



Two weeks daily intake of anthocyanin-rich New Zealand blackcurrant extract enhances whole-body fat oxidation during supine rest in healthy males

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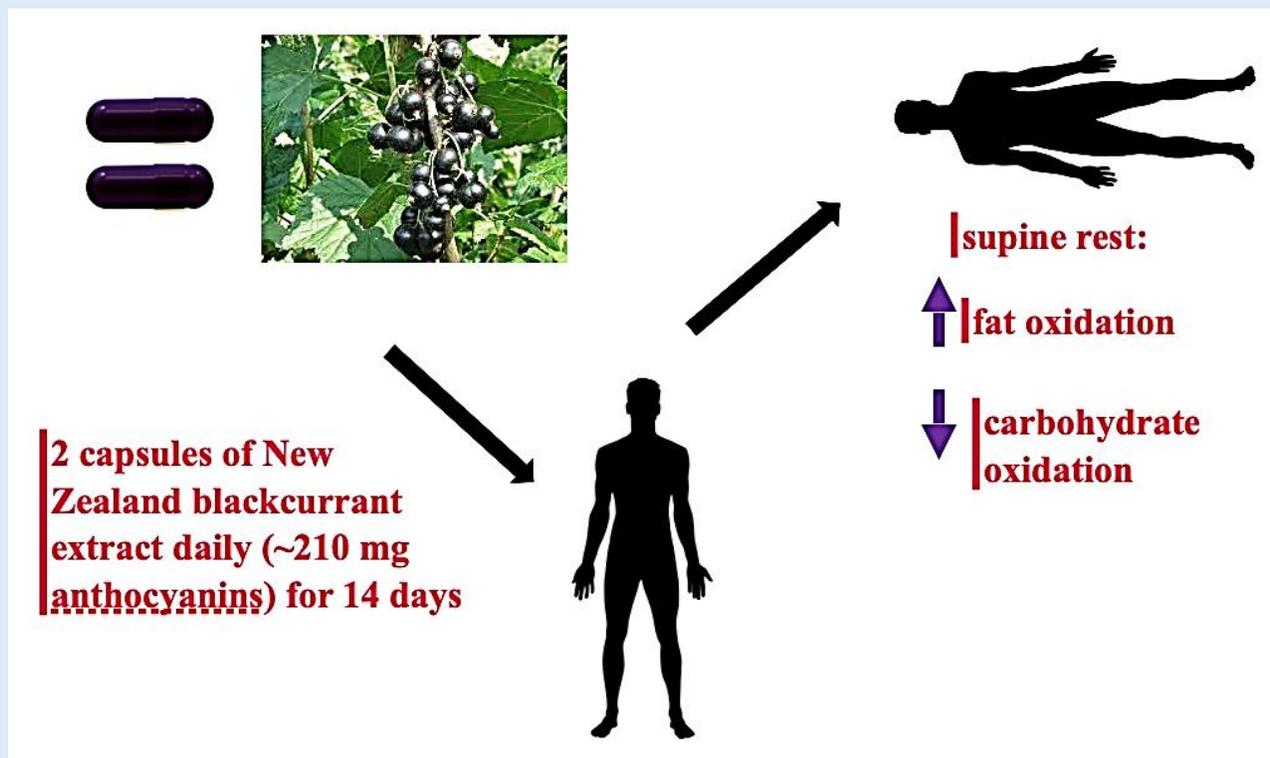
ABSTRACT

Introduction: Increase in exercise-induced fat oxidation has been observed with a 2-week daily intake of New Zealand blackcurrant (NZBC) extract. We examined for supine rest the effects of a 2-week daily intake of NZBC extract on physiological and metabolic parameters in relation to body fat%.

Methods: Healthy active men (n=16, age: 24.1±6.0 yr, body mass: 78.2±15.6 kg, BMI: 24.7±4.2 kg·m⁻²) volunteered. NZBC extract (210 mg of anthocyanins) was consumed with breakfast with a 14-day washout. The final ingestion was 2 hours before testing. Physiological and metabolic responses were measured during supine rest (2x10 min), and observations with the lowest 10-minute ventilation were analyzed. Bioelectrical impedance analysis was used to measure body fat%.

