**Title: Dietary supplementation with New Zealand blackcurrant extract enhances fat oxidation during submaximal exercise in the heat**

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**Running head:** New Zealand blackcurrant alters substrate oxidation during exercise in hot conditions

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## Abstract

**Objectives:** This study investigated the effect of 7 days' supplementation with New Zealand blackcurrant extract on thermoregulation and substrate metabolism during running in the heat.

**Design.** Randomized, double-blind, cross-over study.

**Methods.** Twelve men and six women (mean ± SD: Age 27 ± 6 years, height 1.76 ± 0.10 m, mass 74 ± 12 kg, $V\dot{O}_{2max}$ 53.4 ± 7.0 mL·kg$^{-1}$·min$^{-1}$) completed one assessment of maximal aerobic capacity and one familiarisation trial (18°C, 40% relative humidity, RH), before ingesting 2 x 300 mg day$^{-1}$ capsules of CurraNZ™ (each containing 105 mg anthocyanin) or a visually matched placebo (2 x 300 mg microcrystalline cellulose M102) for 7 days (washout 14 days). On day 7 of each supplementation period, participants completed 60 minutes of fasted running at 65% $V\dot{O}_{2max}$ in hot ambient conditions (34°C and 40% relative humidity).

**Results.** Carbohydrate oxidation was decreased in the NZBC trial [by 0.24 g·min$^{-1}$ (95% CI: 0.21 to 0.27 g·min$^{-1}$)] compared to placebo ($p$ = 0.014, $d$ = 0.46), and fat oxidation was increased in the NZBC trial [by 0.12 g·min$^{-1}$ (95% CI: 0.10 to 0.15 g·min$^{-1}$)], compared to placebo ($p$ = 0.008, $d$ = 0.57). NZBC did not influence heart rate ($p$ = 0.963), rectal temperature ($p$ = 0.380), skin temperature ($p$ = 0.955), body temperature ($p$ = 0.214) or physiological strain index ($p$ = 0.705) during exercise.

**Conclusion.** Seven-days intake of 600 mg NZBC extract increased fat oxidation without influencing cardiorespiratory or thermoregulatory variables during prolonged moderate intensity running in hot conditions.

**Keywords:** Exercise, Hyperthermia, Supplements, Anthocyanin, Substrate oxidation

**Practical implications**

- Heat stress does not reduce the beneficial alterations to substrate metabolism observed following NZBC supplementation in temperate environments.
- Male and female athletes could consider supplementing with 600 mg of NZBC in the 7 days prior to any planned fasted training sessions to further elevate fat oxidation.
- NZBC did not improve thermoregulatory or cardiovascular function during exercise in the heat, but no negative effects of supplementation were found, indicating the supplement is safe to use in both temperate and warm environments.

## Introduction

Polyphenol containing foods and supplements (e.g., green tea, pomegranate, blueberry, montmorency tart cherry, chokeberry, and blackcurrant) are receiving increased interest from the sports nutrition community due to their potential for enhancing aspects of performance and recovery[1]. Berry fruits each contain their own specific make up of anthocyanins, which are responsible for the red, blue, and purple hues of fruits and vegetables and result in fruit-specific metabolic and physiological effects. To date, polyphenols have predominantly been assessed in temperate environmental settings, however, many athletes train and compete in hot environments. Studies investigating whether the reported favourable effects of polyphenol supplementation are maintained under such conditions are therefore warranted.

Exposure to heat requires an increase in blood flow to the skin for heat dissipation purposes, while sufficient blood flow to the exercising skeletal muscle must be maintained to support metabolism[2]. Anthocyanins have been shown to act on the vascular endothelium[3], increase endothelial nitric oxide synthase activity with production of nitric oxide, and increase vasodilation and skin blood flow[4]. As nitric oxide (NO) contributes ~35-45% to cutaneous active vasodilation[5], supplementation with anthocyanins could theoretically increase skin perfusion and aid heat dissipation mechanisms. Several nutritional supplements that act on NO metabolism have been tested for their ability to reduce thermal strain, with mixed results. For example, 7-day supplementation with pomegranate juice containing a high abundance of polyphenol antioxidants (22.5% punicalaguin, 3.5% ellagic acid, 1% anthocyanins) which increase NO bioavailability[6], had no effect on cardiovascular strain, skin blood flow, or exercise performance during a 60 minute cycling bout (31.5ºC, 55% RH)[7]. Beetroot juice, which also increases NO availability and improves temperate performance, improved metabolic efficiency during a simulated desert walk by 6%, but at the cost of an 11% increase in core body temperature[8]. This example illustrates the importance of testing ergogenic aids across a variety of conditions as maladaptive responses are also possible. More recently, we have found that supplementation with curcumin, a polyphenolic compound derived from the spice turmeric, and shown to have vasoactive effects via increasing NO bioavailability, reduced heart rate, core temperature and physiological strain during a 60-minute treadmill run (65% $\dot{V}O_2$max) performed in a hot, dry (37°C, 20% RH) environment[9].

