**Manuscript Title**: **The effects of an acute dose of New Zealand blackcurrant extract on 5 km running performance**

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**Abstract**

This study investigated the effects of an acute dose (900 mg) of New Zealand Blackcurrant (NZBC) extract on 5 km running performance, alongside associated physiological and metabolic responses. Sixteen trained male runners (age 26 ± 5 years, stature 173.4 ± 7.3 cm, body mass, 73.7 ± 6.9 kg, V̇O2max 55.4 ± 6.1 ml.kg-1.min-1) ingested either capsules containing NZBC extract (3 x 300 mg CurraNZTM, 315 mg anthocyanins) or a matched placebo (3 x 300 mg gluten free flour) 2 hours before exercise in a double-blind, randomised, crossover design. Performance time, physiological, and metabolic responses were assessed in a 5-km time-trial, preceded by 10 min exercise at the lactate threshold on a treadmill. NZBC extract did not alter the physiological or metabolic responses to exercise at the lactate threshold (V̇O2, RQ, V̇E, carbohydrate oxidation, fat oxidation, heart rate, blood lactate or Rating of Perceived Exertion, *P*>0.05). The 5-km time-trial was completed in a faster time in the NZBC extract condition compared to placebo (NZBC: 1308.96 ± 122.36 s, Placebo: 1346.33 ± 124.44, *P*=0.001, *d*=-0.23, CI range=-0.46 to 0.00 s). No differences in physiological or metabolic responses were apparent between conditions for the 5-km time-trial (*P*>0.05). Ingesting 900 mg of NZBC extract as an acute dose improves performance in trained male runners without altering physiological or metabolic responses to exercise. Further research is needed to assess a wider range of possible mechanisms (e.g., cardiovascular function, metabolite profiles) to advance insight into improved performance following supplementation.

**Introduction**

The use of dietary supplements to enhance performance is commonplace among sports competitors (Knapik et al., 2016). New Zealand Blackcurrant (NZBC) extract contains high concentrations of anthocyanins; delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyanidin 3-O-rutinoside and cyanidin 3-O-glucoside (Cortez & Gonzalez de Mejia, 2019). Anthocyanins are polyphenols from the flavonoid family comprising the red, blue or purple pigments in fruits, flowers and vegetables (Khoo et al., 2017). Dietary anthocyanins have multiple health benefits, including a reduction in cardiovascular disease risk, anti-inflammatory effects, and anticancer activity (Li et al., 2017). Their value in exercise settings has recently been reviewed, revealing anthocyanin-rich NZBC can benefit cycling and running performance (all ≤ 90 min), in addition to physiological and metabolic responses to exercise (Cook & Willems, 2019).

A primary determinant of high-intensity endurance performance is the ability of the cardiovascular system to maintain blood flow to the periphery for oxygen and nutrient delivery, metabolite removal and lactate shuttling to alternative cells (e.g. the liver, heart and kidneys) (Bassett & Howley, 2000). Investigations have revealed that blackcurrant anthocyanin intake confers positive effects on blood flow (Cook et al., 2015; Cook, Myers, Gault, & Willems, 2017; Matsumoto et al., 2005) femoral artery diameter and cardiac output (Cook, Myers, Gault, & Willems, 2017). These alterations are supported by in-vitro studies, which show that anthocyanins upregulate expression of endothelial nitric oxide synthase (eNOS) and subsequent vasorelaxant agent nitric oxide (NO) release (Xu et al., 2004). Although research findings are somewhat equivocal, these alterations in vascular properties could contribute to improved muscle oxygenation (Fryer et al., 2020) and change the balance of lactate appearance and removal mechanisms to blood lactate accumulation (Willems et al., 2015). Indeed, a rightward and downward shift of the lactate curve at 40, 50, 60 and 70% of maximal cycling power output was observed when NZBC powder (6 g⋅day-1) was supplemented over a 7-day period (Willems et al., 2015); such changes are often associated with enhanced endurance performance. Several other mechanisms for anthocyanins’ effects on endurance are possible. Increases in fat oxidation (17-27%) following 7-days of NZBC extract intake have been reported (Cook et al., 2015; Cook, Myers, et al., 2017b; Strauss et al., 2018), which could promote glycogen sparing (Strauss et al., 2018). Greater NO availability could also favourably affect mitochondrial respiration and sarcoplasmic reticulum Ca2+ handling (Jones, 2014), whilst anthocyanins’ antioxidant properties (De la Cruz et al., 2013) might limit muscle fatigue associated with reactive oxygen species accumulation (Powers & Jackson, 2008).

