations for moderate intensity exercise with the assumption that protein oxidation during exercise was negligible (Jeukendrup and Wallis 2005):

$$\text{Fat oxidation } (g \cdot min^{-1}) = 1.695 \cdot \dot{V}O_2 - 1.701 \cdot \dot{V}CO_2$$

$$\text{Carbohydrate oxidation } (g \cdot min^{-1}) = 4.210 \cdot \dot{V}CO_2 - 2.962 \cdot \dot{V}O_2.$$

Statistical analysis

All statistical analyses were complete using SPSS 20.0 (SPSS, Chicago, USA). Data normality assumptions were assessed using Kolmogorov–Smirnov test. Differences between doses during the 120-min cycling were analysed using a dose (0 vs. 300 vs. 600 vs. 900 mg day^{-1}) by timepoint (15, 30, 45, 60, 75, 90, 105, and 120 min) repeated measures analysis of variance (ANOVA). A Bonferroni post hoc test was used to identify time comparisons. When dose effects were found, average responses over the 120-min protocol were analysed with a repeated measures one-way ANOVA with post hoc pairwise comparisons with Bonferroni correction. Mauchley's test of sphericity was conducted to test for homogeneity of data where violations were present and Greenhouse-Geisser adjustments were made. An a priori power analysis indicated a sample size of 15 would allow detection of a 27% increase in fat oxidation rates with a high statistical power ($1 - \beta = 0.80$: $0.05 = \alpha$ level). To determine the effect size of responses, Cohen's d were calculated (Cohen 1988). All data are reported as mean ± SD, and significance was accepted at $P < 0.05$.

Results

Physiological data, energy expenditure, and rates of substrate oxidation

There was a time effect for $\dot{V}O_2$ ($F_{(1.4267,19.966)} = 7.889$, $P = 0.006$), energy expenditure ($F_{(1.521,\ 21.299)} = 6.490$, $P = 0.010$), and relative intensity ($F_{(7,98)} = 18.062$, $P < 0.001$) with no difference between the doses across the eight collections of the 120-min ride ($P > 0.05$) (Table 3). There was no time or dose effect for $\dot{V}CO_2$ ($P > 0.05$). Mean relative intensity was not different for the doses ($F_{(2.230,31.223)} = 1.101$, $P = 0.360$) (0 mg day^{-1}: 63.9 ± 3.9; 300 mg day^{-1}: 64.6 ± 4.3; 600 mg day^{-1}: 64.8 ± 3.7; and 900 mg day^{-1}: 64.4 ± 3.5% $\dot{V}O_{2max}$).

The RER during the 120-min protocol showed a time ($F_{(3.209,44.924)} = 17.445$, $P < 0.001$) and dose effect ($F_{(3,42)} = 3.984$, $P = 0.014$) with no interaction effect ($F_{(21,294)} = 0.917$, $P = 0.570$) (Fig. 2a). The mean RER (0 mg day^{-1}: 0.86 ± 0.04, 300 mg day^{-1}: 0.85 ± 0.03, 600 mg day^{-1}: 0.83 ± 0.03, 900 mg day^{-1}: 0.84 ± 0.02) showed a dose effect ($F_{(3,42)} = 3.984$, $P = 0.014$) with 600 ($d = 1.01$) and 900 ($d = 0.71$) mg day^{-1} decreasing from 0 mg day^{-1} ($P < 0.05$).