Results: During supine rest, a 14-day daily intake of NZBC extract had no effect on heart rate, minute ventilation, oxygen uptake, carbon dioxide production, and energy expenditure. However, a 14-day daily intake of NZBC extract decreased the respiratory exchange ratio (baseline: 0.840±0.045, 14-day: 0.820±0.058, P=0.03), increased fat oxidation (baseline: 0.079±0.03, 14-day: 0.088±0.043 g·min⁻¹, P=0.05), and decreased carbohydrate oxidation (baseline: 0.178±0.067, 14-day: 0.143±0.071 g·min⁻¹, P=0.03) during supine rest. Nine of the 16 participants had increased fat oxidation by over 10%

during supine rest. For those nine participants, the increases were $26 \pm 16\%$. We did not observe a significant correlation between body fat% and absolute and percentual changes in fat oxidation.



Conclusions: In a healthy male cohort, daily intake of New Zealand blackcurrant extract for two weeks was able to increase fat oxidation during supine rest. Daily intake of New Zealand blackcurrant extract may be an effective anthocyanin-rich supplement for managing body composition and body weight.

Keywords: New Zealand blackcurrant; anthocyanins; metabolic equivalent; rest; fat oxidation; respiratory exchange ratio

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INTRODUCTION

Strategies to lower body weight consider the balance between habitual energy intake and energy expenditure [1]. The primary aim of weight loss interventions is to change body composition by lowering total body fat [2]. Weight loss is most successfully achieved by changes in the habitual dietary intake to lower energy intake and

increases in physical activity levels to enhance energy expenditure [3]. However, it can be challenging for individuals to have 1) a meaningful change in physical activity levels, 2) regular participation in a structured exercise program, and 3) the need to reduce energy intake. Therefore, strategies to optimize whole-body fat oxidation in rest that are not the outcome of an exercise

intervention must be addressed [4]. It is, therefore, of interest to examine interventions with supplements that may change substrate oxidation in rest.

Studies have shown that non-training induced substrate oxidation in rest depends on the composition of habitual food intake [5], ethnicity [6,7], and sex [8,9]. In addition, some ergogenic nutritional aids have shown short-term acute effects on resting fat oxidation, e.g. a thermogenic fitness drink with caffeine [10], and it is likely that they require regular intake for sustained effects. Indeed, Dulloo et al. [11] observed that green tea extract (caffeine and catechins) enhanced fat oxidation in rest by 35% over a 24-hr period, but the adopted dosing strategy required intake with breakfast, lunch, and dinner. In our studies with New Zealand blackcurrant extract, intake once a day for 7 days enhanced cycling and walking-induced fat oxidation [12-14]. Blackcurrant is rich with flavonoid anthocyanin and contains delphinidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-glucoside as the primary anthocyanins [15]. In the study by Strauss et al. [13] with 7-day intake of 210 mg of New Zealand blackcurrant anthocyanins in endurance-trained females, the observations indicated a potential for enhanced adipose tissue lipolysis at rest. In addition, intake for 7-days of 600 g of blackberry (i.e. daily intake of 1500 mg of flavonoids) in overweight and obese males resulted in a lower 24 h respiratory exchange ratio and enhanced fat oxidation of 7% ($P=0.042$) [16]. The daily intake of 2.5 g anthocyanin-rich black soybean extract (i.e. 12.58 mg anthocyanins per day) over 8 weeks by overweight and

obese adults decreased the waist circumference, indicative of a loss of abdominal fat [17]. Excessive fat accumulation over time can create obesity and is a global health problem affecting quality of life and increases overall mortality [18]. The study by Şahin et al. [19] in a male cohort observed that walking-induced fat oxidation was higher with higher levels of body fat%. However, it is not known whether the intake of New Zealand blackcurrant extract can alter fat oxidation at rest.

