Title: Intake duration of anthocyanin-rich New Zealand blackcurrant extract affects metabolic responses during moderate intensity walking exercise in adult males

Authors: Mehmet Akif Şahin[1,2], Pelin Bilgiç[2], Stefano Montanari[1], Mark Elisabeth Theodorus Willems[1]

Affiliations: [1]University of Chichester, Institute of Sport, College Lane, Chichester, PO19 6PE, United Kingdom, [2]Hacettepe University, Department of Nutrition and Dietetics, Ankara, Turkey

Corresponding author: Prof. Mark Willems
University of Chichester
Institute of Sport
College Lane
Chichester, PO19 6PE
United Kingdom

Email: m.willems@chi.ac.uk
ORCID: 0000-0003-4385-8739

Abstract

We examined effects of intake duration of anthocyanin-rich New Zealand blackcurrant (NZBC) extract on physiological and metabolic responses during moderate intensity walking. Healthy men (n=16, age: 24±6 years, body mass: 78±16 kg, BMI: 24.7±4.1 $kg \cdot m^{-2}$, body fat: 15±5%) volunteered. One metabolic equivalent (1-MET: 3.95±0.64 $ml \cdot kg^{-1} \cdot min^{-1}$) was measured during supine rest. Responses during the 30-min walk (n=3: 4-MET; n=13: 5-MET) (speed: 5.7±0.7 $km \cdot hr^{-1}$) were measured at 7-10, 17-20 and 27-30 min and averaged over the time periods. For intake conditions (7-days and 14-days), 2 capsules of NZBC extract (600 mg containing 210 mg of anthocyanins) were taken with breakfast (14-day washout). The final 2 capsules were ingested 2-hr before the morning walk. Intake duration of NZBC extract had no effect on heart rate, minute ventilation, oxygen uptake, and carbon dioxide production. Fat oxidation was enhanced with 7- and 14-day intake by 11±19% and 17±26% (baseline: 0.36±0.12, 7-day: 0.39±0.13, 14-day: 0.41±0.13 $g \cdot min^{-1}$, p=0.007). Only 14-day intake lowered RER (baseline: 0.852±0.046, 7-day: 0.843±0.045, 14-day: 0.837±0.037, p=0.019) and carbohydrate oxidation (baseline: 0.95±0.40, 7-day: 0.91±0.40, 14-day: 0.86±0.33 $g \cdot min^{-1}$, p=0.032). Rating of perceived exertion was lower with 7-day and 14-day intake (baseline: 11.0±2.3, 7-day: 10.5±1.8, 14-day: 10.3±2.1, p=0.002). Longer intake duration (i.e. 14 days) of New Zealand blackcurrant extract seems to enhance fat oxidation more during a 30-min moderate intensity walk than 7 days intake. The intake duration of anthocyanin-rich New Zealand blackcurrant extract may be due to an enhanced bioavailability of anthocyanin-derived metabolites that alter the mechanisms for substrate oxidation during moderate intensity exercise.

KEYWORDS: brisk walking; exercise-induced fat oxidation; respiratory exchange ratio; anthocyanins; metabolic equivalent

Introduction

Polyphenols are the most abundant phytochemicals present in the human diet and can be obtained by ingestion of fruits, vegetables, leaves, seeds and plant-based beverages such as coffee, tea and red wine (Scalbert et al. 2005). Polyphenols have antioxidant activity due to their ability to neutralize free radicals by being an electron or hydrogen atom donor (Apak et al. 2004). In addition, polyphenols have anti-inflammatory effects and ability to promote gut health (Yang and Kortesniemi 2015). Therefore, polyphenols reduce the risk for diseases that are linked with oxidative stress and inflammation (e.g. some cancers, diabetes mellitus, cardiovascular, and neurodegenerative diseases) by reducing oxidative stress, low-density lipoprotein cholesterol oxidation, blood pressure, inflammatory markers and insulin resistance (Hooper et al. 2008; Yang and Kortesniemi 2015). Several thousand polyphenols have been identified and are divided into four major groups: flavonoids, stilbenes, lignans and phenolic acids, with the flavonoid anthocyanins providing berries with pink, red, blue or purple colors (Manach et al. 2004). The berry blackcurrant contains primarily anthocyanins, i.e. delphinidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, and cyanidin-3-*O*-glucoside (Kähkönen et al. 2003).

Health benefits provided by polyphenol intake are associated with the anthocyanin content (Jennings et al. 2013; Lin et al. 2017; Mink et al. 2007). In addition, anthocyanin-rich foods have the potential to affect fat oxidation and obesity (Lee et al. 2016; Solverson et al. 2018). Obesity is a global health problem characterized by excessive fat accumulation in the

human body, it affects quality of life and increases overall mortality (Fontaine and Barofsky 2001). In overweight and obese adults, Lee et al. (2016) observed that 8 weeks daily intake of 2.5 g anthocyanin-rich black soybean extract (12.58 mg anthocyanins) decreased waist circumference, indicating a loss of abdominal fat. In addition, Solverson et al. (2018) observed in overweight and obese adults that 7-day daily intake of 600 g blackberry (1500 mg·day^{-1} flavonoids) decreased the 24 h respiratory exchange ratio, indicating a 7% increase in fat oxidation at rest, and increased exercise-induced fat oxidation by 12% during 30-min of treadmill walking.