While the aforementioned evidence to support a possible endurance performance benefit is promising, studies assessing the effect of NZBC extract on time-trial (TT) performance are minimal, relate principally to cycling, and results are inconsistent. For example, improvements of 2.4% in a 16.1 km cycling TT were reported when trained male cyclists ingested NZBC extract daily (105 mg anthocyanins) for 7 days (Cook et al., 2015). In this study, testing commenced 2 hours after the final dose of NZBC, and so it is not clear if performance was enhanced because of the acute availability of anthocyanins and their metabolites, or some chronic effect of supplementation. However, another study found no performance enhancing effect of NZBC extract during a 7-day supplementation period with cycling TT performance tested on day one, four and seven. Neither, different dosages (105 mg or 210 mg anthocyanin) or the dosing regime (acute or chronic) resulted in faster performance times compared to a placebo. Clearly, further research is required to elucidate whether an acute dose of NZBC extract enhances TT performance in various distances and exercise modalities.

Therefore, the purpose of this study was to investigate the effects of an acute dose of NZBC extract (900 mg, ~315 mg anthocyanin) on 5 km TT performance in trained male runners. The secondary aim was to assess the physiological and metabolic responses to NZBC during exercise performed at the lactate threshold and during a performance TT.

**Methods**

*Participants*

After obtaining institutional ethical approval and informed consent for the conduct of research in accordance with the Declaration of Helsinki, 16 trained male runners or triathletes (age 26 ± 5 years, stature 173.4 ± 7.3 cm, body mass, 73.7 ± 6.9 kg, V̇O2max 55.4 ± 6.1 ml.kg-1.min-1 [range 44.3 – 69.3 ml.kg-1.min-1]), volunteered to participate in the study. All participants engaged in a structured training programme and competed in running events regularly. *A priori* sample size calculations based on an effect size of 0.7 (Cook et al., 2015) with α at 0.05 and power at 0.8 identified a necessary sample size of 15. Exclusion criteria were: 5 km personal best time >25 min, recent (within 6 months) use of nutrition supplements, and disease history that would impact exercise.

*Experimental Design*

A repeated-measures, double-blind, randomised (online programme), cross-over study design was employed, with participants attending the laboratory on three separate occasions at the same time of day (<2 hours difference) with all testing taking place in the morning and early afternoon. On the first visit, participants completed a graded incremental treadmill test to volitional exhaustion to establish V̇O2max and running speed at lactate threshold (LT1) (Jones et al., 1999). In an attempt to avoid learning effects, participants were subsequently familiarised with the 5-km running distance, whereby treadmill speed was controlled manually by each participant.

The two subsequent experimental testing visits included two stages; a 10-min run at the individualised speed corresponding to LT1, followed by a 5-km TT. For all visits, the treadmill was set at a 1% gradient to reflect the energetic cost of outdoor running (Jones & Doust, 1996). Measures of oxygen uptake (V̇O2), Respiratory Exchange Ratio (RER), minute ventilation (V̇E), carbohydrate oxidation, fat oxidation, heart rate (HR), blood lactate (B[La]) and rating of perceived exertion (RPE) were taken for both stages of the protocol and performance splits and overall time was recorded for the TT. The NZBC extract or placebo was administered in a randomised fashion 2 h prior to each experimental visit. The duration between the first and second visit was at least 48 hours to ensure sufficient recovery, with a washout period >10 days and <16 days between the second and third trials. A 14-day washout period was found to allow biochemical and biomarkers of antioxidant status to return to baseline values after one month of daily (306 mg) anthocyanin (strawberry) intake (Alvarez-Suarez et al., 2014). Participants were instructed to arrive at each visit in a euhydrated state, abstain from strenuous exercise and alcohol 24 hours before, and caffeine on the day of testing. Aside from these restrictions, maintenance and consistency of their typical weekly exercise schedule over the course of the study was encouraged. To reduce any effect of diet, participants were informed to ingest only water for 2 h prior to experimental testing and food diaries were issued to complete in the 48 h before visit 2. The diaries were handed back to the participants with instructions to replicate in the 48 hours prior to visit 3 and to indicate any deviation of dietary intake that occurred. Adherence to diet replication was confirmed before subsequent testing. During this period, participants were instructed to follow their typical diet, including their typical pre-race meal (>2 h before arrival) for greater ecological validity. Although some studies restrict food-derived anthocyanin sources before exercise testing (Keane et al., 2018), this is thought to limit the ecological validity of nutrition research (Close et al., 2019) as any effect of supplementation could be exaggerated (Morehen et al., 2021). Participants were asked whether they experienced any side effects of supplementation (e.g., gastrointestinal symptoms including nausea, vomiting, stomach pain, bloating, diarrhoea and constipation) at the end of visit 2 and 3. The ambient laboratory conditions (temperature: 21.5 ± 1.3 ºC, humidity: 63 ± 7 %) were controlled to ensure consistency between visits.