Therefore, the primary aim of the present study was to examine the effects of 2-weeks daily intake of NZBC extract on the physiological and metabolic responses in a resting supine position. We also examined whether there was a relationship between the changes in NZBC-induced fat oxidation at rest and body fat%.

METHODS

Recreationally active healthy Caucasian men ($n=16$, age: 24.1 ± 6.0 yr, body mass: 78.2 ± 15.6 kg, height 178 ± 6 cm, BMI: 24.7 ± 4.2 $\text{kg}\cdot\text{m}^{-2}$, body fat%: $15\pm 6\%$) volunteered and provided written informed consent. The subject number was similar to Solverson et al. [16] with enhanced fat oxidation by blackberry intake. Study approval was provided by the University of Chichester Research Ethics Committee (ethical approval code: 1718_34), and all the study protocols and procedures were adhering to the 2013 Declaration of Helsinki. The participants had to be nonsmokers. In addition, the participants should not have had an allergy to berries or berry products, and they were not allowed to consume additional supplementation for the period of the study.

The study employed a randomized, cross-over experimental design. Participants visited the exercise physiology laboratory on three occasions. For all the laboratory visits, the participants were not allowed to consume caffeine and alcohol intake for 24 hours and should not perform any strenuous exercise for 48 hours. The first visit was mainly a familiarization with the expired air collection methods and measurement of the height, body mass, and body composition (Tanita BC418 Segmental Body Composition analyzer, Tanita, IL, USA). Participants completed a food frequency questionnaire, providing information on the habitual intake (i.e. frequency and amounts) of anthocyanin-containing items (i.e. foods and drinks) that were listed in the Phenol Explorer database [20]. This information was used to estimate the habitual daily anthocyanin intake and was found to be 82 ± 73 mg anthocyanins·day⁻¹. Participants also completed the short version of the International Physical Activity Questionnaire to quantify the physical activity level which was found to be 4385 ± 1635 MET·week⁻¹. Subsequently, the participants were seated for 10 min, followed by lying horizontally on a massage table for familiarization with the procedures of expired air collection. For expired air collection, participants were wearing a mouthpiece for 12 min and expired air collection initiated after 2-min for the remaining time (i.e. 10 min collection) into Douglas bags.

After the familiarization visit, participants visited the laboratory for an additional two visits: baseline (no supplementation) and intake of 14-day NZBC extract (supplementation) intake in randomized order. For the

supplementation condition, participants consumed two capsules of NZBC extract daily with breakfast. One capsule of NZBC extract contains 105 mg of anthocyanins. The anthocyanin composition of the capsules was 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, NZ, Ltd). Participants consumed the last 2 capsules on the day of supplementation testing 2 hours before initiation of the expired air collections. On the non-supplementation and supplementation testing days, the participants were allowed one slice of bread and water for breakfast and 3 hours before initiation of the expired air collections. A 14-day washout period was applied after the supplementation condition. Note that the measurements in the present study did not include parameters for which performance bias is possible [21]. However, bias on fat oxidation during cycling was reported by deceived intake of caffeine [22]. We are not aware of bias on fat oxidation measurements during rest by supplementation. Also, participants recorded 48 h food diary before the first visit and replicated it for the 48 h prior to the remaining baseline and 14-day NZBC extract visits. Analysis of the food diaries with Nutritics (Nutritics LTD., Dublin, Ireland) provided carbohydrate, fat, protein, and total energy intake (Table 1). There were no differences for carbohydrate, fats, or protein in absolute and relative to body mass values and total energy intake between the supplementation conditions (*p*-values for the dietary analyses: carbohydrate: 0.53, fat: 0.80, protein: 0.25, total energy intake: 0.24) for the baseline and 14-day NZBC extract conditions.

Table 1. Absolute and relative values for carbohydrate, fat, protein, and total energy intake for baseline and NZBC extract conditions.