Blackcurrant (*Ribes nigrum*) has one of the highest concentrations of the anthocyanins, is composed primarily of delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside[10], and has been shown to increase peripheral forearm blood flow by 22%[4]. Additionally, a 7-day intake of New Zealand Blackcurrant extract (NZBC; 210 mg anthocyanins per day) increases femoral artery diameter and blood flow during short duration submaximal isometric contractions[11]. The same dosing strategy (7 days NZBC extract intake, 210 mg anthocyanins per day) invokes changes to exercise metabolism, with increasing fat

oxidation during steady state cycling exercise in both men and women a consistent observation[10, 12, 13], and improved 16.1 km cycling time trial performance also noted[12]. The metabolic responses are dose dependant, with fat oxidation increased by 17.5 %, 22% and 24% following 7-days dosing with 300, 600 or 900 mg of NZBC per day respectively[13]. The potential for NZBC to increase cutaneous skin perfusion and reduce the heat-mediated increased reliance on carbohydrate oxidation, offer two potential avenues to mitigate against thermal strain. Therefore, the purpose of this study was to evaluate the effects of 7 days of New Zealand Blackcurrant supplementation on thermoregulatory and metabolic responses during exertional heat stress.

## Methods

Twelve men and six women, all recreationally active (mean ± SD: Age 27 ± 6 yr, height 1.76 ± 0.10 m, mass 74 ± 12 kg, $\dot{V}O_{2max}$ 53.4 ± 7.0 mL·kg$^{-1}$·min$^{-1}$)provided written informed consent prior to completing a double-blind placebo-controlled study with randomised, cross over design. The study was approved by the University Research Ethics Committee with protocols and procedures conforming to the 2013 Declaration of Helsinki. All participants were non-smokers and negative for cardiovascular, pulmonary, or metabolic disease as defined by the American College of Sports Medicine [14].

Each participant completed one maximal exercise test, one familiarisation trial, and two experimental trials. Participants were instructed to abstain from strenuous exercise and alcohol for 48 h prior, and caffeine-containing products on the day of testing. Participants were asked to adhere to their normal exercise training schedule and completed a standard food diary for 48 h prior to each experimental trial. Participants were asked to replicate their diet prior to each experimental visit and intake was quantified using Nutritics software (Nutritics Ltd, Dublin, Ireland). To estimate habitual anthocyanin intake, participants completed a food frequency questionnaire that listed the amount and frequency of anthocyanin-containing foods

and drinks[15]. All testing was conducted in the morning following a 12 hour overnight fast. On the first visit, maximal aerobic capacity was determined from an incremental exercise test to exhaustion and verification exercise bout completed on a motorized treadmill (HP Cosmos, Pulsar, h/p/cosmos Sports & Medical gmbh, Germany). During visit 2, participants were familiarised to all the measurements and procedures completed during visits 3 and 4, but while under thermoneutral conditions (18°C, 40% RH). Prior to visits 3 and 4, participants consumed 2 capsules of concentrated NZBC extract (2 x 300 mg active cassis containing 105 mg of anthocyanins, i.e. 35–50% delphinidin-3-rutinoside, 5–20% delphinidin-3-glucoside, 30–45 % cyanidin-3-rutinoside, 3–10 % cyanidin-3-glucoside per capsule; CurraNZ™, Health Currancy Ltd., Surrey, UK) or 2 capsules of identical looking placebo capsules (2 x 300 mg microcrystalline cellulose M102) with breakfast every morning for 7 days[12, 13]. One experimenter (M.E.T.W) made up visually identical NZBC and placebo pill packets for each participant and left them in the principle investigators office (B.J.L) without any personal interaction. Blinding was not broken until after analysis was completed. Health Currancy Ltd had no role in the collection, analysis, interpretation and dissemination of data. The two experimental conditions (NZBC extract and placebo) were separated by a 14-day washout period (men), or evenly spaced from the proceeding months' mid luteal cycle phase (women). Menstrual cycle phase was monitored in monophasic oral contraception users (n = 3) and those not taking contraception (n = 3) by the three-step method[16]. A 5 mL venous blood sample was collected during the first follicular phase, and on each trial day for determination of progesterone concentration (Enzo Life Sciences Inc., Farmingdale, NY, USA; ADI-901-011; **supplementary table 1**). Trial order was determined using a free online tool (https://www.randomizer.org) and nine participants received NZBC extract as the first condition. All experimental trials were conducted in an environmental chamber (TISS Services UK, Medtead, Hampshire, UK) controlled at 34.1 ± 0.1 °C and 40.8 ± 0.2 % RH.