***Procedures***

*Graded incremental test*

After assessment of anthropometric characteristics, participants completed a graded incremental treadmill test to volitional exhaustion on a motorised treadmill (H/P Cosmos, Pulsar, Nussdorf-Traunstein, Germany) comprising 4-minute stages and increments of 1 km.h-1. As the determination of individualised speed at LT1 was required for subsequent visits, starting speed corresponded to 70% of their 10 km pace, or 80% of marathon pace (Dantas & Doria, 2015); if neither were known then 8 km·h-1 was selected. LT1 was defined as the running speed at which blood lactate first rose above resting values (Jones et al., 1999). This exercise intensity was selected due to a) its association with a predictable steady state metabolic response and b) its ability to appropriately account for individual fitness (more so than a % of V̇O2max) because it demarcates the transition from the moderate to heavy exercise domain. An online gas analyser (Cosmed Quark RMR, Cosmed S.r.l., Rome, Italy) measured breath by breath V̇O2, RER, and HR (Garmin premium HR, Garmin Ltd, Kansas, USA). Rating of perceived exertion (Borg, 1982) and B[La] (Lactate Pro II, Arkray, Kyoto, Japan) were measured during the last 30 seconds of each stage. Criterion for achievement of V̇O2max was defined as a plateau (<3%) in the oxygen uptake exercise intensity relationship, respiratory exchange ratio ≥1.15, HR within 10 b·min-1 of age-predicted maximum, RPE 19-20, BLa >8 mmol-1 and volitional exhaustion (Winter, 2006). Regular verbal encouragement was given to ensure maximum effort.

*Exercise at the lactate threshold*

Upon arrival to the laboratory for the second and third visits, participants assumed a seated position for 5 min before blood pressure of the brachial artery was measured (Omron, M5- I, Omron, Matsusaka Co. Ltd, Japan) to determine systolic, diastolic, and mean arterial pressure (MAP) values, which provided a measure of cardiovascular function at rest. Then, participants completed a 10-minute run at their corresponding LT1 running speed, as previously determined. Heart rate and breath-by-breath analysis were recorded throughout the 10-minute trial to measure effects of NZBC extract on substrate oxidation and gaseous exchange, with measurement of RPE in the final minute and B[La] upon completion.

*Time-trial performance test*

Following 5 min rest, participants completed a self-paced 5 km run as fast as possible from a standing start. Low intra-subject variability of the same distance and protocol has previously been reported (CV 1.5 ± 0.6%, *r* = 0.99) in competitive male runners (Fisher et al., 2017). Fast acceleration to the desired running speed was achieved via a built-in treadmill function, which participants controlled, having been familiarised with the process in their first laboratory visit. Thereafter, participants manually altered their own treadmill speed using up and down arrows but all metrics apart from the cumulative distance were occluded to prevent fixed pacing. Time to complete the 5-km TT, split times of each km, and V̇O2, RER, V̇E, fat oxidation, carbohydrate oxidation, and HR were measured continuously. RPE, B[La] and average running speed were measured on completion of each kilometre. Participants then returned to the laboratory for the third and final visit where they received either placebo or NZBC extract. All procedures as completed during the second visit were then repeated.

*Supplemental protocol*

The supplements were distributed at the end of visit 1 and visit 2 in a randomised and double-blind fashion. A study by Hurst et al. (Hurst et al., 2019) reported peak plasma anthocyanin concentration occurred after 2 hours of ingestion of NZBC extract, therefore in accordance with these findings athletes were instructed to ingest the supplement, 2 h prior to experimental trials, which was confirmed upon arrival. The three NZBC (3 x 300 mg active cassis containing a total of 315 mg anthocyanins) and placebo (3 x 300 mg gluten-free flour) capsules were opaque and hence were not distinguishable by the researcher or participant. The ingredients for NZBC extract (315 mg anthocyanins, 35–50 % delphinidin- 3-rutinoside, 5–20 % delphinidin-3-glucoside, 30–45 % cyanidin-3-rutinoside, 3–10 % cyanidin-3-glucoside; CurraNZTM, Health Currancy Ltd., Surrey, UK) have previously been reported by Cook et al. (2015).

