



**Acute Effects of New Zealand Blackcurrant Extract on Exercise
Performance: Implications of the Dose-Response Relationship and Use
Under Simulated Altitude**

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LIST OF ABBREVIATIONS

ANOVA Analysis of Variance

ADP Adenosine Diphosphate

ATP Adenosine Triphosphate

A-vO₂ Arteriovenous Oxygen Difference

BPM Beats Per Minute

BRJ Beetroot Juice

CO Cardiac Output

CV Cardiovascular

DBP Diastolic Blood Pressure

DNA Deoxyribonucleic Acid

eNOS Endothelial Nitric Oxide Synthase

EPO Erythropoietin

FiO₂ Fraction of Inspired Oxygen

HH Hypobaric Hypoxia

HR Heart Rate

HRmax Maximal Heart Rate

IGF-1 Insulin-like Growth Factor 1

Km Kilometre

[La] Blood Lactate

MAO-B Monoamine Oxidase-B

ml millilitre

mmol/L Millimoles per litre

mOsm/kg Milliosmoles per kg

mRNA Messenger RNA

NH Normobaric Hypoxia

nMol/L Nanomoles per litre

NO Nitric Oxide

NOS Nitric Oxide Synthase

NZBC New Zealand Blackcurrant Extract

OBLA Onset of Blood Lactate Accumulation

ORAC Oxygen Radical Absorbance Capacity

PAR-Q Physical Activity Readiness Questionnaire

PCO_2 Partial Pressure of Carbon Dioxide

PCr (Phosphocreatine / Creatine phosphate)

PDH Pyruvate Dehydrogenase

PO Power Output

PP Pulse Pressure

ROS Reactive Oxygen Species

RPE Rating of Perceived Exertion

SaO_2 Arterial Oxygen Saturation

SpO_2 Peripheral Oxygen Saturation

StO_2 Tissue Oxygen Saturation

SBP Systolic Blood Pressure

SV Stroke Volume

TPR Total Peripheral Resistance

TT Time Trial

VO_2 max Maximal Oxygen Consumption

VO_2 Volume of Oxygen Consumption

WHO World Health Organisation

0.0 ABSTRACT

Purpose: Recreational and elite sporting performers may look to use ergogenic aids as a means of improving their training ability and enhancing recovery and performance on competition days. New Zealand Blackcurrant Extract (NZBC) is an ergogenic aid that has been shown to improve endurance performance in time trial (TT) cycling protocols. Previous research has found significant improvements in medium duration TT performance related disciplines following chronic intake of NZBC over a multi-day period. Currently no research has addressed the potential acute effects of NZBC on similar exercise disciplines or TT performance under simulated altitude. The following research sought to explore the acute effects of NZBC intake on 10 kilometre (km) TT cycling performance. **Methods:** Two experimental studies were carried out as part of this research. The first study examined the acute, dose-response effects of NZBC intake on short term cycling performance following ingestion of 300, 600 or 900 mg of NZBC. The second study examined the influence of acute NZBC intake on TT cycling performance under simulated altitude. **Results:** Acute NZBC intake had a strong, significant trend towards faster TT completion ($P = 0.053$) and a significant improvement in peak power output (PO) following intake of 900 mg when compared to 600 mg NZBC ($P < 0.05$). Additionally, peak blood lactate ([La]) values and [La] removal were greatest following 900 mg of NZBC, indicating post-exercise recovery benefits. Acute NZBC intake had no significant effect on TT cycling performance at simulated altitude or any parameters of endurance performance when compared to a placebo (PLA). **Conclusion:** Although acute NZBC intake has no significant effects on medium duration TT cycling performance, further research is required to determine if these findings would be replicated if a chronic, dose-response intake protocol of NZBC is implemented for exercise under both normoxic and hypoxic conditions.

Keywords: Blackcurrant, Cycling, Time Trial, Dose, Hypoxia

1.0 THESIS OVERVIEW

1.1 THESIS JUSTIFICATION

One of the five primary components of physical fitness is cardiorespiratory endurance, defined as “the ability of the circulatory and respiratory systems to supply fuel during sustained physical activity and to eliminate waste products after supplying fuel” [1]. During prolonged periods of physical activity, cardiorespiratory endurance is determined through the interaction of maximal oxygen uptake (VO_2 max) and the [La] threshold, termed “performance VO_2 ” [2]. The speed or PO that is generated at the point of performance VO_2 determines exercise efficiency in endurance-based exercise disciplines [2]. For this reason, race-based athletic events have become the primary source of determining cardiorespiratory endurance relative to simulated exercise competition [3]. Short and long-term improvements in cardiorespiratory endurance can assist athletes performance when undertaking race-based performance disciplines [4].