Fat oxidation showed time ($F_{(2.799,39.182)} = 21.271$, $P < 0.001$) and dose effects ($F_{(3,42)} = 3.913$, $P < 0.001$), with no interaction effect ($F_{(21,294)} = 0.954$, $P = 0.522$) (Fig. 2b). Mean fat oxidation (0 mg day^{-1}: 0.63 ± 0.20 g min^{-1}, 300 mg day^{-1}: 0.70 ± 0.16 g min^{-1}, 600 mg day^{-1}: 0.74 ± 0.18 g min^{-1}, and 900 mg day^{-1}: 0.74 ± 0.13 g min^{-1}) showed a dose effect ($F_{(3,42)} = 3.913$, $P = 0.015$) with post hoc testing indicating a group mean (i.e., mean of all individual percentage changes of mean fat oxidation) of 21.5% (13 of 15 participants increased) and 24.1% (13 of 15 participants increased) increase in fat oxidation from 0 mg day^{-1} for 600 and 900 mg day^{-1} NZBC, respectively ($P < 0.05$). Between 0 and 300 mg day^{-1}, fat oxidation was 17.5% higher (11 of 15 participants increased); however, this was not different ($P = 0.124$). The effect sizes for increases in average fat oxidation from 0 mg day^{-1} were 0.42, 1.03, and 0.75 for 300, 600, and 900 mg day^{-1} NZBC intake, respectively. Similarly, absolute carbohydrate oxidation during the 120-min protocol showed a time ($F_{(2.635,36.892)} = 9.831$, $P < 0.001$) and dose effect ($F_{(3,42)} = 2.907$, $P = 0.046$), with no interaction effect ($F_{(21,294)} = 0.825$, $P = 0.688$) (Fig. 2c). Mean carbohydrate oxidation (0 mg day^{-1}: 1.78 ± 0.48 g min^{-1}, 300 mg day^{-1}: 1.65 ± 0.45 g min^{-1}, 600 mg day^{-1}: 1.56 ± 0.41 g min^{-1}, and 900 mg day^{-1}:

Table 3 Volume of oxygen uptake and carbon dioxide produced, relative intensity, energy expenditure, glucose, lactate cycling economy, and heart rate during 120-min cycling following a 7-day intake of different doses of New Zealand blackcurrant extract