**Statistical analysis**

Data was analysed using SPSS v26 (SPSS Inc., Chicago, IL, USA). Data normality was assessed using Shapiro-Wilk test. Homogeneity was tested using Mulchey’s Test of Sphericity, with Greenhouse-Geiser adjustments used for violations (P<0.05). Paired samples *t-*tests were used to compare responses between NZBC extract and placebo conditions for the 10-min exercise at LT. A 2-way repeated measures analysis of variance (RM-ANOVA) (condition [2], distance [5]) was used to compare differences between NZBC extract and placebo over each km for all variables, with completion of subsequent post-hoc tests (Bonferroni) for significant effects. Analysis was supplemented by calculation of effect sizes (Cohens *d*) with accompanying 95% confidence intervals, considered as small (0.2-0.59), moderate (0.6-1.19), large (1.2-2.0) and very large (>2.0) (Hopkins et al., 2009). The following equations were used to calculate rates of whole-body fat and carbohydrate oxidation (g·min-1) during exercise: Carbohydrate oxidation = (4.55 × VCO2) – (3.21 × VO2), Fat oxidation = (1.67 × VO2) – (1.67 × VCO2). Equations were modified to account for nitrogen production as recommended (Frayn, 1983). In instances where the RER value ≥1, a value of 0 was inserted for fat oxidation analysis. Data is expressed as mean ± standard deviation whilst significance was set at alpha level *P* ≤ 0.05.

**Results**

***Mean arterial pressure***

Mean arterial pressure after supplementation was not different between conditions (NZBC: 94 ± 4 mmHg, Placebo: 93 ± 4 mmHg, *P*= 0.33). Systolic and diastolic pressures were 125 ± 6 / 77 ± 5 mmHg for the placebo and 125 ± 5 / 79 ± 5 mmHg for the NZBC condition, respectively.

***Exercise at the lactate threshold***

During the run at LT1, there were no significant differences between NZBC and placebo for V̇O2, RER, V̇E, carbohydrate oxidation, fat oxidation, HR, B[La] or RPE, (*P>0.05*; Table 1).

**\*\*\* Insert Table 1 here \*\*\***

***5-km running Time-Trial***

*Completion time and running speed*

The NZBC extract condition reduced the time taken to complete the 5-km TT compared to placebo (*P* = 0.001; NZBC: 1308.96 ± 122.36 s, Placebo: 1346.33 ± 124.44 s, *d*=-0.23, CI range= -0.38 to -0.08) with a group mean reduction of 2.9 ± 2.97 % (range -0.9 to 5.7 %) and 14 of the 16 participants improving their performance time (Fig 1A). Although there were no significant interaction effects, small effect sizes favoured NZBC extract, evidenced by faster times at km 1 (271 ± 25 *cf.* 280 ± 28 s; *d*=-0.25, CI range=-0.47 to -0.02), 2 (258 ± 27 *cf.* 269 ± 28 s; *d*=-0.30, CI range= -0.59 to -0.01) and 4 (265 ± 29 *cf.* 273 ± 27 s; *d*=0.23, CI range=-0.46 to 0.00). Time taken to complete each km alongside running speed can be found in Fig 2a/b.

**\*\*\* Insert Figure 1A and 1B here \*\*\***

**\*\*\* Insert Figure 2A and 2B here \*\*\***

*Physiological and metabolic variables*

No significant main effects for condition or interaction effects were observed for V̇O2 (ml⋅kg-1⋅min-1), RER, VE, HR, B[La], RPE carbohydrate oxidation or fat oxidation (*P*>0.05). However, significant effects for distance were found for all variables (all *P*<0.001 to P=0.017). All data can be found in Table 2.

**Discussion**

The principal finding of the current study is that an acute dose (315 mg anthocyanin) of NZBC extract resulted in faster completion (38 s, ~3%) of a 5-km treadmill running TT when compared to a placebo. Faster running speeds in NZBC were produced without significant changes in the physiological, metabolic and perceptual responses to the TT. There were also no significant differences in responses to exercise at the lactate threshold between conditions. This study is the first to investigate and advocate an acute dose of NZBC extract taken 2 h before exercise to improve 5 km endurance performance in well-trained runners.