Regular training improves cardiorespiratory endurance and significant effects can be seen irrespective of age [5] or training ability [6] from baseline. Combination training interventions such as endurance-resistance exercise protocols can amplify these effects to a greater extent than a single training modality [7] over the same period of time. The benefits of long-term adaption are important for consistent improvements in endurance performance. But many athletes may seek methods of acute physiological stimulation as a means of giving themselves an edge both physically and mentally for upcoming exercise tasks, particularly in race-based performance events. One of these methods is the intake of ergogenic aids, especially nutritional supplements that contain ingredients linked to physiological adaptations leading to improved performance when consumed. One ergogenic aid that has been studied extensively in recent years is NZBC which has been shown to improve endurance performance in different exercise modalities and disciplines. There are still gaps

missing in the current literature, particularly when analysing duration of intake prior to exercise and the environment in which individuals undertake exercise following intake.

1.2 SIGNIFICANCE OF THE RESEARCH

The two experimental studies that form the basis of this thesis aim to build on current areas of research that analyse the effects of NZBC intake on cardiorespiratory performance. Supplement use within the UK's athletic population is widespread with around 48% of young elite athletes taking at least one ergogenic aid [8], a figure identical to a sample of elite Polish athletes [9]. A body of evidence suggests that intake of particular supplements in specific performance-based scenarios leads to a marginal but significant improvement on maximal and submaximal endurance performance [10]. The presented research builds on previous research firstly by including an endurance exercise protocol, typically similar to protocols used in previous research, following ergogenic aid supplementation. Secondly an acute supplementation protocol of NZBC with emphasis on identifying a dose-response effect was implemented, and lastly by analysing the effects that occur following acute NZBC intake on endurance performance in simulated altitude.

The studies aim to produce novel findings in previously unresearched areas. The testing protocols used to achieve this were created following a review of previous exercise data, methodologies, results, ideas and testing procedures from peer reviewed research studies and literature. An extensive online review of the available literature relating to NZBC supplementation and human performance formed the development of the experimental chapters within this thesis. The findings from the experimental studies help to improve an athlete's knowledge of ergogenic aid supplementation when it comes to cardiorespiratory based fitness and performance adaptations.

1.3 THESIS AIMS

The subject of this thesis aims to identify immediate changes in exercise performance following a single bolus of NZBC during submaximal time trial cycling performance in a dose-dependent manner and under simulated altitude. In order for these aims to be met, two studies were conducted:

Study 1: Dose Response Effects of NZBC on Endurance Cycling Performance.

Specific aims for this study were:-

1. To identify the magnitude of any acute physiological effects on cycling performance and parameters of endurance performance, including $\dot{V}O_2$, volume of oxygen uptake ($\dot{V}O_2$) and heart rate (HR) following consumption of a single dose of NZBC at three different doses
2. To identify whether the proposed effects on pre-, during and post-exercise variables, including resting values for HR, blood pressure, cardiac output (CO), total peripheral resistance (TPR) and [La] are greater following higher doses of NZBC

Study 2: Acute effects of NZBC on Endurance Performance in Simulated Altitude.

Specific aims for this study were:-

1. To identify whether acute intake of NZBC has any effects on time trial cycling performance or physiological parameters under simulated altitude

1.4 THESIS STRUCTURE

This current section along with the literature review in section 2 forms the rationale and general basis for the current thesis. In section 3, the first experimental study is presented, designed to examine (i) the acute effects of NZBC and (ii) the dose-response effects of NZBC on endurance cycling performance and physiological parameters of endurance performance. In section 4, the second experimental study

is presented, designed to examine the influence of acute NZBC intake on endurance performance in simulated altitude. Section 5 forms the final part of the thesis and summarises the findings from the two research studies, compares the results relative to specific athletic populations and provides recommendations for future research in this area.