Participants were instructed to drink ~400 mL of water upon waking and arrived for each experimental trial between 06:30 and 08:30. Upon arrival to the laboratory, a urine sample

was taken for assessment of urine osmolality (Osmocheck PAL-OSMO; Vitech Scientific, Partridge Green, West Sussex, UK) and specific gravity (ATAGO 2791, ATAGO, Tokyo, Japan) to ensure participants were euhydrated ($mOsmol^{-1} \leq 600$; USG ≤ 1.020)[17]. Following the recording of nude body mass, participants inserted a polyethylene rectal thermistor (Edale Instruments, Cambridge, UK) 10 cm past the anal sphincter and were fitted with a heart rate monitor. Skin thermistors (Edale Instruments, Cambridge, UK) were attached to the mid-belly of the pectoralis major, triceps brachii, rectus femoris, and gastrocnemius for calculation of mean skin temperature[18]. Mean body temperature and physiological strain index were also calculated during exercise using standard equations[19, 20].

After a 20-minute supine rest period, physiological measurements were noted (HR, skin and rectal temperatures) and participants entered the environmental chamber where they rested for five minutes prior to 60 minutes of treadmill running at 65% $V\square O_{2max}$ (1% incline). Expired air was collected into Douglas bags every 10 minutes to determine rates of substrate oxidation during exercise[21], and inspired air composition within the chamber noted for use in substrate oxidation calculations[22]. Heart rate, rectal temperature, skin temperatures, and rating of perceived exertion were recorded every 10 minutes. Bottled water (chamber temperature) was available *ad libitum* during each trial, and the volume of fluid ingested was recorded. On completion of exercise, participants towel dried and nude body mass was reassessed. The difference in pre-to-post exercise body mass was used to calculate sweat rate (corrected for water ingestion but not respiratory water loss). Participants recovered in an air-conditioned laboratory for 60-minutes post exercise, with further physiological and thermoregulatory measurements taken at 20 and 60-minutes post exercise.

Statistical analysis was performed using IBM SPSS for Windows (Version 23, SPSS, Chicago, Illinois). Data in text and tables are presented as mean (95% confidence intervals); data in figures are displayed as mean ± SD for n = 18. Differences in dietary intake, ambient conditions, urine specific gravity, urine osmolality, fluid intake and sweat rate were determined via paired t-test. Differences in substrate oxidation, cardiorespiratory, and thermoregulation

158 measures were determined using mixed linear models with fixed effects for trial and time. Main
159 effects ($p < 0.05$) were explored using paired t-tests with Bonferroni adjustments. To control
160 for the false discovery rate during multiple comparisons, the procedures of Benjamini and
161 Hochberg (1995) were followed after all post hoc procedures[23]. Differences in the mean
162 exercise responses over the 60-minute exercise bout in Placebo and NZBC were determined
163 using paired t-tests. To quantify the false positive risk (FPR) a prior probability of 0.5 for
164 detecting a change in substrate oxidation was applied. The observed p-value and effect sizes
165 obtained from paired t-tests of data averaged across exercise were used to compute the FPR
166 using an online tool (Colquhoun and Longstaff, http://fpr-calc.ucl.ac.uk:3838). Precise *p*-
167 values are reported, and Cohen's *d* (paired t test data) effect sizes are presented to indicate
168 the magnitude of observed effects[24]. Cohens *d* effect sizes of 0.2, 0.5, and 0.8 are considered
169 small, medium and large, respectively.