The 3% (38 s, *d* = 0.23) improvement in 5-km running performance after ingestion of NZBC extract is likely to be meaningful, given the typical variation in 5 km running time in trained runners (1.5%/14.25 s) and the magnitude of the observed effect size (Fisher et al., 2017). Assessment of individual responses revealed that eleven participants improved by more than 14.25 s (range 34 – 126 s). Percentage improvements were similar to most previous TT studies (1.0 - 2.4%) (Braakhuis et al., 2014; Cook et al., 2015; Murphy et al., 2017) and are consistent with the small significant effect size (0.45) shown in a meta-analysis of NZBC on time-to-fatigue and TT performance tests (Braakhuis et al., 2020). Importantly though, all previous studies reporting performance improvements used a loading phase of daily blackcurrant anthocyanin supplementation (typical dose: 105 – 210 mg) for at least 7 days prior to exercise. In the two studies to test the effects of an acute dose (105 and 210 mg anthocyanin, (Montanari et al., 2020), and 300 mg (Montanari et al., 2023)), no benefit on 16.1 km cycling time-trial performance was reported. Interestingly though, upon completion of a sub-group analysis, the Montanari et al. ( 2023) study found that slower cyclists (<1400 s) performed quicker in the NZBC extract condition with a comparable effect size to that of the present study (*d*=-0.23). Therefore, notwithstanding the potential role that training status might play, these studies suggest that performance enhancement after ingesting a single dose of NZBC extract (3 capsules; 315 mg anthocyanins taken 2 h pre-exercise) is comparable to taking one or two daily capsules for a week up until the day of performance. These findings are valuable to athletes and exercisers, making it possible to confer benefits via a more practical and cost-effective supplementation strategy, without any reported negative side-effects from the prescribed dose.

The faster performance times in the TT were accomplished without participants

experiencing elevated physiological and metabolic responses. The exercise at the lactate threshold, undertaken in an attempt to better understand the possible mechanisms for performance changes, revealed a small reduction in B[La] with NZBC (*d*=-0.22), however the accompanying confidence intervals are compatible with the effect being substantially positive (*d*=0.21) and negative (-0.65). Reductions in B[La] in endurance-trained males supplementing with NZBC have previously been reported (Willems et al., 2015) although another study reported no change (Montanari et al., 2020). Willems et al. (2015) found that NZBC powder (138.6 mg daily anthocyanins for 7 days) mediated a downward and rightward lactate shift (13-27% lower) during a steady-state non-continuous incremental exercise test (40-70% of maximal power output), suggesting that supplementation might influence lactate appearance and removal mechanisms. Acute dosing of blackcurrant anthocyanin can increase peripheral blood flow (Matsumoto et al., 2005) and vasodilation via activation of endothelial nitric oxide synthase and subsequent nitric oxide production in-vitro (Ziberna et al., 2013). Therefore, improved performance outcomes might be explained by arterial dilation and increased blood flow to the working muscle, which is also associated with greater lactate clearance (Willems et al., 2015). Further work should assess the role of NZBC extract specific anthocyanin metabolite profiles, as metabolites in similar compounds (e.g. Montmorency tart cherry) have been identified as possible drivers of change in vasculature (Keane et al., 2016).

We observed no further differences in the physiological, metabolic and perceptual responses to the exercise at lactate threshold and 5 km TT with acute NZBC extract supplementation, therefore the precise mechanisms for improved performance are unclear. Whilst the faster TT performances in NZBC occurring for similar physiological responses could be interpreted as a change in the relationship between exercise intensity and physiological response, we are cautious about interpreting non-significant effects as being statistically equivalent.

Changes in cardiovascular function with chronic NZBC supplementation have been reported; specifically, increases in cardiac output at rest (Cook, Myers, et al., 2017a; Willems et al., 2015) and during submaximal isometric contractions (Cook, Myers, Gault, & Willems, 2017), as well as increases in peripheral blood flow (Matsumoto et al., 2005). However, these findings are not consistent (Willems et al., 2015). Cardiac output and peripheral blood flow were not measured in our study, and it is possible that they contributed to changes in performance; however, we observed similar heart rates between conditions (178 *cf.* 179 b⋅min-1) in the TT and during exercise at the lactate threshold (152 *cf.* 156 b⋅min-1), and so this aspect of cardiovascular function is unlikely to be affected by acute NZBC supplementation. The extent to which cardiovascular changes occur appear to be dose-dependent (Cook, Myers, et al., 2017b). Future studies should therefore examine the effect of a similar acute dose of NZBC extract (315 mg anthocyanins) on cardiovascular function.

Improved muscle oxygenation was recently reported during isometric (Fryer et al., 2020) and submaximal intermittent exercise (Cook, Myers, et al., 2017b) after a 7-day intake of NZBC extract (210 mg anthocyanins). Increased oxygen delivery could increase the contribution of aerobic metabolism and subsequently result in reduced production of blood lactate and associated metabolites (Bassett & Howley, 2000). However, other studies have failed to show that NZBC influences the oxygen cost of exercise (138.6 mg daily anthocyanins for 7 days; (Willems et al., 2015), which agrees with our data during exercise at the lactate threshold and the TT. This suggests that enhanced TT performance with acute NZBC supplementation is not due to a NO-mediated change in oxygen consumption during exercise.