2.0 LITERATURE REVIEW

2.1 SYNOPSIS

The primary aim of this review is to analyse the findings of studies which examine the influence of NZBC on exercise performance, specifically, the purported effects of supplements on exercise performance following intake. Firstly, the definition of endurance performance and parameters that are used to measure this type of performance will be discussed to identify the variables that will be analysed in the upcoming research studies. Following this, an introduction to ergogenic aids and the potential for the enhancement of endurance performance post ingestion will be addressed. Details focusing specifically on NZBC with regards to its nutritional properties and physiological processes that occur following ingestion will be provided. These adaptations will then be linked to the resulting enhancement of endurance performance.

Following this, the effects of NZBC during cycling, particularly with regards to the dose-response relationship is discussed. Finally, the influence of supplements on exercise under simulated altitude is analysed and the potential effects following NZBC intake are taken into consideration. The potential effects of acute intake of NZBC will be taken into account in both situations. During this review, the formation of the study protocols and statistical analysis were formed based on the rationale behind previous research which included observations of exercise protocols included in the selected studies.

During the construction of the literature review, a selection of key search terms were identified. These can be found in the appendices section, accompanying this thesis.

2.2 LITERATURE REVIEW METHODOLOGY

An online search was carried out on the following databases: Google Scholar, ResearchGate, MEDLINE, PubMed, SPORTDiscus and the Web of Science. A total

of 391 studies published in the English language between 1986 and 2019 were analysed. For study inclusion, abstracts were judged against the following eligibility criteria: exercise, supplementation and physiological testing protocols were undertaken in human subjects; supplementation protocols were short term (< 6 weeks) to acute (< 2 intake periods); and the results of NZBC consumption were intended for use and application in the recreational and elite athletic populations.

Systematic review publications were included if the conclusion was defined from the eligibility criteria. A total of 126 abstracts were excluded from the final analysis. Initially, studies were excluded if the full text paper was unavailable, the research was not published in a peer reviewed journal, the paper formed part of a postgraduate thesis or if the study was not published in English language and could not be translated. In terms of specific research methodology, studies initially included in the final analysis were excluded if (1) animals such as laboratory rats or mice were selected as the study sample, (2) polyphenol consumption was not included and (3) the time of the intervention protocols lasted for a duration greater than 6 weeks.. These criteria led to the exclusion of a further 54 studies. Full details of the production of the literature review are presented in figure 1.