	Baseline	14-day NZBC extract intake
Carbohydrate (g)	215 ± 54	210 ± 54
(g · kg body mass⁻¹)	2.85 ± 0.91	2.79 ± 0.87
Fat (g)	83 ± 34	82 ± 35
(g · kg body mass⁻¹)	1.07 ± 0.36	1.05 ± 0.38
Protein (g)	125 ± 43	121 ± 42
(g · kg body mass⁻¹)	1.62 ± 0.58	1.57 ± 0.56
Total energy intake (kcal)	2105 ± 464	2045 ± 487
(g · kg body mass⁻¹)	27.42 ± 6.35	26.59 ± 6.29

Note: NZBC, New Zealand blackcurrant

The experimental procedure consisted of 2x10 min expired air collection in Douglas bags with the participants in rest in the supine position. Heart rate (Polar Vantage NV Polar Electro Oy Kempele Finland) was measured with 15-s intervals in the 7th minute and averaged. Expired air collected in the Douglas bags was analyzed with a three-point calibrated Servomex gas analyzer (Series 1400, Crowborough, UK) for fractions of oxygen and carbon dioxide. The volume of the air was measured (Harvard Apparatus Ltd. Dry gas meter). The gas volumes of oxygen and carbon dioxide were calculated using Haldane transformation and

standardization to standard temperature, pressure and, dry conditions with consideration of the inspired fractions of oxygen and carbon dioxide within the laboratory during the expired air collections. The inspired fractions of oxygen and carbon dioxide were measured halfway through the expired air collections. The ratio between the volumes of carbon dioxide produced and oxygen consumed represents the respiratory exchange ratio (i.e. RER). Rates of whole-body fat and carbohydrate oxidation during rest were calculated with equations from Frayn [23], which has the assumption of negligible protein oxidation.

Statistical analysis: Graphpad Prism (version 5 for Windows, Graphpad Software, San Diego, USA) was used for statistical analyses. In the baseline (no supplementation) and 14-day NZBC intake (supplementation) condition, physiological and metabolic responses at supine rest that provided the lowest minute ventilation of the 2x10-min expired air collections were analyzed. Physiological and metabolic parameters for the baseline and 14-day intake of NZBC extract were analyzed using paired samples student t-tests. Cohens' d effect size was calculated as small: $0.2 \leq d < 0.5$; moderate: $0.5 \leq d \leq 0.79$ and large: $d \geq 0.8$ for those parameters with a significant change with the 14-day intake of NZBC extract. Pearson correlation coefficients were calculated for baseline and 14-day intake observations. Data are reported as mean \pm SD, range and 95% confidence intervals with significance accepted at $P \leq 0.05$.

RESULTS

Physiological responses: During supine rest, 14-day intake of New Zealand blackcurrant extract had no effect on heart rate (baseline: 61 ± 9 (range: 42-75, 95% CI [56, 66] $\text{beats} \cdot \text{min}^{-1}$), 14-day: 61 ± 9 (range: 48-82, 95% CI [56, 66] $\text{beats} \cdot \text{min}^{-1}$), $P=0.95$), minute ventilation (baseline: 8.10 ± 1.43 (range 6.42-11.21, 95% CI [7.34, 8.86] $\text{L} \cdot \text{min}^{-1}$), 14-day: 7.82 ± 0.98 (range: 5.83-9.85, 95% CI [7.30, 8.35] $\text{L} \cdot \text{min}^{-1}$), $P=0.38$), oxygen uptake (baseline: 0.293 ± 0.060 (range: 0.220-0.460, 95% CI [0.261, 0.325] $\text{L} \cdot \text{min}^{-1}$), 14-day: 0.286 ± 0.058 (range: 0.214-0.444, 95% CI [0.255, 0.317] $\text{L} \cdot \text{min}^{-1}$), $P=0.51$), carbon dioxide production (baseline: 0.245 ± 0.050 (range: 0.175-0.356, 95% CI [0.219, 0.273] $\text{L} \cdot \text{min}^{-1}$), 14-day: 0.233 ± 0.041 (range: 0.171-0.319, 95% CI [0.211-0.255] $\text{L} \cdot \text{min}^{-1}$), $P=0.20$) and energy expenditure (baseline: 1.49 ± 0.30 (range: 1.10-2.28, 95% CI [1.33, 1.66] $\text{kcal} \cdot \text{min}^{-1}$), 14-day: 1.44 ± 0.28 (range: 1.07-2.15, 95% CI [1.29, 1.59] $\text{kcal} \cdot \text{min}^{-1}$), $P=0.33$).