Mechanisms for anthocyanin-induced fat oxidation were examined in rodent models. For example, Wu et al. (2013) observed in mice on a high-fat diet with anthocyanin-rich fruit juices (4.83 ml·day^{-1} blueberry and 4.56 ml·day^{-1} mulberry for 12 weeks) suppression of peroxisome proliferator-activated receptor γ (PPARγ) and fatty acid synthase expression and increase of carnitine palmitoyltransferase 1 expression. In addition, Benn et al. (2014) observed that mice fed with blackcurrant extract for 12 weeks had higher expression of PPARα, PPARδ, uncoupling protein 2 and 3 and mitochondrial transcription factor A. Mice fed with cyanidin and cyanidin-3-glucoside) for 12 weeks had enhanced adiponectin and leptin secretion and 5' adenosine monophosphate-activated protein kinase level (Tsuda et al. 2004).

In humans, the effect of anthocyanin-rich blackcurrant extract made from New Zealand grown berries on exercise-induced fat oxidation has been examined with 7-day intake. Cook et al. (2015) observed a 27% increase in fat oxidation in trained male cyclists during 10-min cycling at 65% of maximum oxygen uptake ($\dot{V}O_{2max}$) with 7-day intake of New Zealand blackcurrant (NZBC) extract (105 mg·day^{-1} anthocyanin). In another study, 7-day dose effects of NZBC extract were investigated in male trained cyclists and 22% and 24% increased fat

oxidation were observed during 120-min cycling at 65%$\dot{V}O_{2max}$ for 210 and 315 mg·day^{-1} anthocyanin intake, respectively (Cook et al. 2017). This study protocol was partly replicated in endurance-trained females by Strauss et al. (2018) and an increase of 27% was observed for exercise-induced fat oxidation with intake of 210 mg·day^{-1} anthocyanin for 7 days. However, it is not known whether longer intake of NZBC extract would enhance the metabolic and physiological responses during exercise. After 30-min rowing exercise, 5 weeks daily intake of ~ 240 mg New Zealand-derived blackcurrant extract enhanced the expression of immune factors and cellular anti-inflammatory/antioxidant responses, compared to an acute intake (Hurst et al. 2020). Intake duration studies could provide insight on the bioavailability of the anthocyanin-induced metabolites and the adaptive effects of intake of an anthocyanin-rich extract on exercise metabolism. In addition, intake duration studies could provide observations that will inform future dietary intake guidelines on anthocyanins. Kalt et al (2017) suggested that the handling of anthocyanin in humans changes between 7- and 14-day with daily intake of anthocyanin-rich blueberry juice.

Therefore, the aim of the present study was to examine effects of 7-day and 14-day intake of NZBC extract on physiological and metabolic responses during moderate intensity walking. It was hypothesized that 14-day intake would lead to different substrate oxidation during moderate intensity exercise than 7-day intake. Moderate intensity walking is a popular activity among the general population for weight management and health benefits (Jakicic et al. 2001). In addition, moderate intensity physical activities are recommended by the World Health Organization and American College of Sports Medicine for weight loss and improvement of health status.

Methods

Physically active healthy men (n=16, age: 24±6 years, body mass: 78±16 kg, height: 178±6 cm, BMI: 24.7±4.1 kg·m^{-2}, body fat: 15±5%) volunteered and provided written informed consent. Initially, 18 participants volunteered but the study had one drop-out due to moving away and one for an unknown reason. Participants were nonsmokers, not having known allergy to berries or berry products and advised not to take other supplementation during the study. A randomised, cross-over experimental design was used for and ethical approval was obtained from the University of Chichester Research Ethics Committee (ethical approval code: 1718_34) with protocols and procedures conformed to the 2013 Declaration of Helsinki. Participants had four morning laboratory visits. Before each visit, participants were instructed to refrain from caffeine and alcohol intake for 24 hours and abstain from strenuous exercise for 48 hours.

Preliminary visit

In the first visit, height, body mass, and body composition (Tanita BC418 Segmental Body Composition analyzer, Tanita, IL, USA) were measured. A food frequency questionnaire with anthocyanin-containing foods and drinks listed in the Phenol Explorer data-base (Neveu et al. 2010) was completed by participants to estimate daily anthocyanin intake (82±73 mg anthocyanins·day^{-1}). Participant's physical activity level was quantified with the short version of the International Physical Activity Questionnaire (Lee et al. 2001) (4385±1635 MET· week^{-1}).