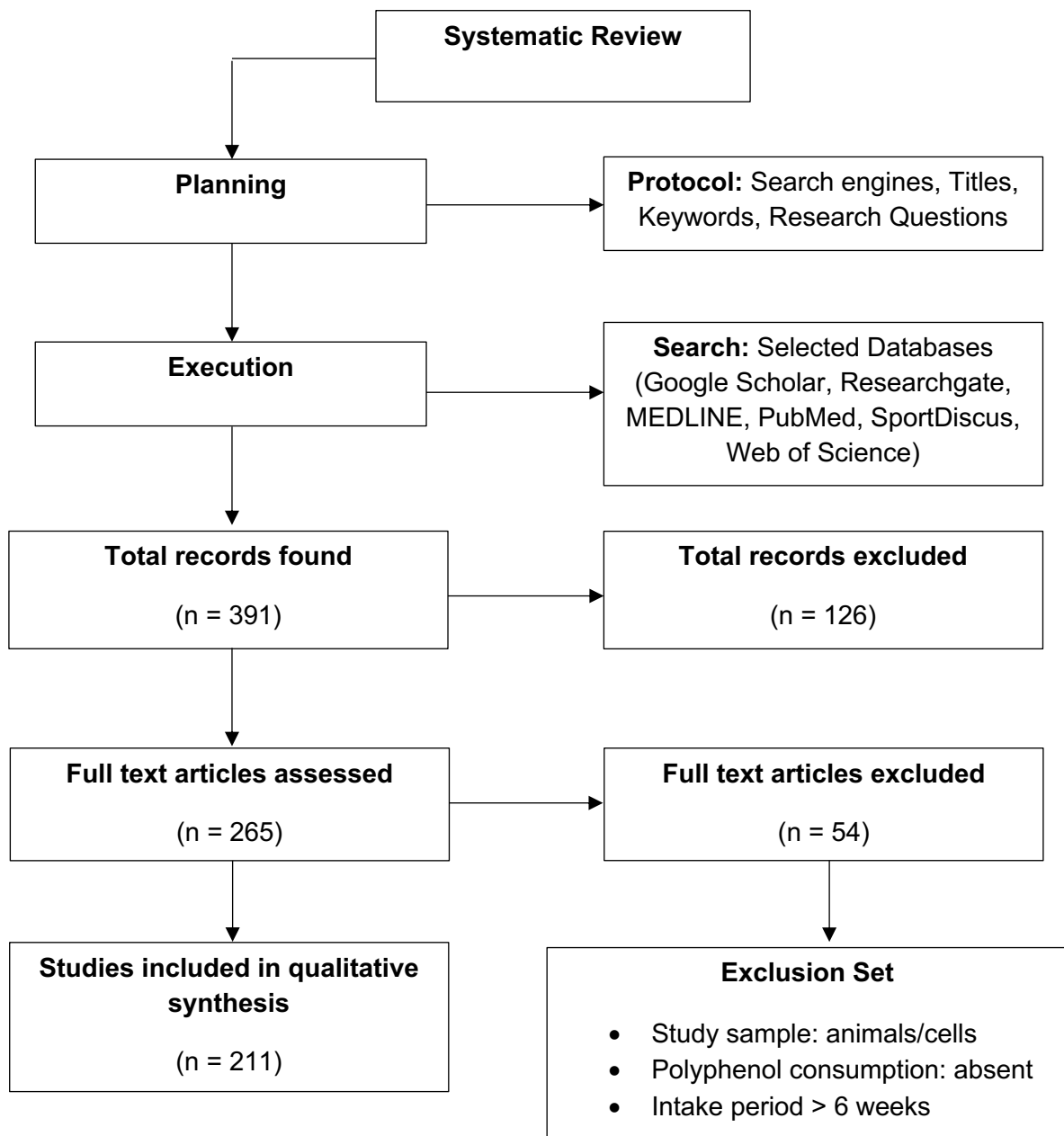


Figure 1. PRISMA flow diagram for studies included in the literature review

Section 2.3 provides a description of cardiorespiratory fitness and how it is defined and measured in different populations. This includes an analysis of physiological factors that determine cardiorespiratory fitness, the type of exercise tests that are undertaken by athletes to measure these factors and how cardiorespiratory performance is interpreted depending on the results of exercise tests.

Following this, section 2.4 focuses on ergogenic aid consumption as a method of improving cardiorespiratory performance. This includes a general overview of literature analysing the effects of different nutritional supplements on cardiorespiratory endurance, how these effects might be different in comparison to power and sprint-based exercise and the rate of supplementation in trained athletic populations. Subsections 2.4.1 and 2.4.2 specifically review antioxidant intake with particular reference to polyphenol intake and the effects this had on exercise performance following both a single intake (acute) and numerous intakes over a multi-day period (chronic).

Section 2.5 provides an analysis of NZBC, an ergogenic aid with extensive, recent literature that has previously been shown to improve cardiorespiratory performance both at rest and during exercise. This includes a specification of the unique chemical properties and nutritional content of NZBC and the physiological adaptations associated with consumption over a particular period of time. Section 2.6 details literature specifically based on post ingestion exercise effects in cycling, running and rowing disciplines along with evidence for dose-response effects.

Section 2.7 provides an analysis of literature based on exercise in hypoxia. This section compares fundamental differences of real altitude against simulated altitude protocols, the cardiorespiratory, musculoskeletal and metabolic adaptations associated with a reduction in VO_2 and whether exercise responses in hypoxia are influenced by individual training status. Finally section 2.8 includes an analysis of research surrounding NZBC intake on exercise performance in hypoxia, antioxidant and polyphenol activity influencing physiological responses and adaptations to hypoxia exposure and the potential for changes in cognitive markers of exercise performance.