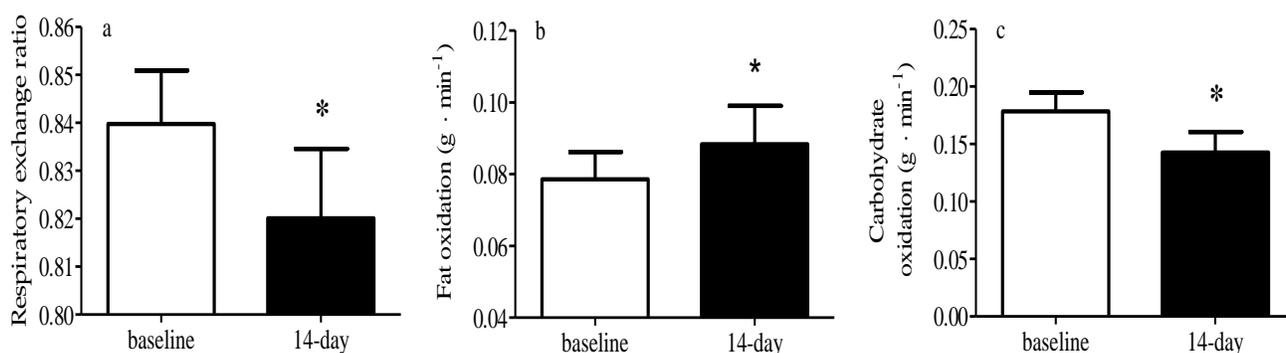


Figure 1. Respiratory exchange ratio (a), fat oxidation (b) and carbohydrate oxidation (c) at supine rest. Data was reported as mean \pm SD from 16 participants. 14-day refers to the daily intake duration of New Zealand blackcurrant extract, * indicates difference with baseline ($P \leq 0.05$).

Metabolic responses: Lower respiratory exchange ratio (baseline - range: 0.774-0.920, 95% CI [0.816, 0.864], 14-day - range: 0.718-0.948, 95% CI [0.789, 0.851], $d=-0.42$, $P=0.03$) (Figure 1a), higher fat oxidation (baseline - range: 0.034-0.174, 95% [0.062, 0.095 $\text{g}\cdot\text{min}^{-1}$], 14-day - range: 0.022-0.209, 95% CI [0.066, 0.111 $\text{g}\cdot\text{min}^{-1}$], $d=0.24$, $P=0.05$) (Figure 1b), and lower carbohydrate oxidation (baseline - range: 0.081-0.288, 95% [0.143, 0.214 $\text{g}\cdot\text{min}^{-1}$], 14-day - range: 0.0253-0.300, 95% [0.105, 0.181 $\text{g}\cdot\text{min}^{-1}$]

1], $d=-0.52$, $P=0.03$) (Figure 1c) were observed with 14-day daily intake of New Zealand blackcurrant extract during supine rest. Twelve participants (75%) had higher fat oxidation during supine rest with an increase of $21\pm 17\%$. There were no significant correlations for the relationship between body fat% and the absolute ($R^2=0.056$), $P=0.38$) and the percentage changes of fat oxidation ($R^2=0.008$), $P=0.75$) with 14-day daily intake (Figures 2ab).