Substrate oxidation remained similar between conditions during exercise at the lactate threshold (NZBC: 0.40 g⋅min−1; PLA: 0.39 g⋅min−1). Our results do not support the augmentation in whole-body fat oxidation during exercise previously reported in trained cyclists after a 7-day daily dosing period (105 mg anthocyanins, 10-120 min, 45-65% VO2 max) (Cook et al., 2015; Cook, Myers, et al., 2017b; Strauss et al., 2018) or when a comparative dose (to the current study; 315 mg anthocyanins) was used for 7 days (Cook, Myers, et al., 2017b). Our fat oxidation values are similar to those studies delivering lower dosages acutely 2 h prior to exercise (300 g 0.34 g⋅min−1; 600 g 0.40 g⋅min−1, (Montanari et al., 2020). Collectively, results suggest that the mechanisms responsible for initiating lipolysis might be dependent on cumulative, or more consistent (e.g., daily) delivery of NZBC. The suggested mechanism that NZBC anthocyanins or their metabolites influence the key proteins regulating lipolysis (Strauss et al., 2018) requires further study.

This study has some limitations. Firstly, it did not control for or analyse anthocyanin intake from meals prior to exercise. Although this ensured that participants consumed and replicated a pre-race meal that was ecologically valid for all visits, knowledge of prior anthocyanin ingestion would have been useful to determine the extent to which the anthocyanin content of foods could have influenced the outcome. However, previous studies show average dietary anthocyanin intake to be low (e.g., 12.5 mg/day in the US (Wu et al., 2006), and 19.8 - 64.9 mg/day and 17.7 – 44.1 mg/day in European males and females (Zamora-Ros et al., 2011), respectively) in relation to the larger dose (~315 mg) provided in the current study. Secondly, our study did not assess all plausible mechanisms that could account for performance differences with administration of the selected dose, which should be assessed in future studies.

In conclusion, this is the first study to demonstrate meaningful improvements in 5 km running performance with ingestion of a single dose (3 capsules; 315 mg anthocyanins) of NZBC extract. Assessment of a wider range of mechanisms (e.g., cardiovascular function measures, metabolite profiles) could advance insight into the enhanced TT performance with an acute dose.

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**Figures:** 2

**Tables:** 2

**Table 1**. Physiological and metabolic responses to 10-minute exercise at the lactate threshold. Data are mean ± SD with effect sizes ± 95% confidence intervals.