2.3 DEFINITION AND MEASUREMENT OF ENDURANCE PERFORMANCE

Endurance based exercises such as running, cycling or rowing, are activities that are undertaken to improve cardiorespiratory endurance. Performing any of these sports involves repetitive actions that use large muscle groups for an extended period of time. Oxidative phosphorylation forms the primary metabolic pathway during this form of exercise to regenerate adenosine triphosphate (ATP) for energy production. Endurance performance is the product of $\dot{V}O_2$ (the amount of oxygen taken around the body in one minute) multiplied by the arteriovenous oxygen difference ($A-vO_2$; the ability of the skeletal muscles to extract oxygen) [11]. Basset and Howley [12] determined that endurance performance is limited by four primary factors, three of which are central and one of which is peripheral. These include (1) pulmonary diffusing capacity, (2) $\dot{V}O_2$ (3) the capacity of the blood to carry oxygen and (4) characteristics of skeletal muscle (figure 2).

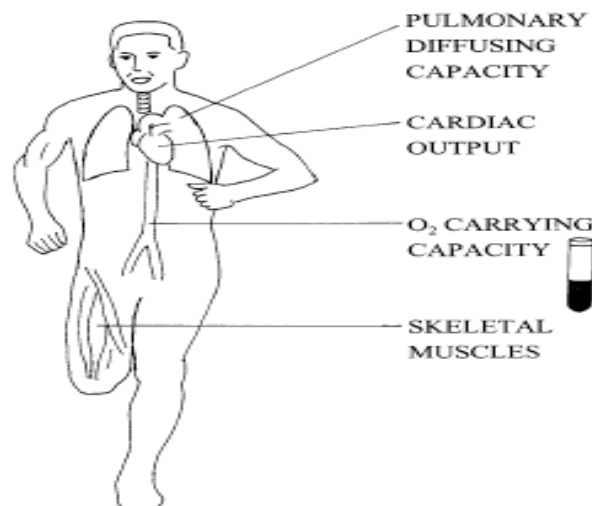


Figure 2. The Primary Physiological Factors that limit endurance performance in humans¹²

Although measurement of $\dot{V}O_2$ max represents the greatest performance velocity that can be achieved during aerobic metabolism [13], cardiorespiratory endurance performance can be predicted to a greater extent through the use of an anaerobic threshold [14]. The anaerobic threshold (determined as $[La]$ at a continuous PO that

does not increase greater than 1 mmol/L within 20 minutes) is deemed more important than onset of blood lactate accumulation (OBLA) alone with regards to endurance performance and exercise velocity [15].

Although used interchangeably, OBLA represents the second significant increase in [La] during intensive exercise and is achieved when [La] reaches a value of 4 millimoles per litre (mmol/L) [16]. VO_2 at the anaerobic threshold contributes to 67% of the variance in endurance performance while the addition of HR increases this by up to 84% for short distance endurance events [15]. Other common thresholds include peak PO and power at the ventilatory and [La] thresholds [17]. Percentage of maximal HR (HRmax) is the most common cardiovascular (CV) threshold used for determining exercise intensity as a strong correlation exists between age and HRmax [18].

Using the formula $208 - (0.7 \times \text{age})$ by Tanaka et al. [18], Percentage of HRmax correlated significantly with [La] threshold, [La] at a running speed of 14.5 km/h and peak running speed performance in recreational athletes [19]. Storen et al [20] found the greatest correlation with cycling TT performance was PO at the [La] threshold, suggesting that the exercise modality predicts which submaximal variables are most appropriate when determining endurance performance.

Borszcz et al. [21] found maximal and submaximal physiological parameters tend to correlate significantly with TT events lasting ~60 minutes in duration in comparison to medium- and short-term endurance events. But evidence also suggests that mean PO during moderate length cycling disciplines, usually around 20 minutes in duration, significantly correlates to a number of similar variables, especially peak PO and VO_2 max [21]. Chronic enhancements in metabolic thresholds are likely to have a positive effect on performance in ultra-endurance stipulations. Overall from an objective basis, a combination of specifically selected CV, respiratory and

biochemical measurements in combination with the most appropriate form of exercise is required when predicting and determining an athlete's maximal performance capacity.

Studies measuring endurance performance focus primarily on data obtained from $\dot{V}O_2$ measurements. From the start of exercise, the affinity of haemoglobin for oxygen is reduced and greater amounts are released from red blood cells into skeletal muscle tissue to increase the rate of oxidative phosphorylation. This results in an increase in the $A-vO_2$ difference with a greater amount of oxygen brought in through increased respiration and less oxygen returning to the heart [22]. Physiological factors leading to reduced blood oxygen levels include a decrease in blood pH (acidosis), increased body temperature, greater levels of 2,3-biphosphoglycerate (2,3-BPG) and an increase in the partial pressure of carbon dioxide (PCO_2) [23].