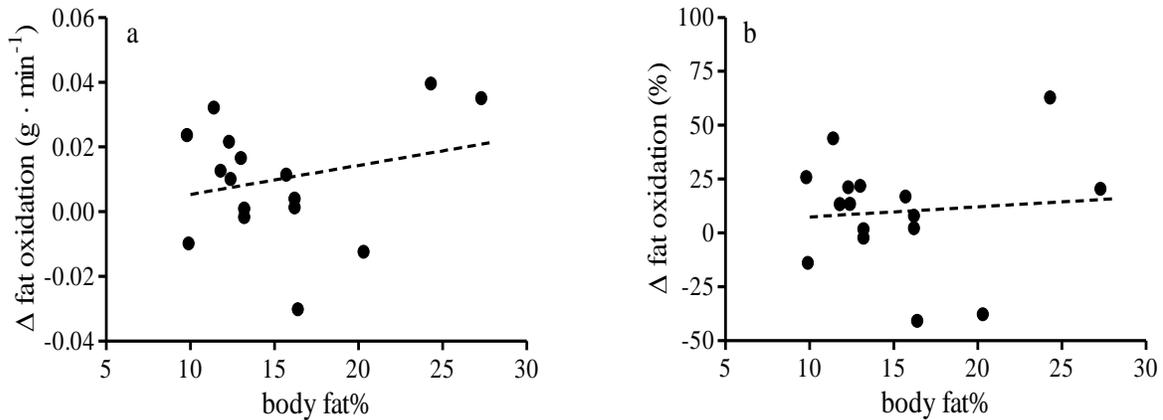


Figure 2. Relationship between body fat (%) and absolute (a) and % change (b) in fat oxidation by 14-day daily intake of New Zealand blackcurrant extract. Correlations were not significant.

DISCUSSION

The present study provides novel observations on the physiological and metabolic responses to 14-day daily intake of an anthocyanin-rich berry extract made from New Zealand blackcurrant (210 mg anthocyanins/day) during supine rest in healthy males. We observed no changes in heart rate, minute ventilation, oxygen uptake, and the production of carbon dioxide. However, notwithstanding the absence of an effect on oxygen uptake and the production of carbon dioxide at supine

rest, we observed a shift in substrate oxidation towards enhanced fat oxidation with substantial interindividual differences. Nine responders (56%) showed enhanced fat oxidation by more than 10% (range: 13% to 61%). If we would assume that the enhanced fat oxidation with continuous intake of New Zealand blackcurrant extract would be present $24\text{ hr}\cdot\text{day}^{-1}$, a decrease in daily energy intake with enhanced fat oxidation would provide opportunities for meaningful changes in body composition and weight loss. The highest responder with

enhanced fat oxidation of 61% would use 20.8 kg of body fat to meet resting energy demands. A weight loss above 5% in overweight and obese individuals is considered clinically meaningful and can provide health benefits [24]. It needs to be noted, however, that 6 out these 9 participants had normal BMIs, and it is not known whether obese individuals would show similar responses as the cohort in the present study. It is also unknown how other activities of daily living and food intake would blunt the effect of New Zealand blackcurrant extract on fat oxidation at rest.

In the study of overweight and obese males by Solverson et al. [16], resting metabolic rate was measured between 2 and 4 am, about 6 hr after dinner, in an attempt to avoid potential confounding effects of food intake and activities of daily living. However, in the period between 2 and 4 am, there were no changes in RER and substrate oxidation. In contrast, observations in the morning (~7:00 am to 11:00 am after the first bite of breakfast), the RER was lowered by 0.0096 units by intake of the blackberry from 0.8512 to 0.8416 with enhanced fat oxidation changing from 0.081 to 0.089 g·min⁻¹ in the blackberry condition [16]. In the present study, expired air samples were collected in the morning about 2 hr after a light breakfast (bread and water) and final intake of the last two capsules, and we observed a lowering of RER of 0.02 units with enhanced fat oxidation of 0.079 to 0.088 g·min⁻¹. The similarity between Solverson et al. [16] and the present study with respect to the enhanced fat oxidation in the morning was obtained with different anthocyanin composition and intake. Blackberry contains