After being seated in a chair for 10 min, participants were asked to lie horizontally on a massage table for resting measurements. Expired air was collected with Douglas bags, two times for 10 min, and the lowest oxygen uptake ($\dot{V}O_2$) was taken as the one-metabolic equivalent (1-MET: 3.95±0.64 ml·kg^{-1}·min^{-1}). Subsequently, participants performed an incremental walking

protocol on a treadmill (HP Cosmos Pulsar Bodycare Products UK) at speeds of 2, 3, 4, 5, and 6 $km \cdot hr^{-1}$, each speed for 8 minutes with a 2-min break between each speed. Expired air was collected in the last 3 min of each speed. The incremental walking test was performed to calculate the linear relationship between walking speed and oxygen uptake to determine the participant's speeds for moderate intensity exercise (i.e. 4- or 5 METs). Participants who felt more comfortable with jogging at the 5-MET treadmill speed were allowed to perform the 30-min treadmill walk (see below for details) at 4-MET in order to ensure that walking was for all participants the exercise modality of the study.

Visits for the 30-min moderate intensity walking exercise

Subsequently, participants visited the laboratory for 3 experimental conditions: baseline (no supplementation), 7-day intake of NZBC extract and 14-day intake of NZBC extract. For the 7-day and 14-day intake conditions, two capsules of NZBC extract (600 mg containing 210 mg of anthocyanins, i.e. 35–50% delphinidin-3-*O*-rutinoside, 5–20% delphinidin-3-*O*-glucoside, 30–45% cyanidin-3-*O*-rutinoside, 3–10% cyanidin-3-*O*-glucoside) (CurraNZ, Health Currancy Ltd., Surrey, UK) were consumed every morning with breakfast. Participants consumed the last 2 capsules two hours before the experimental visits and had one slice of bread and water 3 hours before the visits. A 14-day washout period was applied between each visit (Alvarez-Suarez et al. 2014). No side-effects from the intake of NZBC extract were noted. Also, participants recorded a 48 h food diary before the first experimental visit and were advised to replicate nutritional intake for the 48 h prior to the remaining two visits. Food diaries were analysed using Nutritics (Nutritics LTD., Dublin, Ireland) for carbohydrate, fat, protein, and total energy intake.

The experimental protocol consisted of 30 min of moderate intensity walking on the treadmill at a speed of 4 (n=3) or 5 (n=13) METs (speed: 5.7±0.7 $km \cdot hr^{-1}$). For each participant, the walking speed for the 30-min treadmill walk was the same in each condition. Expired air was collected from 7 to 10, 17 to 20 and 27 to 30 minutes. In addition, heart rate (Polar Vantage NV Polar Electro Oy Kempele Finland) and rating of perceived exertion (15-point Borg scale) were recorded for these three stages. Expired air was analyzed with a three-point calibrated Servomex gas analyser (Series 1400, Crowborough, UK) and volume measured (Harvard Apparatus Ltd. Dry gas meter). Gas volumes were calculated using the Haldane transformation and standardization to STPD conditions with consideration of inspired fractions of oxygen and carbon dioxide in the laboratory during expired air collection. Respiratory exchange ratio (i.e. RER) was calculated as the ratio between the carbon dioxide produced and oxygen consumed. Rates of whole-body fat and carbohydrate oxidation during walking were calculated with equations from Jeukendrup and Wallis (2005), respectively, with the assumption of negligible protein oxidation.

Statistical analyses were completed using Graphpad Prism 5 for Windows. Responses during moderate intensity walking exercise were measured at 7-10, 17-20 and 27-30 min and averaged over the time periods. Normality was checked with a D'Agostino and Pearson omnibus normality test. Physiological and metabolic parameters for the three experimental conditions (i.e. baseline, 7-day and 14-day) during moderate intensity walking exercise were analysed using a one-way repeated measures ANOVA with post-hoc student t-tests. For significant changes, Cohen's d effect sizes were calculated and considered trivial ($d<0.2$), small ($d=0.2$-0.39), moderate ($d=0.4$-0.69) and large ($d\geq0.7$), respectively. The sample size of 16 participants was higher or similar to previous studies with observations of effect of NZBC extract on exercise-

induced fat oxidation [Cook et al. 2015 (n=14); 2017 (n=15), Strauss et al. 2018 (n=16)]. Data are reported as mean±SD and 95% confidence intervals. Significance was accepted at $p<0.05$.

Results

Dietary and energy intake

Table 1 provides the dietary and total energy intake (mean±SD) 48 hr before each experimental visit. There were no differences between conditions for absolute intake of carbohydrate ($p=0.53$), fat ($p=0.80$), and protein ($p=0.25$) (carbohydrate: baseline 95% CI [372, 487 g], 7-days 95% CI [365, 488 g], 14-days 95% CI [364, 478 g]; fat: baseline 95% CI [131, 203 g], 7-days 95% CI [131, 204 g], 14-days 95% CI [128, 201 g]; protein: baseline 95% CI [203, 295 g], 7-days 95% CI [208, 291 g], 14-days 95% CI [197, 286 g]) and intake relative to body mass (carbohydrate: baseline 95% CI [4.74, 6.67 g·kg body mass^{-1}], 7-days 95% CI [4.66, 6.62 g·kg body mass^{-1}], 14-days 95% [4.64, 6.50 g·kg body mass^{-1}]; fat: baseline 95% CI [1.75, 2.52 g·kg body mass^{-1}], 7-days 95% CI [1.74, 2.53 g·kg body mass^{-1}], 14-days 95% [1.70, 2.51 g·kg body mass^{-1}]; protein: baseline 95% CI [2.62, 3.86 g·kg body mass^{-1}], 7-days 95% CI [2.70, 3.76 g·kg body mass^{-1}], 14-days 95% [2.55, 3.74 g·kg body mass^{-1}]). There were also no differences between conditions for total energy intake and the mean±SD of total energy intake are presented in Table 1.