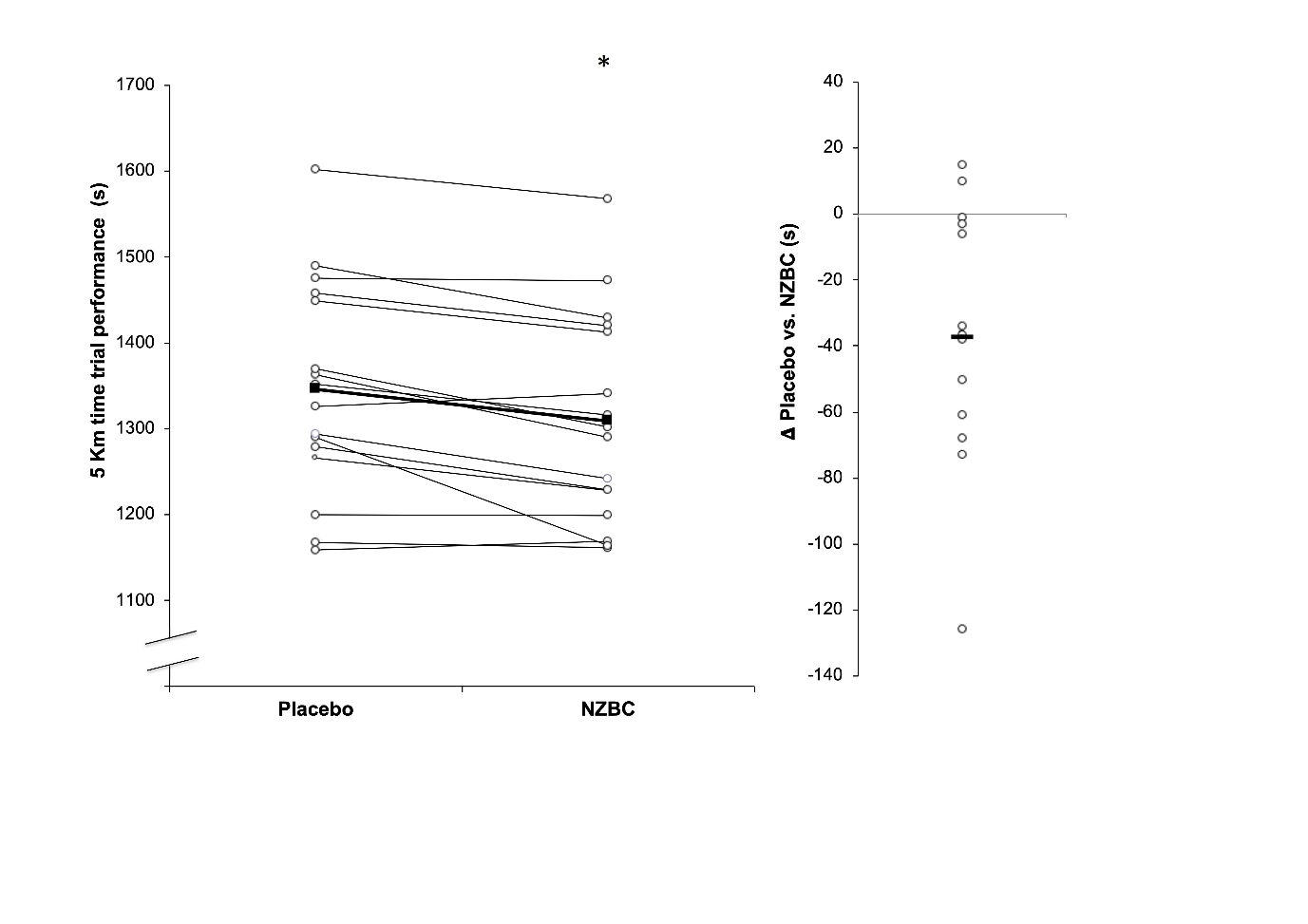
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Placebo** | **NZBC** | ***d*** | **CI (range)** |
| V̇O2 (L⋅min-1) | 3.08 ± 0.26 | 3.03 ± 0.32 | -0.14 | (-0.38 - 0.11) |
| V̇O2 (ml⋅kg-1⋅min-1***)*** | 41.98 ± 4.61 | 41.24 ± 4.43 | -0.12 | (-0.32 - 0.08) |
| V̇O2 (% of max) | 76.01 ± 5.92 | 74.79 ± 7.11 | -0.16 | (-0.43 – 0.12) |
| V̇CO2 (L⋅min-1) | 2.86 ± 0.31 | 2.79 ± 0.28 | -0.16 | (-0.45 – 0.13) |
| RER | 0.93 ± 0.06 | 0.92 ± 0.05 | 0.09 | (-0.60 – 0.41) |
| V̇E (L⋅min-1) | 83.13 ± 12.71 | 83.38 ± 11.50 | 0.02 | (-0.10 – 0.13) |
| CHox (g⋅min−1) | 3.12 ± 0.89 | 2.98 ± 0.56 | -0.12 | (-0.55 –0.31) |
| FATox (g⋅min−1) | 0.39 ± 0.27 | 0.40 ± 0.20 | 0.04 | (-0.41 – 0.50 ) |
| Heart rate (b⋅min-1) | 152 ± 15 | 156 ± 14 | 0.17 | (0.00 – 0.35) |
| Lactate (mmol⋅L-1) | 2.88 ± 1.86 | 2.33 ± 1.11 | -0.22 | (-0.65 – 0.20) |
| RPE (AU) | 11.6 ± 1.41 | 11.4 ± 1.45 | -0.10 | (-0.43 – 0.23) |

