# Title: Daily and not every-other-day intake of anthocyanin-rich New Zealand blackcurrant extract alters substrate oxidation during moderate-intensity walking in adult males

Running title: Blackcurrant and dosing strategy

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Abstract

Daily intake of anthocyanin-rich New Zealand blackcurrant (NZBC) extract can enhance exercise-induced fat oxidation. It is not known whether habitual dietary anthocyanin intake and body composition affects blackcurrant-induced fat oxidation or if daily intake is required. We examined effects of daily and every-other-day intake of NZBC extract on metabolic and physiological responses during moderate-intensity walking. Sixteen physically active males (age: 24±6 yr, body mass: 78±16 kg, BMI: 24.7±4.2 kg·m-2, body fat: 15.2±5.0%) volunteered. A randomised, cross-over design with a control condition was used and habitual dietary anthocyanin intake quantified. For intake conditions, participants consumed 2 capsules of NZBC extract (210 mg of anthocyanins) daily or every-other-day for 14 days (14-D and 14-EOD) with 14-days washout. Final two capsules were taken 2-hr before the walk (speed: 5.7±0.7 km·hr-1). There was a trend for lower respiratory exchange ratio and carbohydrate oxidation with changes only for 14-D. Fat oxidation was increased only for 14-D (P<0.05) with 50% of the participants more than a 10% change. In 14-D, there was a positive correlation for BMI and body fat % with the absolute change in fat oxidation but not with dietary anthocyanin intake. Daily intake of NZBC extract is required to enhance exercise-induced fat oxidation. Enhanced exercise-induced fat oxidation by daily intake of NZBC extract is related to body composition but not to habitual dietary anthocyanin intake in physically active males. Daily anthocyanin intake seems to allow the gradual build-up and maintenance of anthocyanin-derived metabolites required to alter mechanisms for exercise-induced substrate oxidation.

**Key words**: blackcurrant; dosing strategy; substrate oxidation; exercise; metabolic equivalent

**Introduction**

The dark purple blackcurrant berries are rich in color-giving anthocyanins (Neveu et al. 2010). Anthocyanins are phytochemicals and part of the flavonoid family, a subclass of polyphenols. The anthocyanin composition of blackcurrant consists primarily of delphinidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, and cyanidin-3-*O*-glucoside (Overall et al. 2017). In berry plants and bushes, color-providing anthocyanins are secondary metabolites and function also to offer protection from oxidative stress and pathogens (Manach et al. 2004). In humans, regular anthocyanin intake can provide substantial health benefits (Li et al. 2017; Zafra-Stone et al. 2007). Higher anthocyanin intake was associated with lower insulin resistance, lower inflammation, reduced arterial stiffness, reduced blood pressure, and lower risk for cardiovascular disease and type 2 diabetes as the intake may have improved endothelial function, blood flow, and is linked with the anthocyanin-modulating effects on the antioxidant defense system (Cassidy et al. 2016; Jacques et al. 2013; Jennings et al. 2014; Mink et al. 2007). When epidemiological studies provide quantitative information on anthocyanin-induced health benefits (Cassidy et al. 2016), it is assumed that the benefits are obtained with daily intake, in line with dietary guidelines of daily intake for other nutrients.

Studies have also shown beneficial effects of long-term anthocyanin intake on body weight and body composition and possibly linked with an enhancement of fat oxidation. For example, in overweight and obese Korean adults, Lee et al. (2016) observed lower abdominal fat, indicated by lower waist circumference (1.9 cm) and hip circumference (1.3 cm) after 8 weeks daily intake of 2.5 g anthocyanin-rich black soybean extract (12.58 mg anthocyanins). Solverson et al. (2018) observed that 7-day daily intake of 600 g blackberry (361 mg·day-1 anthocyanins) decreased the 24 h respiratory exchange ratio by 0.007 units, indicating a 7% increase in fat oxidation at rest. In addition, exercise-induced fat oxidation by treadmill walking was enhanced by 12% in overweight and obese males (Solverson et al. 2018). Observations in animals and animal tissues have also supported an effect of anthocyanins on fat oxidation. Treatment of isolated rat adipocytes for 24 hr with cyanidin-3-*O*-glucoside, an anthocyanin present in blackcurrant, enhanced the release of adiponectin and leptin which can have implications for the prevention of obesity (Tsuda et al. 2004). Anthocyanin-rich fruit juice (i.e. blueberry and mulberry) in mice fed a high-fat diet inhibited body weight gain, attenuated lipid accumulation and enhanced the expression of the mRNA for carnitine palmitoyltransferase l, involved in translocation of fatty acids into the mitochondria (Wu et al. 2013).