Measuring endurance performance presents some complications when it comes to the environment in which it is undertaken and characteristics of the individual undertaking exercise. One factor that has been studied is body mass. A study by Jobson et al [24] determined that cyclists with lower body mass (<65kg) can produce valid findings in a field-based environment. Laboratory based testing is recommended for heavier cyclists however due to the absence of environmental factors that decrease the external validity of exercise results to a greater extent than in cyclists of average mass. With the present research including participants with an average mass greater than 70kg, laboratory-based testing protocols were employed during the research testing period.

2.4 ERGOGENIC AIDS AND THE USE OF BERRIES AS A SUPPLEMENT

Athletes may aim to improve endurance performance further through the use of supplements, commercially available items, commonly advertised as having a

positive influence on multiple physical and psychological health factors [25]. Supplements designed to improve sports performance are generally termed ergogenic aids, which are defined as “any training technique, mechanical device, nutritional practice, pharmacological method or psychological technique that can improve exercise performance capacity and/or enhance training adaptations” [26].

Following oral ingestion, nutritional supplements induce a series of hormonal, biochemical and musculoskeletal changes with the aim of stimulating physiological preparation prior to exercise, increasing efficiency during exercise and reducing recovery time following training [27]. These changes may occur in an acute or chronic manner depending on the chemical properties of the supplement and the length of time taken for stimulation of the affected physiological system. Based on limited data, maximal plasma anthocyanin concentration occurs 0.5-4 hours post ingestion at doses ranging between 68-1300 mg [28].

Ergogenic aids may include, but are not limited to vitamins, minerals, herbal remedies, amino acids, whey protein and caffeine [29]. Evidence suggests that supplementation rates are significantly higher amongst elite athletes in comparison to non-elite performers [30] with 58.8% of elite UK performers reporting the use of at least one ergogenic aid and 48.6% using more than one nutrition supplement for a single period of time [31]. At the elite level, the margin between success and failure is smaller, often by just a matter of seconds in some endurance events. A cross-sectional analysis by Zdesar Kotnik et al. [32] revealed the primary reasons for supplement use included improvements in sports performance and skeletal muscle enhancement by male adolescents while female adolescents cited improvements in immune system function.

Exercise duration has a big influence on the efficacy of a supplement. Ergogenic aids designed to improve performance in endurance sports, for example beetroot

juice (BRJ), aim to stimulate blood flow, improve mitochondrial respiration efficiency and VO_2 kinetics whilst reducing ATP cost during muscle force production [33]. In contrast, ergogenic aids designed to improve performance in exercise events lasting <150 seconds, for example creatine monohydrate, stimulate the re-synthesis of phosphocreatine (PCr) and the conversion of adenosine diphosphate (ADP) to ATP whilst increasing the expression of Insulin-like growth factor I (IGF-I) mRNA levels [34].

Ergogenic aid supplementation is not without risk given that intake with regards to sports performance is not endorsed and detrimental effects on long term health may occur with substantial, prolonged doses [35]. Intake of illegal ergogenic aids is especially prominent in track and field athletic events where the use of non-steroidal anti-inflammatory drugs (NSAIDS) is twice as high in comparison to team-based sports [36]. This makes the risk of testing positive for a banned substance considerably higher due to possible contamination from unchecked manufacturing processes [35]. Alongside physiological changes, psychological factors may present another risk with regards to supplementation.

In a two-part study, Hurst et al. [37] concluded that athletes who use ergogenic aids regularly with the strong belief that they are effective are more likely to commit doping than short term users. Literature has concluded that when used appropriately, there are no safety issues or adverse effects with short term use of antioxidants [38], creatine [39] and multi-ingredient pre-workout supplements [40] over a period of a few days or weeks.