170

171 **Results**

172 There were no differences in dietary intake, and macronutrient profile **(Supplementary Table**
173 **2**), pre-exercise body mass, or participant hydration status between the placebo and NZBC
174 conditions (all $p > 0.05$; **Table 1**).

175 Heart rate, rectal temperature, mean skin temperature, mean body temperature, and
176 physiological strain increased with exercise in both study conditions (main effect of time all $p$
177 $< 0.0001$) with no difference being shown between conditions and no interaction effect (all $p$
178 $> 0.05$). Fluid intake ($p = 0.938$) and whole body sweat rate ($p = 0.465$) were also not different
179 between study conditions **(Table 1**).

180 **** Please Insert Table 1 near here ****

181 Both oxygen consumption (main effect for time, $F = 9.900$, $p < 0.0001$) and carbon dioxide
182 production (main effect for time, $F = 3.536$, $p = 0.004$) increased throughout each condition,
183 and no condition x time interaction was observed for either variable (**Table 1**). The respiratory

exchange ratio (RER) was lower during the first 50 minutes of exercise in NZBC compared to placebo (Main effect of condition, F = 26.365, $p < 0.0001$, **Figure 1A**), and mean exercise RER was lower during NZBC [0.88 (95% CI: 0.77 to 0.99)] compared to placebo [0.90 (95% CI: 0.82 to 0.99); t(17) = 2.222, $p = 0.04$, $d = 0.06$]. The small effect size and observation of $p = 0.04$ implies a false positive risk of at least 58%, so these results are no more than suggestive. There was a main effect for time (F = 2.653 p = 0.024), however when corrected for multiple comparisons the differences became less apparent and there was no condition x time interaction (F = 0.045, $p = 0.999$). Carbohydrate oxidation was lower throughout exercise in NZBC (main effect of condition, F = 22.62, p < 0.0001, **Figure 1B**), translating to a mean exercise decrease of 0.24 $g \cdot min^{-1}$ (95% CI: 0.21 to 0.27 $g \cdot min^{-1}$) versus placebo (t(17) = 2.751, $p = 0.0136$, $d = 0.46$). The observation of p = 0.0136 and medium effect size implies a false positive risk of 28%, so these results are also no more than suggestive. There was no main effect for time (F = 1.108, p = 0.358) or condition x time interaction for carbohydrate oxidation (F = 0.122, $p = 0.987$). Fat oxidation was elevated between minutes 10 – 50 of exercise in NZBC compared to placebo (main effect of condition, F = 55.64, $p < 0.0001$, **Figure 1C**), equal to a mean exercise increase of 0.12 $g \cdot min^{-1}$ [95% CI: 0.10 to 0.15 $g \cdot min^{-1}$; (t(17) = 2.980, $p = 0.008$, $d = 0.58$, **Figure 1D]**. The observation of p = 0.008 and effect size of 0.58 implies a false positive risk of 5%, suggesting a strong effect of NZBC extract on exercise-induced fat oxidation in the heat. Fat oxidation increased over time during the exercise bout (main effect for time, F = 4.813, $p = 0.0003$), but no condition x time interaction was observed (F = 0.483, $p = 0.788$).

**** Please insert figure 1 near here ****

## Discussion

This study investigated the effects of 7 days (600 mg per day) New Zealand Blackcurrant extract supplementation on thermoregulatory and metabolic responses during fasted running exercise in the heat. We observed no alterations in thermoregulatory responses or physiological strain throughout exercise but did observe enhanced fat oxidation alongside a

moderate reduction in CHO oxidation. Our results suggest that short term intake of NZBC extract has ergogenic potential for men and women exercising in the heat. In total, 9 out of 12 men (75%), and 4 out of 6 women (67%) demonstrated increased fat oxidation, supporting previous work showing effects in both sexes[10, 13]. Of these 13 individuals, 11 experienced increases in fat oxidation exceeding the inter-individual variability observed for our protocol (CV = 8%; determined during test-retest performed ~20 days apart to match study conditions).