primarily cyanidin-3-glucoside (i.e. 77% of total anthocyanin content) and no delphinidin (www.phenol-explorer), whereas anthocyanin-rich blackcurrant contains less than 10% cyanidin-3-glucoside and primarily delphinidins (up to 70%). In addition, the observations in the present study were obtained with about 58% less anthocyanin intake (i.e. 210 mg vs 361 mg [16]). This may suggest that blackcurrant is more effective for enhancing fat oxidation in rest than blackberry. However, other differences in methodology should be taken into consideration, e.g. the controlled high-fat diet intake and body composition of the participants [16] and the limitation in the present study not to provide a controlled-diet for the 14-day dosing period. A recent study by Pilolla et al. [25] with a 7-day intake of wild blueberry powder (375 mg of anthocyanins) enhanced exercise-induced fat oxidation showing the potential of different berries to alter substrate oxidation. Future studies may want to address in the same cohort the effectiveness of berries with different anthocyanin compositions to enhance fat oxidation with the berry intake matched for total anthocyanin content. The outcome of such studies on berry-induced fat oxidation may inform future dietary guidelines on the effectiveness of the natural anthocyanin composition of berries.

In a previous study with a 14-day intake of New Zealand blackcurrant extract in adult males [19], it was observed that enhanced fat oxidation during 30-min of 5-MET walking was positively correlated with body fat%. However, the enhanced fat oxidation at rest in the

present study was not significantly correlated with body fat%. This may suggest that the mechanisms to enhance fat oxidation by intake of New Zealand blackcurrant are different during exercise than for rest. However, the mechanism for enhanced fat oxidation during exercise is not clear, but may be due to enhanced lipolysis and blood flow with enhanced uptake potentially by internal changes in the mitochondria. It is possible that requirements for metabolic responses during exercise, such as enhanced availability of non-esterified free fatty acids by increases in blood flow, are needed for the effects of intake of New Zealand blackcurrant extract.

In general, the optimal intake of supplements includes the timing, dose, and intake duration, and the optimal intake strategy for New Zealand blackcurrant extract to affect substrate oxidation in rest is not completely known. Most studies on the effects of polyphenol intake on physiological, metabolic, cardiovascular, and anthropometric responses examine at the end of the intervention period. Only a few studies examined the effects of intake duration of polyphenols on fat oxidation and body composition [26–28]. Low ($78 \text{ mg}\cdot\text{day}^{-1}$) and high ($593 \text{ mg}\cdot\text{day}^{-1}$) dose tea catechin beverages were consumed for 12 weeks by healthy males with observations of 8 hr postprandial fat oxidation at 4, 8, and 12 weeks [26]. Postprandial fat oxidation increased continuously throughout the test period and was the highest at the 12-week time-point [26]. In the study by Kobayashi et al. [27], visceral, subcutaneous, and total fat areas were decreased in a cohort of moderately obese adults with the intake for 12 weeks of catechins-enriched green tea beverage ($280 \text{ mg}\cdot\text{day}^{-1}$) at 8 and 12-weeks. There were no differences between the 8 and 12-week time points. In contrast, capsaicinoid supplementation (2 mg and $\text{mg}\cdot\text{day}^{-1}$) for 12 weeks had no effect on body composition [28]. Future studies may

want to address the potential effectiveness of long-term intake of New Zealand blackcurrant extract in overweight and obese cohorts on fat oxidation during rest and exercise, body weight loss, and changes in body composition. Note that other flavonoids, e.g. genistein, have also been suggested to have anti-obesity properties [29].

CONCLUSIONS

It is concluded that a 14-day daily intake of NZBC extract is able to enhance fat oxidation at rest in healthy males. Therefore, chronic intake of New Zealand blackcurrant extract may be considered for body weight management combined with reduced daily energy intake.

Abbreviations: BMI: body mass index, NZBC: New Zealand blackcurrant, RER: respiratory exchange ratio

Author Contributions: METW, PB, and MAS conceived and designed research. MAS and SM conducted experiments. METW and MAS analyzed data. METW wrote the manuscript. All authors read and approved the manuscript.

Data Availability: Data is available on request to the corresponding author.

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