In humans, the enhancement of whole-body fat oxidation by nutritional ergogenic aids may be beneficial for weight management. Enhanced whole-body fat oxidation has been observed after 7-days daily intake of anthocyanin-rich New Zealand blackcurrant (NZBC) extract intake. For example, Cook et al. (2015) observed in trained male cyclists a 27% increase in fat oxidation during 10-min of ergometer cycling at 65%*V̇*O2max with 7-day daily intake of NZBC extract (105 mg·day-1 anthocyanin). In addition, during 120-min of cycling at 65%*V̇*O2max, fat oxidation was enhanced by 22% in males and 27% in females with 7-day daily NZBC extract (210 mg·day-1 anthocyanins) (Cook et al. 2017; Strauss et al. 2018). However, it was not clear in these studies whether daily intake was required to enhance exercise-induced fat oxidation (Cook et al. 2015, 2017; Strauss et al. 2018). Although the mechanisms for enhanced exercise-induced fat oxidation by anthocyanin intake have not been established, a causal link with the bioavailability of anthocyanins and anthocyanin-derived metabolites is likely. Czank et al. (2013) and De Ferrars et al. (2014) observed that anthocyanin metabolites are still present in plasma up to 48-h and 96 hr, respectively, after ingestion of the blackcurrant anthocyanin cyanidin-3-*O*-glucoside. Therefore, intake of anthocyanins every-other-day may still accumulate anthocyanin-derived metabolites in plasma over time and result in adaptations responsible for changes in exercise-induced substrate oxidation.

Whole-body resting fat oxidation has been correlated with body mass index after 4-weeks intake of capsinoids (Inoue et al. 2007). The relationship between body mass index, and body fat, and potential to enhance exercise-induced fat oxidation by blackcurrant intake has not been examined. In addition, many studies implemented a wash-out dietary strategy that would lower the intake of polyphenols before dosing (Bell et al. 2015; Czank et al. 2013). The wash-out dietary strategy before testing of supplement-induced responses seems to suggest a potential blunting of habitual dietary intake of polyphenols on those responses. It is not known whether habitual dietary intake of anthocyanins can affect the responses to an anthocyanin-rich supplement.

Therefore, the primary aim of the present study was to examine the effects of daily intake and every-other-day intake of anthocyanin-rich New Zealand blackcurrant extract on the physiological and metabolic responses during moderate-intensity exercise. The secondary aim was to examine whether body mass index, body fat percentage and habitual dietary anthocyanin intake was related to the blackcurrant-induced response on fat oxidation during exercise.

**Materials and methods**

**Participants**

Sixteen recreationally active healthy adult Caucasian men volunteered [age: 24±6 years, height: 178±6 cm, body mass: 78±16 kg, BMI: 24.7±4.1 kg·m-2 (range: 19.5 to 38.1 kg·m-2, 8 normal weight, 7 overweight and 1 severely obese), body fat: 15±5% (range: 9.9 to 27.3%)] and provided written informed consent. Participant’s physical activity level was quantified with the short version of the International Physical Activity Questionnaire (Craig et al. 2003), and all had a high score (IPAQ score: 4385±1635 MET·week-1). Participants had no known allergy to berries or berry products, were non-smokers, and were not allowed to take other nutritional ergogenic aids during the study. A randomised ([www.random.org](http://www.random.org) using sequences), repeated measures, cross-over experimental design with a control condition was used. Ethical approval was obtained from the University of Chichester Research Ethics Committee with protocols and procedures conformed to the 2013 Declaration of Helsinki.