Condition	Time (min)							
	15	30	45	60	75	90	105	120
$\dot{V}O_2$ (L min^{-1})†								
0 mg day^{-1}	2.63 ± 0.39	2.70 ± 0.40	2.68 ± 0.37	2.68 ± 0.41	2.73 ± 0.37	2.71 ± 0.41	2.77 ± 0.37	2.82 ± 0.36
300 mg day^{-1}	2.63 ± 0.41	2.67 ± 0.43	2.73 ± 0.44	2.69 ± 0.43	2.79 ± 0.46	2.79 ± 0.47	2.84 ± 0.38	2.85 ± 0.38
600 mg day^{-1}	2.65 ± 0.35	2.70 ± 0.43	2.75 ± 0.41	2.77 ± 0.41	2.76 ± 0.42	2.75 ± 0.37	2.82 ± 0.37	2.85 ± 0.40
900 mg day^{-1}	2.65 ± 0.37	2.69 ± 0.35	2.68 ± 0.38	2.72 ± 0.40	2.77 ± 0.40	2.76 ± 0.39	2.82 ± 0.42	2.85 ± 0.33
$\dot{V}CO_2$ (L min^{-1})								
0 mg day^{-1}	2.30 ± 0.36	2.36 ± 0.35	2.34 ± 0.32	2.33 ± 0.36	2.32 ± 0.31	2.32 ± 0.36	2.34 ± 0.36	2.37 ± 0.35
300 mg day^{-1}	2.27 ± 0.39	2.29 ± 0.38	2.34 ± 0.40	2.28 ± 0.39	2.35 ± 0.40	2.32 ± 0.41	2.38 ± 0.36	2.36 ± 0.36
600 mg day^{-1}	2.27 ± 0.34	2.29 ± 0.38	2.33 ± 0.36	2.34 ± 0.37	2.31 ± 0.34	2.29 ± 0.31	2.32 ± 0.35	2.31 ± 0.35
900 mg day^{-1}	2.29 ± 0.34	2.28 ± 0.33	2.27 ± 0.37	2.31 ± 0.38	2.31 ± 0.38	2.29 ± 0.36	2.32 ± 0.40	2.33 ± 0.38
Relative intensity (%$\dot{V}O_{2max}$)†								
0 mg day^{-1}	62.1 ± 4.9	63.8 ± 4.2	63.4 ± 4.1	63.2 ± 4.6	64.5 ± 4.4	64.3 ± 3.9	63.9 ± 4.1	65.4 ± 4.2
300 mg day^{-1}	62.2 ± 4.4	63.1 ± 4.8	64.3 ± 4.6	63.6 ± 4.8	65.7 ± 4.5	65.7 ± 5.3	65.7 ± 4.2	66.2 ± 4.3
600 mg day^{-1}	62.5 ± 3.9	63.6 ± 3.6	64.9 ± 4.1	65.2 ± 3.6	65.1 ± 4.2	65.0 ± 4.1	65.2 ± 3.9	65.9 ± 4.4
900 mg day^{-1}	62.4 ± 3.3	63.5 ± 3.4	63.1 ± 3.5	64.2 ± 4.3	65.2 ± 4.9	65.0 ± 4.0	66.0 ± 4.0	65.9 ± 3.4
EE (kJ min^{-1})†								
0 mg day^{-1}	54.0 ± 8.0	55.4 ± 8.1	55.0 ± 7.4	54.8 ± 8.2	55.7 ± 7.5	55.3 ± 8.2	56.4 ± 7.6	57.2 ± 7.4
300 mg day^{-1}	53.8 ± 8.6	54.5 ± 8.8	55.6 ± 9.0	54.8 ± 8.9	56.6 ± 9.3	56.4 ± 9.6	57.5 ± 7.8	57.7 ± 7.9
600 mg day^{-1}	54.0 ± 7.8	55.0 ± 8.8	56.0 ± 8.4	56.2 ± 8.4	55.9 ± 8.4	55.7 ± 7.4	56.9 ± 7.6	57.3 ± 8.1
900 mg day^{-1}	54.1 ± 7.7	54.7 ± 7.2	54.5 ± 8.1	55.4 ± 8.3	56.1 ± 8.4	55.8 ± 8.1	57.6 ± 7.4	58.0 ± 8.4
Glucose (mmol L^{-1})†								
0 mg day^{-1}	4.07 ± 0.61	4.44 ± 0.50	4.43 ± 0.56	4.18 ± 0.58	4.21 ± 0.49	3.95 ± 0.72	4.21 ± 0.42	4.12 ± 0.40
300 mg day^{-1}	3.68 ± 0.66	4.04 ± 0.85	3.96 ± 0.59	4.10 ± 0.46	4.11 ± 0.53	3.90 ± 0.53	3.91 ± 0.43	3.60 ± 0.71
600 mg day^{-1}	3.84 ± 0.48	4.04 ± 0.67	4.06 ± 0.90	3.75 ± 0.92	3.76 ± 0.68	3.67 ± 0.61	3.78 ± 0.36	3.69 ± 0.35
900 mg day^{-1}	3.72 ± 0.90	4.24 ± 0.56	4.21 ± 0.62	4.10 ± 0.54	3.93 ± 0.49	3.92 ± 0.58	3.77 ± 0.66	3.96 ± 0.44
Lactate (mmol L^{-1})								
0 mg day^{-1}	1.24 ± 0.52	1.25 ± 0.63	1.12 ± 0.61	1.23 ± 0.61	1.21 ± 0.53	1.26 ± 0.68	1.10 ± 0.49	1.21 ± 0.48
300 mg day^{-1}	1.26 ± 0.39	1.12 ± 0.41	1.21 ± 0.60	1.21 ± 0.60	1.27 ± 0.53	1.26 ± 0.75	1.26 ± 0.47	1.31 ± 0.92
600 mg day^{-1}	1.19 ± 0.37	1.32 ± 0.79	1.11 ± 0.50	1.11 ± 0.25	1.04 ± 0.29	1.14 ± 0.52	1.13 ± 0.29	1.14 ± 0.50
900 mg day^{-1}	1.37 ± 0.60	1.48 ± 0.55	1.17 ± 0.37	1.15 ± 0.46	1.21 ± 0.55	1.12 ± 0.38	1.00 ± 0.29	1.07 ± 0.34
Cycling economy (mL kg^{-1} W^{-1})†								
0 mg day^{-1}	11.1 ± 2.2	11.4 ± 2.0	11.4 ± 2.0	11.3 ± 1.9	11.6 ± 2.5	11.5 ± 2.1	11.4 ± 2.0	11.5 ± 2.3
300 mg day^{-1}	11.1 ± 2.1	11.3 ± 2.1	11.6 ± 2.1	11.3 ± 2.0	11.7 ± 2.0	11.7 ± 2.0	11.5 ± 1.8	11.6 ± 2.0
600 mg day^{-1}	11.1 ± 2.0	11.3 ± 1.9	11.5 ± 1.9	11.6 ± 2.0	11.5 ± 2.1	11.6 ± 2.2	11.4 ± 2.0	11.6 ± 2.0
900 mg day^{-1}	11.2 ± 2.3	11.3 ± 2.1	11.3 ± 2.2	11.5 ± 2.1	11.6 ± 2.0	11.6 ± 2.4	11.4 ± 1.5	11.7 ± 2.5
Heart rate (b min^{-1})†								
0 mg day^{-1}	128 ± 12	130 ± 12	132 ± 12	135 ± 14	136 ± 15	137 ± 15	138 ± 16	139 ± 17
300 mg day^{-1}	128 ± 12	131 ± 13	133 ± 14	134 ± 12	136 ± 15	138 ± 15	140 ± 15	142 ± 16
600 mg day^{-1}	127 ± 12	130 ± 13	133 ± 13	134 ± 13	137 ± 11	137 ± 12	138 ± 13	139 ± 12
900 mg day^{-1}	132 ± 17	135 ± 17	136 ± 16	137 ± 15	138 ± 16	140 ± 17	141 ± 17	143 ± 17