2.4.1 ANTIOXIDANT INTAKE ON EXERCISE PERFORMANCE

The topic of supplementation is covered extensively when it comes to analysing the effects of antioxidants on sporting performance [41]. Antioxidants are substances that are produced in the presence of radicals and other reactive oxygen species

(ROS) to delay the oxidation of substrates that lead to oxidative stress [42]. Fruit extracts are becoming popular as supplements in scientific literature due to trends in antioxidant capacity improvement following consumption. Naderi et al. [43] however argued that data supporting improvements in exercise performance are not as clear cut as initially thought, suggesting poor clarity when it comes to the purity and consistency of the extracts provided in these studies.

2.4.2 THE USE OF BERRIES AS A SUPPLEMENT

Polyphenols are a class of antioxidant, found primarily in plants, and are present in multiple food and drink products including green tea, wine, chocolate and berries [44]. The effects of polyphenol intake are well documented in both clinical and trained populations [45,46,47]. Rodriguez-Mateos et al. [45] found total cranberry polyphenol intake (up to 1238 mg) was associated with a dose-response effect on flow mediated dilation, peaking at 4 hours post intake. Following ingestion, sixty new metabolites were identified within plasma, twelve of which were linked to increased skeletal muscle blood flow. A meta-analysis from Marx et al. [46] concluded that there is preliminary evidence to support polyphenol-rich interventions in patients undergoing haemodialysis to reduce or manage cardiovascular disease risk. However no intervention is currently superior due to limited studies in this area.

In a double study, Venables et al. [48] found acute supplementation with green tea extract increased fat oxidation by 17% during moderate intensity exercise and increased insulin sensitivity by 13% in healthy male participants. The focus of this section will include an analysis of polyphenols in berries with respect to the chemical structure and intake. A meta-analysis from Somerville et al. [47] consisting of 14 studies showed mean improvements in maximal and submaximal endurance performance of 1.9% following 7 days of polyphenol intake. The primary samples in the selected studies included trained male athletes on an intervention dose of 688 ± 478 mg per day.

Over the same time period, intake of Quercetin, a plant based flavanol found primarily in apples, cranberries and blueberries [49], improved physical performance in running and cycling based events by 2.8%. In one particular study involving inactive but healthy individuals, Khan et al. [50] found an increase in flow mediated dilation and plasma vitamin C concentration following intake of low and high polyphenol concentrated blackcurrant juice. This, in combination with a reduction in F₂-isoprostane levels, demonstrates the potential for polyphenol intake to improve cardiorespiratory exercise performance by attenuating the accumulation of waste products in peripheral muscle.

Although the current review involves studies that demonstrated the beneficial aspects of chronic polyphenol supplementation, very few studies have analysed the acute effects of polyphenol consumption (a single intake point) on short term exercise performance (< 30 minutes). Despite this, an updated review from Bowtell and Kelly [51] highlighted timing of polyphenol intake as a particularly important factor in acute studies. Studies where exercise was undertaken up to 60 minutes following supplement ingestion had evidenced ergogenic effects on performance [52] but no performance improvements were present for studies where intake was > 2 hours prior to exercise despite an acute increase in total antioxidant capacity [53].

Polyphenols are categorised into one of four groups (1) phenolics, (2) flavonoids, (3) stilbenes and (4) lignans [54]. Anthocyanins (Greek *Anthos*, Flower and Greek *kyanos*, dark blue) are one of six classes of flavonoids that make up the largest group of natural, water soluble pigments in plants [55]. Primarily these molecules are glycosides that bind to a sugar group including glucose, galactose, rhamnose, xylose or arabinose attached to an aglycon [56]. Up until 2015, more than 700 derivatives had been identified from 27 aglycon groups [57]. Large quantities of anthocyanins are found in red, blue and purple fruits including bilberries, chokeberries, raspberries and blackcurrants [58].

Anthocyanins contain both anti-inflammatory and anti-oxidant properties [59] which may attenuate depression of sodium-potassium pump activity, one of the primary biochemical factors in ROS production [60]. Clinically, anthocyanin consumption is linked to a reduction in markers of CV disease risk [61], attributed to the reduction of low-density lipoproteins and the increase in cholesterol efflux [62], and the inhibition of cancer cell growth [63], demonstrating the benefits of polyphenol intake on cellular disease prevention. In an alternative finding, Giordano et al. [64] found no differences in cellular markers of platelet and leukocyte activation following one month of blood orange juice supplementation.