**Study design**

Participants attended the laboratory for four morning visits. In the first visit, height, body mass, and body fat % (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 database (Neveu et al. 2010) was completed to estimate daily anthocyanin intake (82±73 mg·day-1) (range: 0.1 to 206.8 mg·day-1). Before each visit, participants were instructed to refrain from the intake of alcohol and caffeine for 24 hours and abstain from strenuous exercise for 48 hours.

**Standardization of moderate-intensity walking**

After arrival for the first visit, participants were seated in a chair for 10 min, and subsequently required to lie supine on an examination couch for collection of expired air. Expired air was collected two times for 10 min with Douglas bags and the lowest oxygen uptake (see below for analysis of expired air) was taken to represent the one-metabolic equivalent (1-MET). The 1-MET was 3.95±0.64 ml·kg-1·min-1 (95% CI [3.61, 4.92]). Subsequently, the participants performed an incremental walking test on a treadmill (HP Cosmos Pulsar Bodycare Products, UK) with walking speeds of 2, 3, 4, 5, and 6 km·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 for each participant the linear relationship between walking speed and oxygen uptake to determine the participant’s moderate-intensity walking speeds (i.e. the walking speed at 4- or 5- METs). The moderate-intensity walking speed for three participants was at 4-MET because those participants indicated that the treadmill speed at 5-MET would require jogging.

**Experimental protocols**

Participants visited the laboratory for three experimental conditions: control (no supplementation), 14-day daily intake of NZBC extract (14-D) and 14-day every-other-day intake of NZBC extract (14-EOD). For the supplementation conditions (i.e. 14-D and 14-EOD), participants consumed 2 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) (Health Currancy Ltd., Surrey, UK; CurraNZ Ltd, NZ) with breakfast daily for 14 days or every-other-day for 14 days. On the morning of the final intake in both 14-D and 14-EOD conditions, participants ingested the last two capsules 2 hours before the visits and had one slice of bread and water 3 hours before the visits. The dose of 600 mg NZBC extract was based on dose-response observations of whole-body exercise-induced fat oxidation in males by Cook et al (2017). A 14-day washout period was applied between each visit (Alvarez-Suarez et al. 2014). No supplement was provided for the control condition and between the supplement days in the 14-EOD condition (Figure 1). Participants recorded a 48 hr food diary before the first experimental visit and were advised to replicate the 48 hr dietary intake prior to the remaining two visits. Food diaries were analysed using Nutritics (Nutritics LTD., Dublin, Ireland) for carbohydrate, fat, protein, and total energy intake. There were no differences in absolute or relative values per kilogram of body mass for carbohydrate, fat, protein, or total energy intake between the experimental conditions (p>0.05) (Table 1).

For the 30-min moderate-intensity walk, participants walked on the treadmill at a speed of 4 (n=3) or 5 (n=13) METs (speed: 5.7±0.7 km·hr-1). Expired air was collected from 7 to 10, 17 to 20 and 27 to 30 minutes. 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, UK). Gas volumes were calculated using the Haldane transformation and standardized to STPD conditions with consideration of inspired fractions of oxygen and carbon dioxide within 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 were calculated with equations (1) and (2) from Jeukendrup and Wallis (2005) and the assumption of negligible protein oxidation.