Values are mean ± SD, $n = 15$

$\dot{V}O_2$ oxygen uptake, $\dot{V}CO_2$ carbon dioxide produced, *EE* energy expenditure

† Significant main effect for time ($P < 0.05$)

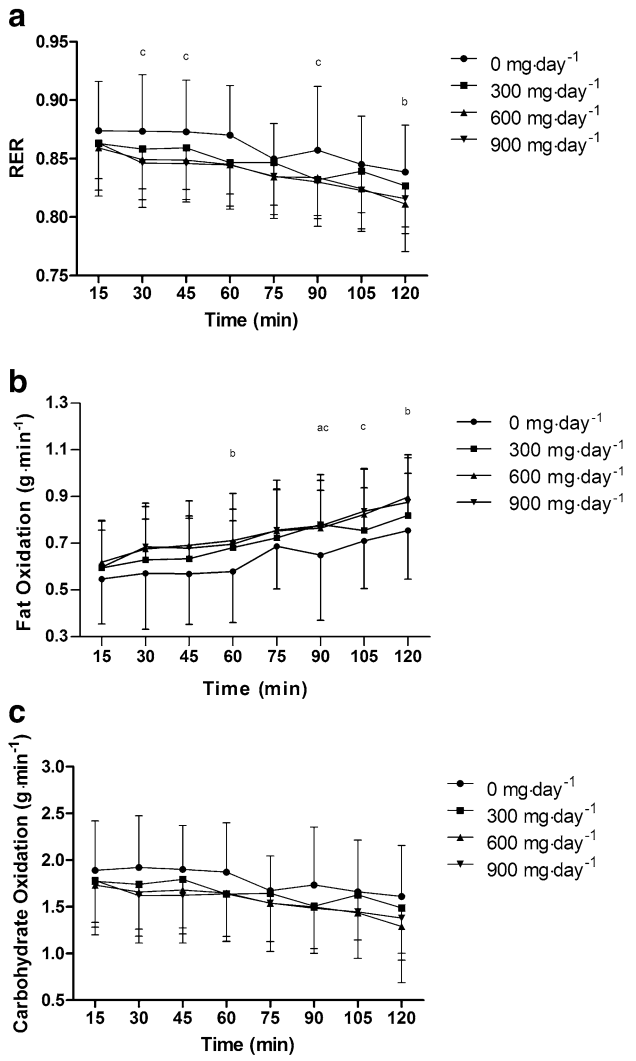

Fig. 2 Respiratory exchange ratio (RER) (**a**), fat oxidation (**b**), and carbohydrate oxidation (**c**) during 120-min cycling at ~65% $\dot{V}O_{2max}$ following 0, 300, 600, and 900 mg day^{-1} New Zealand blackcurrant extract. Vales are presented as mean ± SD. **a** 0 and 300 mg day^{-1} different; **b** 0 and 600 mg day^{-1} different; and **c** 0 and 900 mg day^{-1} different ($P < 0.05$)

1.56 ± 0.46 g min^{-1}) showed a dose condition effect ($F_{(3,42)} = 2.907$, $P = 0.046$), with post hoc testing indicating no differences between the doses ($P > 0.05$).

Blood parameters

There was a main time effect for plasma glucose ($F_{(3.511,2.405)} = 4.049$, $P = 0.009$), with no differences between the dosing conditions ($P > 0.05$). There was no dose or time effect for plasma lactate ($P > 0.05$) (Table 3).