In the present study we chose to examine cardiovascular and thermoregulatory function during exertional heat stress because anthocyanins present in NZBC have been shown to increase NO bioavailability and increase skin blood flow[4, 11, 25]. Given that NO has important roles in cutaneous blood flow, thermoregulatory control of sweating, and skeletal muscle blood flow, we hypothesised that NZBC might reduce cardiovascular strain and improve thermoregulatory function. Contrary to our hypothesis, no changes were observed in skin temperature, rectal temperature, or whole body sweat rate. As such, these data suggest heat loss was not increased following NZBC supplementation. However, it is important to highlight that evaporation of sweat and thus heat loss is impaired in uncompensible heat stress conditions. Compensable conditions, which allow for a more complete evaporation of sweat, may be a more appropriate experimental model for determining whether increases in peripheral blood flow increase heat loss. While we observed no changes to thermoregulatory variables, improvements in blood flow and vascular function following anthocyanin ingestion have also been linked with an increase in fat oxidation during exercise, likely as a result of a greater availability of plasma fatty acids[26]. In the present study, we observed an increase in mean fat oxidation rates (~30%), comparable to the 27% and 22% increases observed during prolonged (i.e. 2 hr) cycling exercise at 65% $V\square O_{2max}$ in temperate conditions using the same dosing strategy[12, 13]. Our results suggest that the beneficial effects of NZBC extract on substrate oxidation observed during cycling in temperate environmental conditions, are maintained when tested in an uncompensable exertional heat stress model. The observed increase in fat oxidation of ~ 30% is to date the highest reported after NZBC intake, and compares favourably

238 to other supplements (for example green tea extract, 17-24% increase[27]) and endurance
239 training programmes (+0.12 – +0.22 g·min$^{-1}$ [28]) in terms of magnitude of fat oxidation increase.
240 We present some evidence for a reduction in CHO oxidation during exercise, however the
241 high FPR (28%) suggest this result needs further replication. The 0.24 g·min$^{-1}$ decrease in
242 CHO oxidation is similar to the ~ 0.22 g·min$^{-1}$ observed in previous work utilizing a 7 day, 600
243 mg/day dosing period[13], however others have reported no difference in CHO oxidation vs
244 placebo[10, 12].

245

246 Previous studies using NZBC extract have been performed in the post prandial state[10, 12, 13],
247 albeit 2 hr following low calorie intake unrepresentative of performance nutrition practices,
248 therefore a lower rate of fat oxidation is to be expected as prior CHO ingestion can limit
249 lipolysis[29]. Although completing each trial after an overnight fast may preclude the application
250 of our results to situations representative of performance in the heat, it allowed for greater
251 standardization between conditions, which alongside 48-hour dietary control, can increase the
252 reliability of fat oxidation measurements[30]. Altering nutrient availability before and/or during
253 training in order to commence a session with low exogenous CHO, or commencing training
254 with low muscle glycogen, has been shown to augment the cellular responses to training[31].
255 For example, training in the fasted state increases free fatty acid mobilization and
256 phosphorylation of peroxisome proliferator-activated receptor (PPAR) and downstream
257 targets, amplifying the skeletal muscle signalling responses to training[31]. Whether the use of
258 NZBC supplementation during fasted/low glycogen availability training would further stimulate
259 the adaptive pathways warrants consideration. While it is well established that endurance
260 training increases fat oxidation at a given absolute workload, there is limited evidence
261 supporting the notion that increased fat oxidation directly improves endurance performance
262 when exercise duration is below 2-3 hours. Our data may be relevant to those competing in
263 ultra-endurance events > 4 hours, in which maximal fat oxidation has been shown to be
264 associated with performance[32]. However, the duration of our exercise protocol (60 minutes),

and the intensity employed (65% $\dot{V}O_2$max) cannot be readily applied to the longer duration (> 4 hours), lower intensity work that characterizes ultra-endurance events. In addition, prolonged ultra-endurance performance cannot be sustained on water alone, and requires exogenous fuel ingestion (for example, ingestion of carbohydrate gels and beverages). Future work attempting to determine the efficacy of anthocyanin/NZBC supplementation on exercise performance effects will therefore need to consider how food and supplement interactions impact upon substrate oxidation [33].

## Conclusions

In summary, 7 days of supplementation with 600 mg of NZBC extract increased whole-body fat oxidation during fasted running at a moderate intensity in hot climatic conditions compared to placebo, without having any beneficial or negative effects on thermoregulatory measurements. Future research should aim to determine whether the NZBC mediated alterations to substrate metabolism confer a performance benefit during endurance and ultra-endurance performance, performed in both temperate and hot environments, while incorporating more ecologically valid exogenous fuelling strategies.