(1)

(2)

**Statistical analysis**

Statistical analysis was completed using Graphpad Prism 5 for Windows (GraphPad Software, San Diego California USA). Responses during moderate-intensity walking were measured at 7-10, 17-20 and 27-30 min and averaged. Normality was tested with the Shapiro-Wilks normality test. Physiological and metabolic parameters for the three conditions (i.e. control, 14-D, and 14-EOD) were analysed using one-way repeated measures ANOVA with post-hoc Tukey’s multiple comparison test. Pearson correlation coefficients were calculated and tested for relationships between body mass index, body fat % and habitual dietary anthocyanin intake (not considering supplementation) and changes of exercise-induced fat oxidation in the 14-D condition. All data are reported as mean±SD umless stated otherwise and significance was accepted at P<0.05. When significance was obtained, Cohen’s d effect sizes were calculated (small: 0.2≤d<0.5; moderate: 0.5≤d≤0.79; large: d≥0.8). A power analysis for sample size indicated recruitment of 13 to allow a detection of a 15% increase in fat oxidation from a normal value of fat oxidation of 0.24 g·min-1 (Dasilva et al. 2011) with high statistical power (1−β = 0.80: 0.05 = α level). Our sample size of 16 was comparable to studies with blackcurrant effects on exercise-induced fat oxidation (e.g. Cook et al. 2015: n=14; Strauss et al. 2018: n=16).

**Results**

**Intensity of the 30-min treadmill walk and physiological responses**

For the treadmill walking conditions, there were no differences in oxygen uptake (control: 1.50±0.30 L·min-1, 95% CI [1.33, 1.66]; 14-D: 1.51±0.34 L·min-1, 95% CI [1.33, 1.70]; 14-EOD: 1.52±0.32 L·min-1, 95% CI [1.35, 1.69]; one-way ANOVA: P=0.49). As a consequence, the exercise intensity expressed as a MET value for the 30-min treadmill walk was similar for the experimental conditions (control: 5.2±0.7, 95% CI [4.8, 5.5]; 14-D: 5.2±0.8, 95% CI [4.8, 5.6]; 14-EOD: 5.3±0.7, 95% CI [4.9, 5.7]; Kruskal-Wallis test: P=0.67), indicating the planned matched moderate-intensity for the 30-min treadmill walk.

During the 30-min of moderate-intensity treadmill walking, there were no effects for daily and every-other-day intake of New Zealand blackcurrant extract on heart rate (control: 101±17 beats·min-1, 95% CI [92, 111]; 14-D: 101±18 beats·min-1, 95% CI [91, 111]; 14-EOD: 101±18 beats·min-1, 95% CI [91, 111]; P=0.93), minute ventilation (control: 30.4±5.8 L·min-1, 95% CI [27.3, 33.5], 14-D: 30.8±6.5 L·min-1, 95% CI [27.4, 34.3]; 14-EOD: 31.2±6.1 L·min-1, 95% CI [28.0, 34.5]; P=0.21), and carbon dioxide production (control: 1.28±0.29 L·min-1, 95% CI [1.13, 1.43]; 14-D: 1.27±0.30 L·min-1, 95% CI [1.11, 1.43]; 14-EOD: 1.29±0.29 L·min-1, 95% CI [1.14, 1.45]; P=0.41).

**Metabolic responses and energy expenditure during the 30-min treadmill walk**

Whole-body fat oxidation during the 30-min treadmill walk was only enhanced for the 14-day daily intake condition (Fig 2A) (control: 95% CI [0.30, 0.42 g·min-1]; 14-D: 95% [0.34, 0.48 g·min-1]; 14-EOD: 95% CI [0.32, 0.43 g·min-1], one-way ANOVA: P=0.04) with an increase of 17±26% (post-hoc, P<0.05), albeit with moderate effect size (d=0.40). Thirteen out of the 16 participants (~81%) had higher whole-body fat oxidation during walking with 14-day daily intake of NZBC extract, with 8 of those more than 10% change. In addition, there was a significant correlation between body mass index and body fat % and the absolute change in whole-body fat oxidation (r2=0.3968, P=0.009 and r2=0.5662, P<0.001, respectively), but not between habitual dietary anthocyanin intake and the absolute change in whole-body fat oxidation (r2=0.0010, P=0.91) (Fig. 3ABC). The habitual dietary intake of anthocyanins did not include anthocyanins obtained from NZBC extract supplementation.