Cycling power, economy, and heart rate

A time effect for cycling economy ($F_{(7,98)} = 3.114$, $P = 0.005$) and heart rate ($F_{(1.675,23.446)} = 17.070$, $P < 0.001$) was observed with no effect of dose ($P > 0.05$) (Table 3). The power was fixed for the 120-min protocol, with the average power for the four dosing conditions (0 mg day^{-1}: 193 ± 31 W, 300 mg day^{-1}: 193 ± 30 W, 600 mg day^{-1}: 194 ± 32 W, and 900 mg day^{-1}: 193 ± 31 W) not different ($P > 0.05$).

Discussion

The principal finding from this study was that there was a dose-dependent effect of NZBC on fat oxidation during 120-min cycling in trained male cyclists at ~65% $\dot{V}O_{2max}$. This is the first study to examine the effect of different doses of NZBC on fat oxidation during long duration exercise in trained cyclists. The results indicate a 21.5 and 24.1% ($P < 0.05$) increase in mean fat oxidation rates, and absolute increases of 0.11 and 0.10 g min^{-1}, for 600 and 900 mg day^{-1} NZBC intake, respectively, with the calculated effect sizes indicating moderate-to-large effects.

The changes in mean fat oxidation rates observed in this study are lower than the 27% increase (0.37 ± 0.15 placebo vs. 0.44 ± 0.12 NZBC) reported by Cook et al. (2015) during 10-min cycling at 65% $\dot{V}O_{2max}$ following 300 mg day^{-1} NZBC (105 mg day^{-1} anthocyanin). The group mean increases of 21.5 and 24.1% in the present study occurred following 600 and 900 mg day^{-1} of NZBC, doses which are twice and three times that of Cook et al. (2015) with 300 mg day^{-1} demonstrating no change in average fat oxidation in this study.

This may represent a lack of statistical power to detect a difference (i.e., 0 vs. 300 mg day^{-1}), despite a group mean increase of 17.5%, an identical absolute increase of 0.07 g min^{-1}, and effect size of 0.42. Furthermore, the 27% increase observed by Cook et al. (2015) occurred during an incremental 30-min protocol (3 blocks of 10 min at 45, 55, and 65% $\dot{V}O_{2max}$) and it has been noted that during an incremental protocol, the work completed in the previous stage may influence fat oxidation in the next stage (Achten et al. 2002). In Cook et al. (2015), fat oxidation with NZBC extract at 65% $\dot{V}O_{2max}$ was 0.44 ± 0.12 g min^{-1}. The present study used cycling exercise of 120 min and this would explain the higher absolute fat oxidation values (e.g., 0.70 ± 0.16 g min^{-1} with 300 mg day^{-1}) as fat oxidation increases over time during prolonged exercise (Romijn et al. 1993). As the power was fixed for the 120-min protocol, the observations of a time effect for heart rate can be explained by

the cardiovascular drift effect observed during prolonged exercise (Fritzsche et al. 1999). Similarly, the time effect for $\dot{V}O_2$ would explain the time effect for relative intensity and cycling economy and is likely to result from $\dot{V}O_2$ drift caused by an increase in body temperature, recruitment of additional muscle fibres, and fat oxidation (Ishijima et al. 2011). Despite this $\dot{V}O_2$ drift, the mean oxygen cost elicited a relative intensity of ~65% $\dot{V}O_{2max}$ with no differences between the doses. This also indicates that intake of NZBC has no adverse physiological responses on $\dot{V}O_2$ that could diminish performance. However, future studies should examine the implications for the performance of endurance exercise modalities from an increase in fat oxidation by NZBC.