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368 **Table 1.** Mean (95 % CI) pre-trial hydration status and mean exercise cardiorespiratory,
369 thermoregulatory, and subjective data recorded during the 60-minute run at 65% $V\square O_{2max}$
370 following 7 days of placebo, or 7 days of NZBC extract supplementation. Data are averaged
371 across 6 time points for 12 healthy men and 6 healthy women (n = 18).

| Variable | Placebo | NZBC |
|---|---|---|
| **Pre-trial measurements** | | |
| Body mass (kg) | 73.38 (67.52 – 79.23 | 73.31 (67.33 – 79.28) |
| Urine osmolality (mOsm·kg$^{-1}$) | 328 (253 – 404) | 266 (202 - 329) |
| Urine specific gravity | 1.009 (1.007 – 1.011) | 1.007 (1.005 – 1.009) |
| **Cardiorespiratory** | | |
| Heart rate (bts·min$^{-1}$) | 173 (157 to 190) | 174 (156 to 192) |
| $V\square O_2$ (L·min$^{-1}$) | 2.75 (1.72 to 3.78) | 2.75 (1.80 to 3.70) |
| Relative intensity (% $V\square O_{2max}$) | 69 (60 to 78) | 70 (60 to 80) |
| $V\square CO_2$ (L·min$^{-1}$) | 2.47 (1.53 to 3.41) | 2.42 (1.60 to 3.23) |
| RER | 0.90 (0.82 to 0.98) | 0.88 (0.77 to 0.99)$^{+}$ |
| CHO oxidation (g·min$^{-1}$) | 2.24 (1.07 to 3.40) | 2.00 (0.81 to 3.20)$^{+}$ |
| Fat oxidation (g·min$^{-1}$) | 0.53 (0.18 to 0.87) | 0.65 (0.28 to 1.02)* |
| **Thermoregulation** | | |
| $T_{rectal}$ (°C) | 38.49 (37.80 to 39.17) | 38.46 (37.77 to 39.16) |
| Change in $T_{rectal}$ (°C) | 1.70 (0.81 to 2.60) | 1.50 (0.62 to 2.38) |
| $T_{skin}$ (°C) | 35.03 (33.62 to 36.44) | 35.01 (33.71 to 36.32) |
| $T_{body}$ (°C) | 36.99 (34.79 to 39.20) | 37.22 (36.43 to 38.01) |
| WBSR (L.hr$^{-1}$) | 2.0 (1.7 – 2.3) | 2.2 (1.9 – 2.5) |
| Fluid intake (mL) | 899 (732 - 1067) | 908 (710 - 1106) |
| PSI (A.U) | 7.5 (6.2 to 8.7) | 7.4 (5.8 to 9.0) |

CHO: Carbohydrate. RER: Respiratory exchange ratio. RPE: Rating of perceived exertion PSI: Physiological strain index. $V\square O_2$: Oxygen consumption. $V\square CO_2$: Carbon dioxide production. WBSR: whole-body sweat rate. + denotes $p < 0.05$ vs, placebo. * denotes $p < 0.01$ vs. placebo.

## Figure legends

**Figure 1.** Respiratory exchange ratio **(A)** and carbohydrate oxidation **(B)** were lower throughout the NZBC trial versus placebo (*p < 0.01, mixed linear model with Bonferroni *post hoc* test). Fat oxidation **(C)** was increased for the first 50 minutes of the NZBC trial (# p < 0.001, mixed linear model with Bonferroni *post hoc* test), but no different from placebo at 60 minutes. These data are further illustrated by overall substrate utilization **(D)** throughout the 60-minute steady state run (paired t-tests). Figure insets show the mean exercise value for each participant (lines) and mean group response (bars). Values are mean ± standard deviation for n = 18. CHO = carbohydrate.