For whole-body carbohydrate oxidation, there was a trend for a difference between the experimental conditions (Fig. 2B) (control: 95% CI [0.74, 1.17 g·min-1]; 14-D: 95% [0.68, 1.03 g·min-1]; 14-EOD: 95% CI [0.68, 1.03 g·min-1], one-way ANOVA: P=0.069) with only 14-day daily intake having decreased carbohydrate oxidation of 8±13% (post-hoc, 14-D: P<0.05). Twelve out of 16 participants (75%) had lower carbohydrate oxidation during moderate-intensity treadmill walking with 14-day daily intake of NZBC extract.

There was a trend for RER to be different between the experimental conditions (Fig. 2C) (control: 95% CI [0.827, 0.876]; 14-D: 95% [0.817, 0.857]; 14-EOD: 95% CI [0.831, 0.868], one-way ANOVA, P=0.064) with only the 14-day daily intake 0.015 lower units (post-hoc, 14-D: P<0.05). Twelve out of 16 participants (75%) had lower RER values during moderate-intensity treadmill walking with 14-day daily intake of NZBC extract.

During the 30-min of moderate-intensity walking, there was no effect of daily and every-other-day intake of New Zealand blackcurrant extract on energy expenditure (Fig. 2D) (control: 95% CI [6.59, 8.20 kcal·min-1]; 14-D: 95% CI [6.56, 8.34 kcal·min-1]; 14-EOD: 95% CI [6.67; 8.35 kcal·min-1]; one-way ANOVA, P=0.54).

**Rating of perceived exertion during the 30-min treadmill walk**

Rating of perceived exertion was lower with 14-day daily and every-other-day intake of NZBC extract during moderate-intensity treadmill walking (control: 11.1±2.2, 95% CI [9.9, 12.2]; 14-D: 10.3±2.0, 95% CI [9.2, 11.3]; 14-EOD: 10.5±2.0, 95% CI [9.4, 11.6] (ANOVA: P=0.002) (post-hoc, 14-D and14-EOD both P<0.05).

**Discussion**

This is the first study that addressed whether every-other-day intake of an anthocyanin-rich supplement was able to be effective on physiological and metabolic responses during moderate-intensity walking compared to daily intake. The rationale for examining intake every-other-day was based on the observations of bioavailability of anthocyanin-derived metabolites (Czank et al. 2013; De Ferrars et al. 2014). Czank et al. (2013) provided the evidence in humans that anthocyanin-derived metabolites by intake of cyanidin-3-glucoside, an anthocyanin present in blackcurrant, were detectable up to 48 hrs. In addition, De Ferrars et al. (2014) showed that some metabolites (e.g. ferulic acid) had half-lives up to 96 hr. Interestingly, dietary intake of ferulic acid has been suggested to be useful for obesity and obesity related diseases (Wang et al. 2019), and could reduce adipocyte tissue mass (Koh et al. 2017). However, ferulic acid is just one of many cyanidin-3-*O*-glucoside derived metabolites (De Ferrars et al. 2014). The maximal concentration or continued presence of the anthocyanin-derived metabolites play likely a decisive role to alter the mechanisms responsible for the changes in exercise-induced substrate oxidation. In the present study, intake every-other-day of NZBC extract (i.e. 7 doses over a period of two weeks) was not effective. This may suggest that the potential stimulus of anthocyanin-derived metabolites when NZBC extract was taken every-other-day was not sufficient to alter mechanisms involved with changing exercise-induced substrate oxidation. A limitation of the present study, however, was the absence of observations on anthocyanin-derived metabolites. In addition, because the final dose of NZBC extract, in both conditions (i.e. daily and every-other-day) was taken 2 hr before testing, the absence of an effect in the every-other-day condition suggests that the final dose of 210 mg of blackcurrant anthocyanins does not seem to contribute to the 14-day daily intake effects. Furthermore, the absence of enhanced whole-body fat oxidation in the 14-every-other-day condition indicates that there is no acute effect of NZBC extract. The principal findings from the present study are (1) daily NZBC extract intake is required to enhance exercise-induced fat oxidation, decrease carbohydrate oxidation, and lower RER during moderate-intensity walking, (2) the enhanced fat oxidation during moderate-intensity walking in adult males by daily intake of NZBC extract was related to body mass index and body fat % but not to habitual dietary intake of anthocyanins. It needs to be noted that no supplementation was taken for the control condition, but we are not aware of fat and carbohydrate oxidation being affected intake of placebo supplementation. However, it is possible that the absence of a placebo condition affected the responses of the participants for the rating of perceived exertion during the 30-min walk.