The coefficient of variation (CV) of fat oxidation during exercise lasting greater than 1 h is reported between 3 and 6% (Hodgson et al. 2013) with the day-to-day variation reported to be as high as 9.6% (Achten and Jeukendrup 2003). The much larger group mean 21.5 and 24.1% increases from 600 and 900 mg day^{-1}; NZBC was, therefore, attributed to the NZBC intake. This may result from effects of the anthocyanins in NZBC on fat metabolism. For example, in C57BL/6J mice fed a high fat diet, blackcurrant anthocyanins increased the mRNA expression of 633 genes involved in energy expenditure and mitochondrial biogenesis including peroxisome proliferator-activated receptor alpha, proliferator-activated receptor delta, uncoupling protein 2 and 3, and mitochondrial transcription factor A (Benn et al. 2014). Anthocyanin has also been observed to increase AMP-activated protein kinase (AMPK) in skeletal muscle of mice (Takikawa et al. 2010) and fatty acid oxidation of human HepG2 cells following in vitro incubation (Guo et al. 2012). The activity of acetyl-CoA carboxylase (ACC) 1 and ACC-2 is inhibited by AMPK, which leads to increased fatty acid oxidation and decreased fatty acid synthesis (Towler and Hardie 2007). Using biopsies, Roepstorff et al. (2005) demonstrated that following 60-min cycling at 65% $\dot{V}O_{2max}$ in moderately trained men, there was a decrease in muscle malonyl-CoA concentration, which was associated with an increased activity of AMPK and inhibition of acetyl-CoA carboxylase resultant from its phosphorylation by AMPK. It has also been reported that AMPK activation can induce translocation of FAT/CD36 allowing increased fatty acid uptake (Luiken et al. 2003). It is, therefore, possible that the interaction of the physiological responses during exercise and alterations in fat oxidation mechanisms following anthocyanin intake leads to increased fat oxidation during exercise. However, various factors should be considered when comparing these studies to in vivo human conditions. For example, Takikawa et al. (2010) fed mice a very high intake of anthocyanins (10 g kg^{-1} diet) while Guo et al. (2012) incubated for 1 h with only cyanidin-3-glucoside.

Blackcurrant can increase peripheral blood flow during an MVC of the trapezius muscle following typing activity (Matsumoto et al. 2005) Therefore, anthocyanin-induced increases in peripheral blood flow may also explain the higher fat oxidation rates through greater delivery of free fatty acids, as an increase in plasma fatty acids has shown to increase fat oxidation (Romijn et al. 1993). The increase in peripheral blood flow may occur by increasing nitric oxide availability, as shown by anthocyanins ability to inhibit NADPH oxidase (Rodriguez-Mateos et al. 2013). It should be noted, however, that mode of exercise, intensity, and tissue mass within the study by Matsumoto et al. (2005) were very different compared to the present study. To develop a greater understanding of the potential mechanisms involved, future studies should examine plasma glycerol as an indirect marker of lipolysis and free fatty acids during exercise following intake of NZBC.

Rodriguez-Mateos et al. (2016) observed non-linear dose-dependent changes in FMD to cranberry polyphenols, with 409 mg of polyphenols having no effect, whereas responses to 787 and 1238 mg increased linearly and plateaued after 1238 mg. The results in this study with NZBC indicate similar dose-dependent changes, as it appears that there may be a minimum NZBC dose required to elicit physiological effects. For example, fat oxidation was only increased after 600 mg day^{-1} (210 mg day^{-1} anthocyanin) and 900 mg day^{-1} (315 mg day^{-1} anthocyanin), with no difference between 600 and 900 mg day^{-1}. These responses may also represent that an upper limit in substrate utilisation changes by NZBC was reached or that changes in substrate utilisation were limited, because mechanisms for anthocyanin absorption were limited (Kurilich et al. 2005).

Upon ingestion, anthocyanins are reported to have poor bioavailability, with studies reporting uptake of 12.4 ± 1.4% of the ingested dose (Czank et al. 2013). However, beneficial vascular responses following anthocyanin intake have been associated with a peak in phenolic metabolites such as ferulic acid, isoferulic acid, vanillic acid, 2-hydroxybenzoic acid, benzoic acid and caffeic acid in the plasma (Rodriguez-Mateos et al. 2013). Furthermore, anthocyanin metabolites have been observed in plasma up to 48 h following intake (Kay et al. 2005); therefore, a 7-day intake, as in this study, may represent a build-up of anthocyanin metabolites over time which resulted in the increased fat oxidation. The use of multiple days of supplementation before an exercise with a supplement taken on the day of the test is consistent with previous studies supplementing with cherry anthocyanins (Bell et al. 2015). However, this approach does not allow separation of the acute or chronic effects of the supplementation.

Furthermore, as anthocyanins may act synergistically with other dietary polyphenols (Niki et al. 1988), future studies may implement anthocyanin wash out periods before testing similar to Bell et al. (2015) (i.e., no fruits, vegetables, tea coffee, alcohol, chocolate, cereals, whole meal bread, and grains 4 days before and 3-day post exercise). However, this approach should be used with caution as such experimental design would be problematic for ecological validity. The duration responses (i.e., if changes occur following 1 day of supplementation) to NZBC intake on fat oxidation are unknown. This is an area where future research should focus on, considering that complete anthocyanins are detectable in the plasma at 1 h after consumption (Zhu et al. 2011).