Moderate intensity walking exercise: Physiological and metabolic responses

During 30-min of moderate intensity walking exercise, the 7-day and 14-day intake of NZBC extract had no effect on heart rate (baseline: 101±17, 95% CI [92, 111 beats·min^{-1}]; 7-day: 102±17, 95% CI [93, 111 beats·min^{-1}]; 14-day: 101±18, 95% CI [91, 111 beats·min^{-1}],

p=0.87), minute ventilation (baseline: 30.4±5.8, 95% [27.3, 33.5 L·min^{-1}]; 7-day: 30.8±6.3, 95% CI [27.4, 34.2 L·min^{-1}]; 14-day: 30.8±6.5, 95% CI [27.4, 34.3 L·min^{-1}], *p*=0.59), oxygen uptake (baseline: 1.50±0.30, 95% CI [1.33, 1.66 L·min^{-1}]; 7-day: 1.52±0.32, 95% CI [1.35, 1.69 L·min^{-1}]; 14-day: 1.51±0.34, 95% CI [1.33, 1.69 L·min^{-1}], *p*=0.57), carbon dioxide production (baseline: 1.28±0.29, 95% CI [1.13, 1.43 L·min^{-1}]; 7-day: 1.29±0.30, 95% CI [1.13, 1.45 L·min^{-1}]; 14-day: 1.27±0.30, 95% CI [1.11, 1.43 L·min^{-1}], *p*=0.64), and energy expenditure (baseline: 7.40±1.51, 95% CI [6.59, 8.20 kcal·min^{-1}]; 7-day: 7.51±1.59, 95% CI [6.66, 8.36 kcal·min^{-1}]; 14-day: 7.45±1.67, 95% CI [6.56, 8.34 kcal·min^{-1}], *p*=0.59).

Exercise-induced fat oxidation during moderate intensity walking exercise was increased with 7-day and 14-day NZBC intake by 11±19% (95% CI [0.0, 21%], d=0.24) and 17±26% (95% CI [2, 30%], d=0.38), respectively. Exercise-induced fat oxidation values for baseline, 7-days and 14-days were 0.36±0.12 g·min^{-1} (95% CI [0.30, 0.42 g·min^{-1}]), 0.39±0.13 (95% CI [0.32, 0.46 g·min^{-1}]), and 0.41±0.13 (95% CI [0.34, 0.48 g·min^{-1}]) (one-way ANOVA: *p*=0.007) (Fig. 1a). Compared to baseline, 11 (69%) and 13 (81%) of the participants had higher values of exercise-induced fat oxidation with 7-day and 14-day intake of NZBC extract, but the mean responses for fat oxidation at 7-day and 14-day were not different. However, ten participants (63%) had higher values of exercise-induced fat oxidation with 14-day intake compared to 7-day intake of NZBC extract.

Only 14-day intake of NZBC extract resulted in decreased exercise-induced carbohydrate oxidation by 9±13% (95% CI [-15, -1%], d=-0.27) (one-way ANOVA: *p*=0.032) (Fig. 1b), with 12 participants having lower values compared to baseline. The exercise-induced carbohydrate oxidation values for baseline, 7-days and 14-days were 0.95±0.40 (95% CI [0.74, 1.17 g·min^{-1}]), 0.91±0.40 (95% [0.70, 1.13 g·min^{-1}]), and 0.86±0.33 (95% CI [0.68, 1.03 g·min^{-1}]). In addition,

only 14-day intake of NZBC extract provided lower RER by 0.016 units (baseline: 0.852±0.046 (95% CI [0.827, 0.876], 7-day: 0.843±0.045 (95% CI [0.819, 0.867], 14-day: 0.837±0.037 (95% CI [0.817, 0.857], one-way ANOVA: p=0.019) (d=-0.35) (Fig. 1c) compared to baseline during moderate intensity walking exercise, with 12 participants (75%) having lower RER values at 14-days compared to baseline.

Rating of perceived exertion was lower with 7-day and 14-day NZBC intake (baseline: 11.0±2.3 (95% CI [9.9 to 12.2], 7-day: 10.5±1.8 (95% CI [9.6, 11.4], 14-day: 10.3±2.1 (95% CI [9.2, 11.3], one-way ANOVA: p=0.002) during moderate intensity walking exercise (Fig. 1d).