Seven-day daily intake of NZBC extract (i.e. 7 doses) enhanced exercise-induced fat oxidation in endurance trained males by 27% during 10-min of cycling at 65% of *V̇*O2max (Cook et al. 2015) and by 22% and 24% during 2-hr of cycling at 65%*V̇*O2max in endurance trained males (Cook et al. 2017) and females (Strauss et al. 2018), respectively. The observations in the present study confirms an enhancement of fat oxidation by 17% during moderate-intensity walking in recreationally active males with 14-day daily intake of NZBC extract (i.e. 14 doses over a period of two weeks). Regular moderate-intensity walking is recommended to obtain health benefits as it can change body composition. A recent systematic review on polyphenols and physical activity on body weight and fat did not contain studies with intake of anthocyanin-rich supplements (Llaha et al. 2020). Future studies should examine long-term intake of anthocyanin-rich blackcurrant on body composition, in addition to whether fat oxidation at rest can be changed by intake of NZBC extract.

In the present study, the relationship between BMI and body fat % and changes in exercise-induced fat oxidation indicates a potential larger benefit of daily intake of NZBC extract for those individuals with unfavourable body composition. This may suggest that the sensitivity of adipose tissue to respond to anthocyanin and anthocyanin-derived metabolites differs between individuals. It is also possible that interindividual differences exist for anthocyanin-derived metabolites due to the role of gut microbiota in metabolism of anthocyanins (Eker et al. 2020). Nevertheless, future work should address the potential of intake of NZBC extract in participants with a much a broader range of BMI and body fat % values, as our convenience sample only included one participant in the obese category.

Epidemiological studies have quantified the anthocyanin dietary intake in the general population and the importance of anthocyanin intake to obtain health benefits is recognized (Cassidy et al. 2011). Habitual dietary anthocyanin intake, however, did not affect the changes in exercise-induced fat oxidation by daily intake of blackcurrant anthocyanins. This seems to suggest that having a low habitual dietary anthocyanin intake makes individuals not more responsive to anthocyanin-rich blackcurrant extract.

**Conclusions**

It was concluded that 14-days daily intake of anthocyanin-rich New Zealand blackcurrant extract intake and not every-other-day in 14-days is required for enhancing fat oxidation during moderate-intensity walking. Our observations also indicate that there is no acute effect of anthocyanin-rich New Zealand blackcurrant extract intake on fat oxidation during moderate-intensity walking. Daily intake of anthocyanin-rich New Zealand blackcurrant extract will probably increase the bioavailability of anthocyanin-derived metabolites that are required to alter the mechanisms responsible for metabolic responses during moderate-intensity exercise.

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**Declaration of interest**

Health Currancy (UK) Ltd and CurraNZ (NZ) Ltd provided supplementation. However, Health Currancy (UK) Ltd and CurraNZ (NZ) Ltd but had no role in any aspect of the study and manuscript.