Note: CHox = Carbohydrate oxidation;FATox = Fat oxidation

**Table 2** **Physiological and metabolic data recorded during the 5 km running time-trial. Data are mean ± SD.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Condition** | **1 km** | **2 km** | **3 km** | **4 km** | **5 km** | **Overall** | ***d* and CI (range)** |
| **V̇O2**(ml⋅kg-1⋅min-1)  NZBC  Placebo | 43.66 ± 4.16  44.55 ± 4.15 | 50.47 ± 4.76  49.81 ± 5.19 | 50.85 ± 5.14  50.42 ± 5.44 | 51.20 ± 5.55  50.45 ± 5.29 | 52.24 ± 5.54  51.54 ± 5.39 | 49.68 ± 4.87  49.35 ± 4.91 | 0.05  (-0.15 – 0.25) |
| **V̇O2**(L⋅min-1)  NZBC  Placebo | 3.20± 0.30  3.27 ± 0.27 | 3.70 ± 0.28  3.65 ± 0.30 | 3.72 ± 0.27  3.69 ± 0. 30 | 3.75 ± 0.26  3. 69 ± 0.27 | 3.82 ± 0.26  3.77 ± 0.27 | 3.64 ± 0.26  3.61 ± 0.26 | 0.07  (-0.21 – 0.35) |
| **V̇CO2**(L⋅min-1)  NZBC  Placebo | 3.04 ± 0.35  3.07 ± 0.37 | 3.65± 0.36  3.56 ± 0.36 | 3.63± 0.32  3.56± 0.30 | 3.62± 0.31  3.53± 0.25 | 3.74± 0.33  3.64 ± 0.27 | 3.53 ± 0.30  3.47 ± 0.28 | 0.17  (-0.16 – 0.49) |
| **RER** (AU)  NZBC  Placebo | 0.95 ± 0.08  0.94 ± 0.07 | 0.99 ± 0.06  0.98 ± 0.05 | 0.98 ± 0.06  0.97 ± 0.05 | 0.97 ± 0.05  0.96 ± 0.05 | 0.98 ± 0.06  0.97 ±0.05 | 0.97 ± 0.06  0.96 ± 0.05 | 0.15  (-0.40 – 0.69) |
| **V̇E** (L⋅min-1)  NZBC  Placebo | 95.31 ± 12.78  94.02 ± 13.84 | 119.12 ± 18.63  113.80 ± 15.24 | 125.31 ± 19.91  119.81 ± 16.21 | 129.43 ± 19.28  124.10 ± 17.64 | 138.17 ± 18.12  132.57 ± 16.69 | 121.46 ± 17.06  116.86 ± 15.14 | 0.23  (0.10 – 0.59) |
| **Heart rate** (b⋅min-1)  NZBC  Placebo | 164 ± 12  164 ± 13 | 178 ± 11  177 ± 12 | 181 ± 11  181 ± 11 | 184 ± 10  182 ± 11 | 188 ± 10  185 ± 11 | 179 ± 10  178 ± 11 | 0.09  (-0.08 – 0.30) |
| **Blood Lactate** (mmol⋅L-1)  NZBC  Placebo | 6.54 ± 3.0  5.62 ± 3.55 | 8.61 ± 4.85  7.52 ± 5.17 | 9.16 ± 4.75  10.02 ± 6.52 | 9.88 ± 3.11  8.96 ± 3.59 | 12.21 ± 3.34  10.48 ± 3.57 | 9.28 ± 3.15  8.52 ± 3.86 | 0.15  (-0.08 – 0.38) |
| **RPE** (AU)  NZBC  Placebo | 13 ± 1  13 ± 2 | 15 ± 1  14 ± 1 | 16 ± 1  15 ± 2 | 17 ± 1  17 ± 2 | 19 ± 1  19 ± 2 | 16 ± 2  16 ± 2 | 0.18  (-0.35 – 0.26) |
| **Carbohydrate oxidation** (g⋅min−1)  NZBC  Placebo | 3.56 ± 1.03  3.49 ± 1.10 | 4.72 ± 1.08  4.47 ± 0.97 | 4.57 ± 0.95  4.36 ± 0.81 | 4.45 ± 0.90  4.23 ± 0.75 | 4.73 ± 1.03  4.43 ± 0.86 | 4.40 ± 0.90  4.19 ± 0.82 | 0.19  ( -0.31 – 0.69) |
| **Fat oxidation** (g⋅min−1)  NZBC  Placebo | 0.32 ± 0.24  0.36 ± 0.30 | 0.18 ± 0.23  0.22 ± 0.25 | 0.22 ± 0.21  0.24 ± 0.27 | 0.26 ± 0.23  0.29 ± 0.27 | 0.23 ± 0.22  0.26 ± 0.29 | 0.24 ± 0.21  0.27 ± 0.26 | -0.10  (-0.57 – 0.37) |

**List of Figures**

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**Figure 1A.** Overall time during a 5-km running time-trial. Bold black line and squares show group mean ± SD. Individual responses are shown with lighter black lines. \* indicates significantly faster 5-km time-trial in NZBC (*P*=0.001). **1B.** shows individual differences (circles) in seconds between placebo and NZBC.



**Figure 2A**. Running speed (km/ h) and **2B.** completion time per km (s) throughout the 5 km time-trial.

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