Discussion

This is the first study that examined the effects of intake duration of an anthocyanin-rich berry extract made from New Zealand blackcurrant on the physiological and metabolic responses during moderate-intensity exercise. The main findings from this study are:1) enhanced fat oxidation with 7- and 14-day intake of NZBC extract during 30-min of moderate intensity walking exercise with small effect size, 2) reduced perceived exertion with 7-day and 14-day intake of NZBC extract during 30-min of moderate intensity walking exercise, 3) NZBC extract is more effective to alter metabolic responses with 14-day intake than 7-day intake during 30-min moderate intensity walking exercise, as it lowered RER and carbohydrate oxidation, albeit with small effect size. The different metabolic responses with 14-day intake of NZBC extract during moderate intensity exercise compared to 7-day intake indicates that the effects of NZBC extract on metabolic responses are not just due to the final intake of NZBC extract on the day of testing. In other words, if the 6-days preloading (in the 7-day condition) and 13-days preloading (in the 14-day condition) was not able to cause any metabolic adaptation, then the metabolic

responses in both conditions would have been expected to be similar and due to the final intake of NZBC extract on the day of testing, but that was not the case.

In previous studies with 7-day intake of NZBC extract, exercise-induced fat oxidation was enhanced in endurance trained male and female cyclists with 10-min cycling bouts at different intensities (Cook et al. 2015), and 2 hr of cycling at 65%$\dot{V}O_{2max}$ (Cook et al. 2017; Strauss et al. 2018). Increasing fat oxidation during exercise may be a nutritional ergogenic strategy to affect exercise performance. However, the present study employed an exercise modality with a moderate exercise intensity (i.e. between 3 and 6 METs) that is used by the general population to obtain health benefits (Jakicic et al. 2001). Our observation of enhanced fat oxidation during 30-min moderate intensity walking exercise with 7-day and 14-day NZBC extract by 11% and 17%, respectively, may have applications for those that exercise to manage body weight and get health benefits. However, the implication of enhanced exercise-induced fat oxidation with intake of NZBC extract should not be overstated. In Cook et al. (2015), 7-days NZBC extract increased fat oxidation by 15%, 13% and 27% at 45%, 55% and 65%$\dot{V}O_{2max}$ during cycling with heart rates of 106±11, 118±13 and 132±15 beats·min^{-1}, respectively. In the present study, 7-days NZBC intake increased fat oxidation by 11% during moderate intensity walking exercise and a heart rate of 102±17 beats·min^{-1}. This may indicate that the effect of NZBC intake extract on exercise-induced fat oxidation may depend on the type of exercise. In support of this is the observation that running and cycling at the same relative intensity can have a different effect on fat oxidation according to a different muscle mass being involved in the exercise (Cheneviere et al. 2010). Future studies should examine whether there is a relationship between exercise intensity and exercise modality on the effects of NZBC extract on exercise-induced fat oxidation.

In general, optimal intake of supplements needs consideration of the timing, dose, and intake duration (Naderi et al. 2016). The optimal intake strategy for New Zealand blackcurrant extract to affect exercise-induced substrate oxidation is not known. Only a few studies examined the effects of intake duration of polyphenols on fat oxidation and body composition (Harada et al. 2005; Kobayashi et al. 2016; Urbina et al. 2017). High (593 mg·day^{-1}) and low (78 mg·day^{-1}) dose tea catechin beverages were consumed for 12-weeks by healthy males with observations of 8 hr postprandial dietary fat oxidation at 4, 8 and 12-weeks (Harada et al. 2005). Postprandial dietary fat oxidation increased continuously throughout the test period with the high dose of catechins and was the highest at the 12-week time-point. Moreover, Kobayashi et al. (2016) observed that intake for 12-weeks of catechins-enriched green tea beverage (280 mg·day^{-1}) decreased visceral, subcutaneous and total fat areas in moderately obese adults at 8 and 12-week intake compared to control with no differences between 8 and 12-week intake. In another study, capsaicinoid supplementation (2 and 4 mg·day^{-1}) had no effect on body composition after the 6 and 12-week intervention (Urbina et al. 2017). Future studies should address whether long intake duration (i.e. 3 months or longer) of effective short-term acting anthocyanin-rich products with known affect on exercise-induced fat oxidation can alter fat oxidation at rest, body composition and postprandial substrate oxidation. In addition, information is needed on the tissue availability of anthocyanins and the bioavailability of anthocyanin-derived metabolites, as it is likely that both can affect the long-term intake effects on exercise-induced metabolic responses.

In animal models, anthocyanin-rich foods increase fat oxidation via a combination of different mechanisms and are linked with gene, enzyme and transcription factor expressions that regulate energy and fat metabolism. In the present study, we used the indirect calorimetry method to quantify substrate oxidation and did not examine potential causal mechanisms for fat

and energy metabolism. However, Strauss at al. (2018) observed that 7-day intake of NZBC extract increased plasma non-esterified fatty acids and glycerol concentrations, at least at rest. Therefore, it is possible that the 14-day intake of anthocyanin-rich NZBC extract may have a higher effect on lipolysis than 7-day intake. It is possible that the present study had not enough power to show higher values for whole-body fat oxidation in the 14-day intake condition compared to the 7-day intake condition. However, it is likely though that the decrease in carbohydrate oxidation combined with a lowering of the RER in the 14-day condition was due to combined effects of an increase in lipolysis and metabolic handling of fatty acids in the muscle, reducing the contribution of carbohydrate oxidation in meeting the energy demands of the moderate intensity exercise. Future work is needed on even longer duration intake of NZBC extract combined with an analysis of hormonal responses and bioavailability of anthocyanin-derived plasma metabolites. It is worth noting also that substrate oxidation cannot be affected by bias, and a double-blind design was therefore not required. Because the present study was not double-blind, caution is required to interpret the RPE observations. However, it is interesting that the perceived exertion score during a self-motivated treadmill walk tended to be lower with intake of New Zealand blackcurrant juice in a double-blind design (Lomiwes et al. 2019).