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**Figure legends**

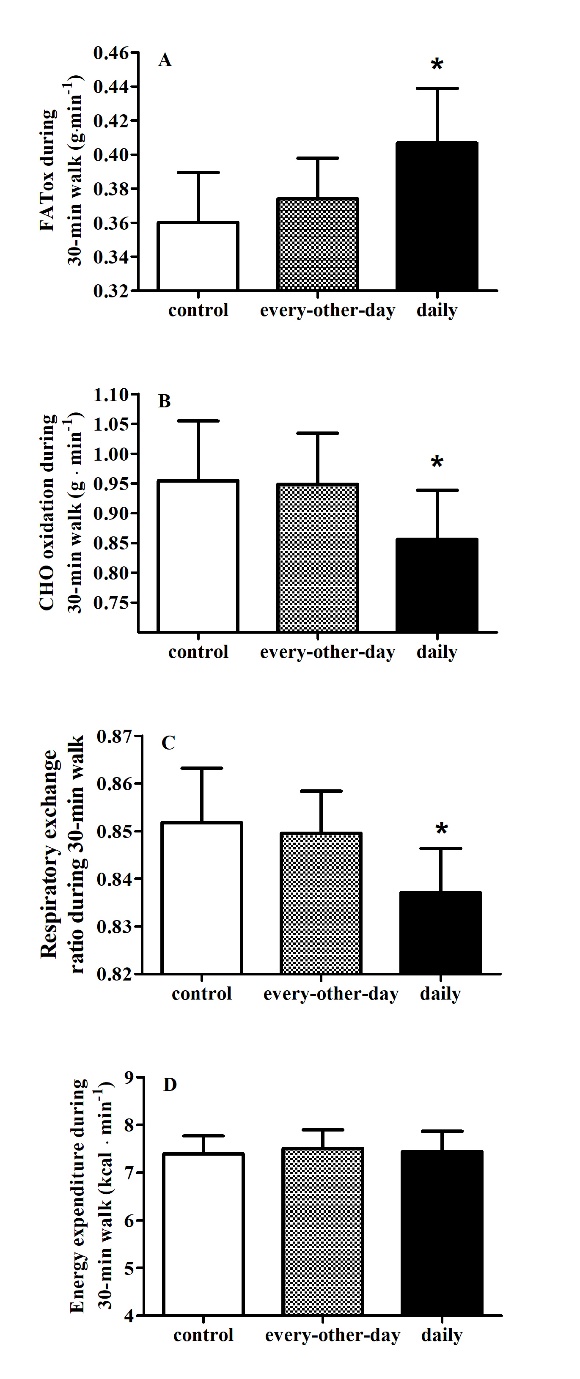
Figure 1. Schematic diagram of the randomized, cross-over study design with a control condition. NZBC indicates dosing with 600 mg of New Zealand blackcurrant extract (i.e. 210 mg of anthocyanins). 14-EOD indicates dosing every other day for two weeks. 14-D indicates dosing every day for two weeks. On testing day, the dose was taken two hours before the moderate-intensity 30-min walk.

Figure 2. Fat oxidation (A), carbohydrate oxidation (B), respiratory exchange ratio (C), and energy expenditure (D) during 30-min of moderate-intensity walking. Data reported as mean ± SEM from 16 participants. Every-other-day and daily refers to the intake of New Zealand blackcurrant extract over a two-week period. \* indicates different from control (*p*<0.05).