Anthocyanins are a sub-class of flavonols. The daily intake of anthocyanins calculated from the food frequency questionnaire was 67 ± 47 mg day^{-1}. This is comparable to the intake of flavonols (including anthocyanins) in men within the United Kingdom of 51 mg day^{-1} (Zamora-Ros et al. 2011). It also highlights that the lowest dose of NZBC (105 mg day^{-1}) used is likely to be considerably higher than their habitual intake and represents a substantial increase in daily consumption of anthocyanins. However, this dose results in no changes in substrate utilisation, but an even higher dose of 600 mg day^{-1} NZBC extract is required, indicating that these effects would be difficult to achieve from consuming unprocessed blackcurrants, whereby each capsule was equivalent to ~80 blackcurrants.

Conclusions

Seven days intake of New Zealand blackcurrant increases fat oxidation during 120-min cycling at ~65% $\dot{V}O_{2max}$ in endurance-trained individuals and this occurs in a dose-dependent manner. High-dose intake of New Zealand blackcurrant does not have adverse physiological effects in trained cyclists. To elucidate mechanisms of the observed findings from this study, future research should examine fat oxidation with measures of circulating fatty acids and peripheral blood flow.

Acknowledgements Supply of supplement (CurraNZ™) for this study was obtained from Health Currancy Ltd (United Kingdom). The authors declare no other conflict of interest.

References

Achten J, Jeukendrup AE (2003) Maximal fat oxidation during exercise in trained men. Int J Sports Med 24(3):603–608

Achten J, Gleeson M, Jeukendrup AE (2002) Determination of the exercise intensity that elicits maximal fat oxidation. Med Sci Sports Exerc 34(1):92–97

Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Di Stefano G, González-Paramás AM, Santos-Buelga C, Busco F, Quiles JL, Cordero MD, Bompadre S, Mezzetti B, Battino M (2014) One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. J Nutr Biochem 25(3):289–294

Bell PG, Walshe IH, Davison GW, Stevenson EJ, Howatson G (2015) Recovery facilitation with Montmorency cherries following high-intensity, metabolically challenging exercise. Appl Physiol Nutr Metab 40(4):414–423

Benn T, Kim B, Park YK, Wegner CJ, Harness E, Nam TG, Kim DO, Lee JS, Lee JY (2014) Polyphenol-rich blackcurrant extract prevents inflammation in diet-induced obese mice. J Nutr Biochem 25(3):1019–1025

Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Lawrence Erlbaum Associates, Hillsdale, p 567

Cook MD, Myers SD, Blacker SD, Willems MET (2015) New Zealand blackcurrant extract improves cycling performance and fat oxidation in cyclists. Eur J Appl Physiol 115(11):2357–2365

Czank C, Cassidy A, Zhang Q, Morrison DJ, Preston T, Kroon PA, Botting NP, Kay CD (2013) Human metabolism and elimination of the anthocyanin, cyanidin-3-glucose: a (13)C-tracer study. Am J Clin Nutr 97(5):995–1003

Eichenberger P, Colombani PC, Mettler S (2009) Effects of 3-week consumption of green tea extracts on whole-body metabolism during cycling exercise in endurance-trained men. Int J Vitam Nutr Res 79(1):24–33

Fritzsche RG, Switzer TW, Hodgkinson BJ, Coyle EF (1999) Stroke volume decline during prolonged exercise is influenced by the increase in heart rate. J Appl Physiol 86(3):799–805

Guo H, Liu G, Zhong R, Wang Y, Wang D, Xia M (2012) Cyanidin-3-O-β-glucoside regulates fatty acid metabolism via an AMP-activated protein kinase-dependent signaling pathway in human HepG2 cells. Lipids Health Dis 11:10

Hodgson AB, Randell RK, Jeukendrup AE (2013) The effect of green tea extract on fat oxidation at rest and during exercise: evidence of efficacy and proposed mechanisms. Adv Nutr 4(2):129–140

Holloway G, Bezaire V, Heigenhauser GJ, Trandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL (2006) Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. J Physiol 571(1):201–210

Howley ET, Bassett DR, Welch HG (1995) Criteria for maximal oxygen uptake: review and commentary. Med Sci Sports Exerc 27(9):1292–1301