A limitation of the present study was that participants recorded 48 h food diary before the first experimental visit and replicated for the following visits instead of having a standard similar diet a few days before experimental visit. Polyphenols may interact with other phytochemicals or nutrients synergistically or antagonistically and affect the bioavailability of anthocyanin-derived metabolites that are potential linked with adaptative mechanisms. For instance, Serra et al. (2010) observed that carbohydrate-rich food can repress the absorption of procyanidins. Therefore, it is possible that individual differences for physiological and metabolic responses to

the NZBC extract may be due to variations in their normal diet and affecting the bioavailability of anthocyanins and anthocyanin-derived metabolites. However, variation in responses due to intake of a nutritional supplement is common (Burke 2017). Another limitation is that the habitual physical activity was not controlled in the present study and an acute exercise bout may affect the translocation of gut-derived phenolics by flavonoid intake into the circulation (Nieman et al. 2018).

Conclusions

7-day and 14-day NZBC extract intake enhanced fat oxidation during 30-min of moderate intensity walking exercise. In addition, 14-day NZBC extract intake is more effective than 7-day intake for metabolic responses during 30-min moderate intensity walking exercise. Intake duration of anthocyanin-rich supplementation may affect the build-up of plasma and tissue anthocyanin-derived metabolites over time with gradual effect on the mechanisms that alter exercise-induced substrate oxidation. However, the intake duration of anthocyanin-rich blackcurrant that will maximize exercise-induced fat oxidation is not known.

Acknowledgement

Mehmet Akif Şahin was supported by the Scientific and Technological Research Council of Turkey to conduct the study at the University of Chichester (United Kingdom). Health Currancy (United Kingdom) Ltd and CurraNZ (New Zealand) Ltd provided supplementation.

Declaration of interest

Health Currancy (United Kingdom) Ltd and CurraNZ (New Zealand) Ltd provided supplementation. However, Health Currancy (United Kingdom) Ltd and CurraNZ (New Zealand) Ltd but had no role in any aspect of the study and manuscript.

References

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. https://doi.org/10.1016/j.jnutbio.2013.11.002

Apak R, Güçlü K, Özyürek M, Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem 52(26): 7970-7981. https://doi.org/10.1021/jf048741x

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(10):1019-1025. https://doi.org/10.1016/j.jnutbio.2014.05.008

Cheneviere X, Malatesta D, Gojanovic B, Borrani F (2010) Differences in whole-body fat oxidation kinetics between cycling and running. Eur J Appl Physiol 109(6):1037-1045. https://doi.org/10.1007/s00421-010-1443-5

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. https://doi.org/10.1007/s00421-015-3215-8

Cook MD, Myers SD, Gault ML, Edwards VC, Willems MET (2017) Dose effects of New Zealand blackcurrant on substrate oxidation and physiological responses during prolonged cycling. Eur J Appl Physiol 117(6):1207-1216. https://doi.org/10.1007/s00421-017-3607-z

Fontaine KR, Barofsky I (2001) Obesity and health-related quality of life. Obes Rev 2(3):173-182. https://doi.org/10.1046/j.1467-789x.2001.00032.x

Harada U, Chikama A, Saito S, Takase H, Nagap T, Tokimitsu I (2005) Effects of the long-term ingestion of tea catechins on energy expenditure and dietary fat oxidation in healthy subjects. J Health Sci 51(2):248-252. https://doi.org/10.1248/jhs.51.248

Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr 88(1):38-50. https://doi.org/10.1093/ajcn/88.1.38

Hurst RD, Lyall KA, Wells RW, Sawyer GM, Lomiwes D, Ngametua N, Hurst SM (2020) Daily Consumption of an Anthocyanin-Rich Extract Made From New Zealand Blackcurrants for 5 Weeks Supports Exercise Recovery Through the Management of Oxidative Stress and Inflammation: A Randomized Placebo Controlled Pilot Study. Front Nutr 7:16. https://doi.org/10.3389/fnut.2020.00016.