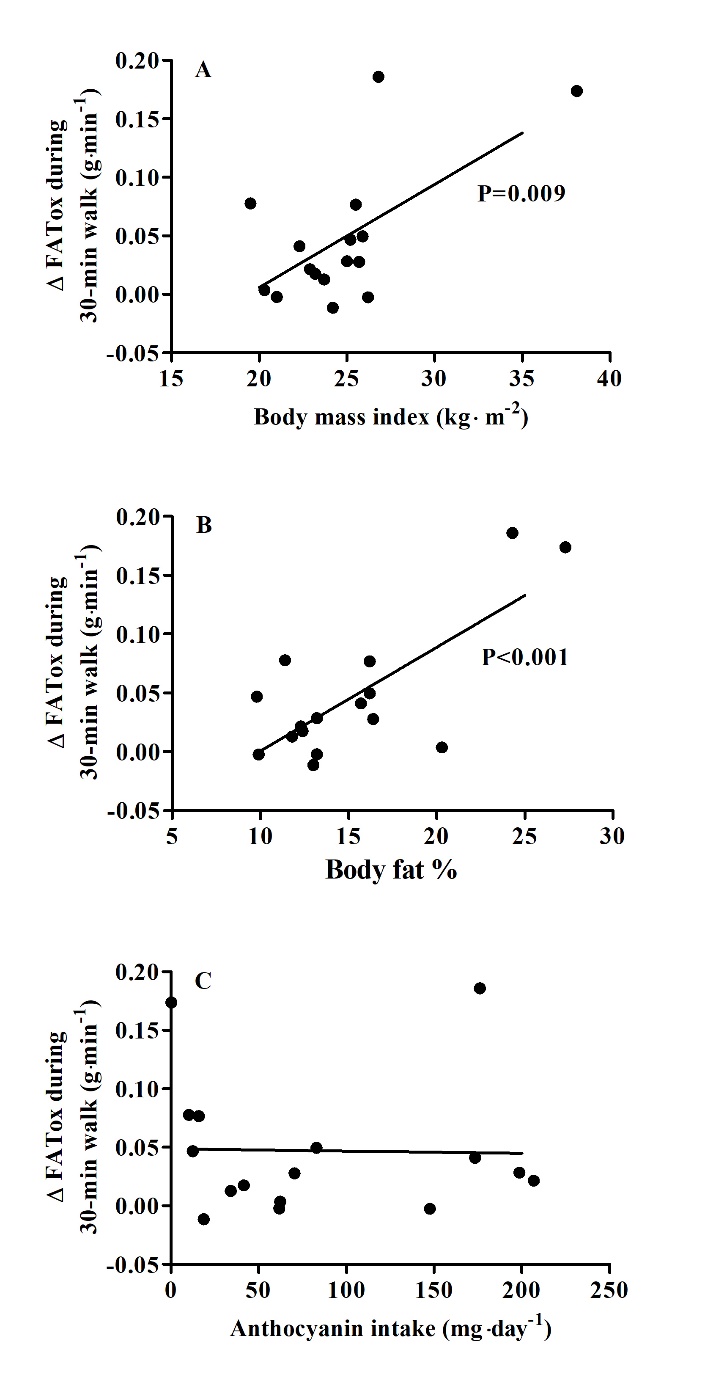
Figure 3. Relationship between body mass index (A), body fat % (B), and habitual anthocyanin intake (C) and changes in fat oxidation in the 14-day daily intake of New Zealand blackcurrant extract during 30-min of moderate-intensity walking. The correlation was significant for body mass index and body fat %.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dosing period in days | | | | | | | | | | | | | | | Testing day |
| Randomized cross-over | Condition | Day1 | Day2 | Day3 | Day4 | Day5 | Day6 | Day7 | Day8 | Day9 | Day10 | Day11 | Day12 | Day13 | Day14 |
| **Control** |  |  |  |  |  |  |  |  |  |  |  |  |  | 30-min walk |
| 14-Days Wash-out | | | | | | | | | | | | | | |
| **14-EOD** |  | NZBC |  | NZBC |  | NZBC |  | NZBC |  | NZBC |  | NZBC |  | NZBC and 30-min walk |
| 14-Days Wash-out | | | | | | | | | | | | | | |
| **14-D** | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC | NZBC and 30-min walk |

Figure 1. Schematic diagram of the randomized, cross-over study design with a control condition. NZBC indicates dosing with 600 mg of New Zealand blackcurrant extract (i.e. 210 mg of anthocyanins). 14-EOD indicates dosing every other day for two weeks. 14-D indicates dosing every day for two weeks. On testing day, the dose was taken two hours before the moderate-intensity 30-min walk.

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**Figure 2**

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**Figure 3**