Ishijima T, Fukunaga T, Sakamoto S, Higuchi M (2011) Drift in oxygen consumption during prolonged sub-maximal exercise in subject of different training status. Int J Sport Health Sci 9:64–72

Jeukendrup AE, Wallis GA (2005) Measurement of substrate oxidation during exercise by means of gas exchange measurements. Int J Sports Med 26(Suppl 1):S28–S37

Jeukendrup AE, Raben A, Gijsen A, Stegen JHCH, Brouns F, Saris WHM, Wagenmakers AJM (1999) Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. J Physiol 515(2):579–589

Kay CD, Mazza GJ, Holub BJ (2005) Anthocyanins exist in the circulation primarily as metabolites in adult men. J Nutr 135(11):2582–2588

Kurilich AC, Clevidence BA, Britz SJ, Simon PW, Novotny JA (2005) Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. J Agric Food Chem 53(16):6537–6542

Luiken JJ, Corrt SL, Willems J, Coumans WA, Bonen A, van der Vusse GJ, Glatz JF (2003) Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated

through AMP-activated protein kinase signaling. Diabetes 52(7):1627–1634

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 work in humans. Eur J Appl Physiol 94(1–2):36–45

Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford) Article ID bap024

Niki E, Yamaoto Y, Takahashi M, Yamamoto K, Yamamoto Y, Komuro E et al (1988) Free radical-mediated damage of blood and its inhibition by antioxidants. J Nutr Sci Vitaminol 34(5):507–512

Ota N, Soga S, Shimotoyodome A, Haramizu S, Inaba M, Murase T, Tokimitsu I (2005) Effects of combination of regular exercise and tea catechins intake on energy expenditure in humans. J Health Sci 51(2):233–236

Perkins IC, Vine SA, Blacker SD, Willems MET (2015) New Zealand blackcurrant extract improves high-intensity intermittent running. Int J Sport Nutr Exerc Metab 25(5):487–493

Pojer E, Mattivi F, Johnson D, Stockley CS (2013) The case for anthocyanin consumption to promote human health: a review. Compr Rev Food Sci Food Sav 12(5):483–508

Rodriguez-Mateos A, Rendeiro C, Bergillos-Meca T, Tabatabaee S, George TW, Heiss C, Spencer JP (2013) Intake and time dependence of blueberry flavonoid-induced improvement in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity. Am J Clin Nutr 98:1179–1191

Rodriguez-Mateos A, Feliciano RP, Boeres A, Weber T, Dos Santos CN, Ventura MR, Heiss C (2016) Cranberry (poly)phenol metabolites correlate with improvements in vascular function: a double-blind, randomized, controlled, dose-response, crossover study. Mol Nutr Food Res 60(10):2130–2140. doi:10.1002/mnfr.201600250

Roepstorff C, Halberg N, Hillig T, Saha AK, Ruderman NB, Wojtaszewski JF, Richter EA, Kiens B (2005) Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. Am J Physiol Endocrinol Metab 288(1):133–142

Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, Wolfe RR (1993) Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol 265(3):E380–E391

Takikawa M, Inoue S, Horio F, Tsuda T (2010) Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. J Nutr 140(3):527–533

Towler MC, Hardie DG (2007) AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 100(3):328–341

Venables MC, Hulston CJ, Cox HR, Jeukendrup AE (2008) Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. Am J Clin Nutr 87(3):778–784

Vøllestad NK, Blom PC (1985) Effect of varying exercise intensity on glycogen depletion in human muscle fibres. Acta Physiol Scand 125(3):395–405

Willems MET, Myers SD, Gault ML, Cook MD (2015) Beneficial physiological effects with blackcurrant intake in endurance athletes. Int J Sport Nutr Exerc Metab 25(4):367–374

Zamora-Ros R, Knaze V, Luján-Barroso L, Slimani N, Romieu I, Fedirko V et al (2011) Estimated dietary intakes of flavonols, flavanones and flavones in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24 h dietary recall cohort. Br J Nutr 106(12):1915–1925

Zhu Y, Xia M, Yang Y, Liu F, Li Z, Hao Y, Mi M, Jin T, Ling W (2011) Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. Clin Chem 57(11):1524–1533

Ziberna L, Lunder M, Tramer F, Drevenšek G, Passamonti S (2013) The endothelial plasma membrane transporter bilitranslocase mediates rat aortic vasodilation induced by anthocyanins. Nutr Metab Cardiovasc Dis 23(1):68–74