Jakicic JM, Clark K, Coleman E, Donnelly JE, Foreyt J, Melanson E, Volek J, Volpe SL (2001) American College of Sports Medicine Position stand. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 33(12):2145-2156. https://doi.org/10.1097/00005768-200112000-00026

Jennings A, Welch AA, Spector T, Macgregor A, Cassidy A (2013) Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. J Nutr 144(2):202-208. https://doi.org/10.3945/jn.113.184358

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. https://doi.org/10.1055/s-2004-830512

Kähkönen MP, Heinämäki J, Ollilainen V, Heinonen M (2003) Berry anthocyanins: isolation, identification and antioxidant activities. J Sci Food Agric 83(14):1403-1411. https://doi.org/10.1002/jsfa.1511

Kalt W, McDonald JE, Vinqvist-Tymchuk MR, Liu Y, Fillmore SAE (2017) Human anthocyanin bioavailability: effect of intake duration and dosing. Food Funct 8(12):4563-4569. https://doi.org/10.1039/c7fo01074e

Kobayashi M, Kawano T, Ukawa Y, Sagesaka UM, Fukuhara I (2016) Green tea beverages enriched with catechins with a galloyl moiety reduce body fat in moderately obese adults: a randomized double-blind placebo-controlled trial. Food Funct 7(1):498-507. https://doi.org/10.1039/c5fo00750j

Lee PH, Macfarlane DJ, Lam TH, Stewart SM (2011) Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): a systematic review. Int J Behav Nutr Phys Act 8, 115. https://doi.org/10.1186/1479-5868-8-115

Lee M, Sorn SR, Park Y, Park HK (2016) Anthocyanin rich-black soybean testa improved visceral fat and plasma lipid profiles in overweight/obese korean adults: A randomized controlled trial. J Med Food 19(11):995-1003. https://doi.org/10.1089/jmf.2016.3762

Lin BW, Gong CC, Song HF, Cui YY (2017) Effects of anthocyanins on the prevention and treatment of cancer. Br J Pharmacol 174(11):1226-1243. https://doi.org/10.1111/bph.13627

Lomiwes D, Ha B, Ngametua N, Burr NS, Cooney JM, Trower TM, Sawyer G, Hedderley D, Hurst RD, Hurst SM (2019) Timed consumption of a New Zealand blackcurrant juice support

positive affective responses during a self-motivated moderate walking exercise in healthy sedentary adults. J Int Soc Sports Nutr 16(1):33. https://doi.org/10.1186/s12970-019-0300-0.

Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79(5):727-747. https://doi.org/10.1093/ajcn/79.5.727

Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr (2007) Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 85(3):895-909. https://doi.org/10.1093/ajcn/85.3.895

Naderi A, De Oliveira EP, Ziegenfuss TN, Willems MET (2016) Timing, optimal dose and intake duration of dietary supplements with evidence-based use in sports nutrition. J Exerc Nutrition Biochem 20(4):1-12. https://doi.org/10.20463/jenb.2016.0031

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) 2010:bap024. https://doi.org/ 10.1093/database/bap024

Nieman DC, Kay CD, Rathore AS, Grace MH, Strauch RC, Stephan EH, Sakaguchi CA, Lila MA (2018) Increased Plasma Levels of Gut-Derived Phenolics Linked to Walking and Running Following Two Weeks of Flavonoid Supplementation. Nutrients 10(11):1718. https://doi.org/10.3390/nu10111718.Scalbert A, Johnson IT, Saltmarsh M (2005) Polyphenols: antioxidants and beyond. Am J Clin Nutr 81(1 Suppl):215S-217S. https://doi.org/10.1093/ajcn/81.1.215S

Serra A, Macià A, Romero MP, Valles J, Bladé C, Arola L, Motilva MJ (2010) Bioavailability of procyanidin dimers and trimers and matrix food effects in in vitro and in vivo models. Br J Nutr 103(7):944-952. https://doi.org/10.1017/S0007114509992741

Solverson P, Rumpler W, Leger J, Redan BW, Ferruzzi MG, Baer DJ, Castonguay TW, Novotny JA (2018) Blackberry feeding increases fat oxidation and improves insulin sensitivity in overweight and obese males. Nutrients 10(8):pii E1048. 10.3390/nu10081048

Strauss JA, Willems MET, Shepherd SO (2018) New Zealand blackcurrant extract enhances fat oxidation during prolonged cycling in endurance-trained females. Eur J Appl Physiol 118(6):1265-1272. https://doi.org/10.1007/s00421-018-3858-3

Tsuda T, Ueno Y, Aoki H, Koda T, Horio F, Takahashi N, Kawada T, Osawa T (2004) Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. Biochem Biophys Res Commun 316(1):149-157. https://doi.org/10.1016/j.bbrc.2004.02.031

Urbina SL, Roberts MD, Kephart WC, Villa KB, Santos EN, Olivencia AM, Bennett HM, Lara MD, Foster CC, Prupura M, Jäger R, Taylor LW, Wilborn CD (2017) Effects of twelve weeks of capsaicinoid supplementation on body composition, appetite and self-reported caloric intake in overweight individuals. Appetite, 113:264-273. https://doi.org/ 10.1016/j.appet.2017.02.025

Wu T, Tang Q, Gao Z, Yu Z, Song H, Zheng X, Chen W (2013). Blueberry and mulberry juice prevent obesity development in C57BL/6 mice. PLoS One, 8(10):e77585. https://doi.org/ 10.1371/journal.pone.0077585