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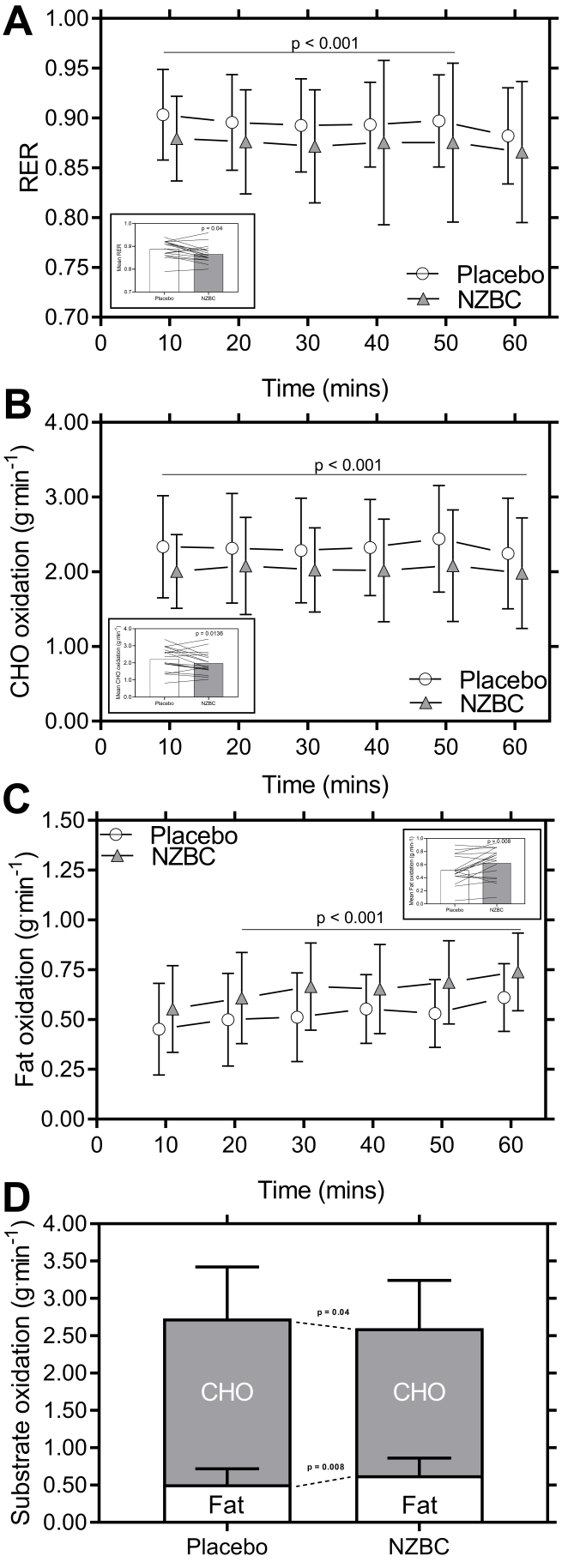
A
RER
p < 0.001
Placebo
NZBC
Time (mins)
B
CHO oxidation (g·min-1)
p < 0.001
Placebo
NZBC
Time (mins)
C
Fat oxidation (g·min-1)
Placebo
NZBC
p < 0.001
Time (mins)
D
Substrate oxidation (g·min-1)
p = 0.04
p = 0.008
CHO
CHO
Fat
Fat
Placebo
NZBC

**Supplementary Table 1.** The Mean ± SD menstrual cycle and hormone characteristics for the 6 women participants.

| | Placebo | | NZBC | |
|---|---|---|---|---|
| | OC (*n* = 3) | Non-OC (*n* = 3) | OC (*n* = 3) | Non-OC (*n* = 3) |
| Cycle length (days) | 28 ± 0 | 29 ± 4 | 28 ± 0 | 27 ± 1 |
| Positive ovulation (day) | N/A | 16 ± 1 | N/A | 13 ± 3 |
| Test day | 20 ± 4 | 22 ± 2 | 21 ± 4 | 23 ± 1 |
| Progesterone ($ng \cdot mL^{-1}$) | 0.28 ± 0.02 | 12.88 ± 4.26 | 0.25 ± 0.004 | 10.19 ± 4.3 |

OC – oral contraceptive users. Non-OC – natural menstrual cycle.

**Supplementary Table 2.** The mean ± SD absolute macronutrient intake 48 h prior to each condition (n = 15*).

| Variable | Placebo | NZBC |
|---|---|---|
| **Nutritional status** | | |
| Total energy intake (kJ) | 8051 ± 1911 | 8134 ± 1926 |
| Carbohydrate (g) | 197 ± 41 | 198 ± 45 |
| Fat (g) | 93 ± 40 | 108 ± 50 |
| Protein (g) | 75 ± 23 | 75 ± 22 |
| Habitual anthocyanin intake (mg $day^{-1}$) | 64 ± 32 | 61 ± 38 |

* Due to insufficient information provided by 1 female subject, and 2 male subjects, only 15/18 food diaries were analysed.