Yang B, Kortesniemi M (2015) Clinical evidence on potential health benefits of berries. Curr Opin Food Sci, 2:36-42. https://doi.org/10.1016/j.cofs.2015.01.002

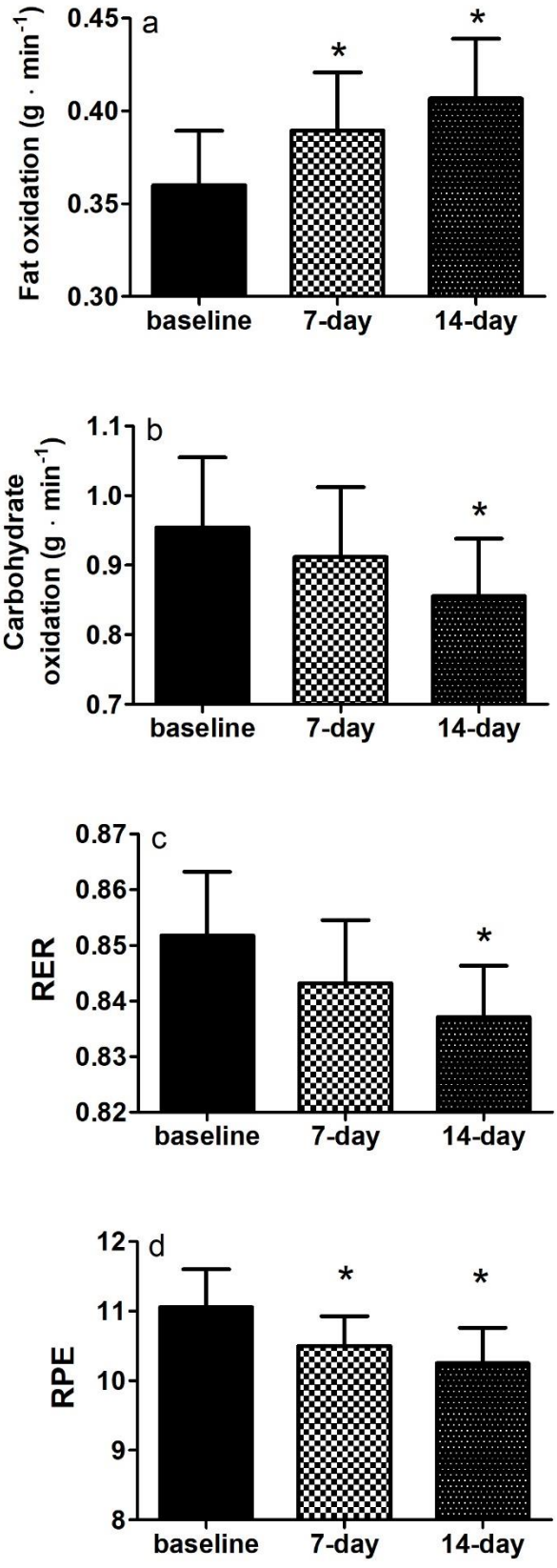
a
Fat oxidation (g · min-1)
0.45
0.40
0.35
0.30
*
*
baseline
7-day
14-day
b
Carbohydrate oxidation (g · min-1)
1.1
1.0
0.9
0.8
0.7
*
baseline
7-day
14-day
c
RER
0.87
0.86
0.85
0.84
0.83
0.82
*
baseline
7-day
14-day
d
RPE
12
11
10
9
8
*
*
baseline
7-day
14-day

Figure 1. Fat oxidation (a), carbohydrate oxidation (b), respiratory exchange ratio (c), and rating of perceived exertion (d) during 30-min of moderate intensity walking exercise. Data reported as mean ± SD from 16 participants. 7-day and 14-day refers to the intake duration of New Zealand blackcurrant extract. RER, respiratory exchange ratio; RPE, rating of perceived exertion. * indicates different from baseline ($p<0.05$).

Table 1. Dietary intake 48 h before each experimental visit of 30-min moderate intensity exercise. NZBC, New Zealand blackcurrant.

	Baseline	7-day NZBC extract intake	14-day NZBC extract intake
Carbohydrate (g)	430 ± 107	426 ± 116	421 ± 107
(g · kg body mass^{-1})	5.7 ± 1.8	5.6 ± 1.8	5.6 ± 1.8
Fat (g)	167 ± 67	167 ± 69	164 ± 69
(g · kg body mass^{-1})	2.1 ± 0.7	2.1 ± 0.7	2.1 ± 0.8
Protein (g)	249 ± 86	249 ± 78	241 ± 84
(g · kg body mass^{-1})	3.2 ± 1.2	3.2 ± 1.0	3.1 ± 1.1
Total energy intake (kcal)	4210 ± 928	4207 ± 992	4091 ± 974
(kcal · kg body mass^{-1})	55 ± 13	55 ± 13	53 ± 13
Total energy intake (kJ)	17615 ± 3882	17602±4150	17117 ± 4076
(kJ · kg body mass^{-1})	230 ± 53	229±55	223 ± 53