With regards to recommended intake of anthocyanins, the Food and Agriculture organisation and the world health organisation (WHO) expert committee established a safe intake of 2.5 mg/kg per day of grape skin extracts [57]. However this does not include total anthocyanins, and values are likely to differ between other fruits depending on the type, quantity and quality of anthocyanins. Geographical location and cultural diet also affects rate of anthocyanin intake. Zamora-Ros et al. [65] determined that mean anthocyanin consumption was highest in Turin, Italy with values of 64.9 mg/day for males and 44.1 mg/day for females. The primary reason for this is the Mediterranean diet, adopted in Italy which includes a high portion of berries, red coloured fruit and vegetables and red wine [66].

The anti-oxidant properties in anthocyanins stimulate the release of nitric oxide (NO), a molecule synthesised from the amino acid L-Arginine [67] by NO synthase (NOS) enzymes. Neuronal and endothelial NOS stimulate the production of low levels of NO which help to maintain or increase vasodilation, vascular homeostasis, antiplatelet aggregation and neurotransmission (figure 3) [68]. In effect, NO increases circulating blood flow, delivering more oxygen to exercising tissues, leading to a reduction in oxidative stress and subsequent physical fatigue.

Acute intake of blueberry polyphenols increases flow-mediated dilation and pulse wave velocity in the brachial artery accompanied by a reduction in Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [69]. This is a biochemical reaction that increases production of NO [70] and correlates with levels of circulating polyphenol metabolites and greater flow mediated dilation. L-citrulline, a non-essential amino acid produced alongside NO, is converted back to L-arginine to repeat the cycle (figure 3) [68].

Following intake of blackcurrant anthocyanins, two biochemical events take place in human umbilical veins. The first is the release of NO via activation of the AKT pathway and phosphorylation of endothelial NO synthase (eNOS) [71]. The second is stimulation of the nuclear erythroid 2-related factor 2 (NRF2) pathway; NRF2 is a transcription factor that regulates gene expression of antioxidant proteins [72]. NRF2 increases oxidant signalling rates leading to greater regulation of mitochondrial biogenesis and cellular autophagy in the event of severe, prolonged oxidative stress [73]. NO has a half-life between 0.1-2 seconds [74], illustrating the speed at which this molecule acts to stimulate the desired CV and neurological events. The CV effects of increased NO concentration are evident in multiple studies.

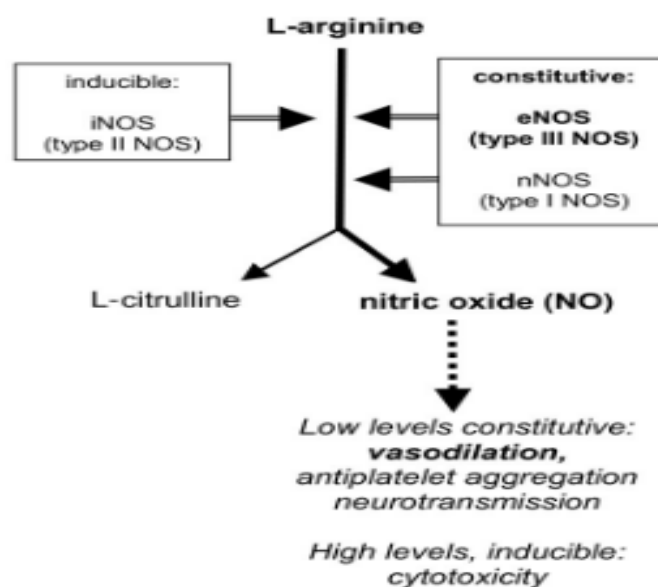


Figure 3. Generation of Nitric Oxide (NO) from L-Arginine and Physiological Effects Depending on Tissues Levels⁶⁸

Matsumoto et al. [75] observed a 22% increase in peripheral blood flow following blackcurrant intake in nine healthy male subjects. Although no differences were present in keyboard typing performance in both groups, biochemically anthocyanin intake attenuated the reduction of oxy-haemoglobin and muscular stiffness. The increase in NO production following blackcurrant intake leads to an increase in peripheral blood flow as a result of anthocyanin induced vasorelaxation and vasodilation [76], increasing skeletal muscle oxygen saturation (StO₂) and attenuating the effects of muscle fatigue. This suggests a potentially ergogenic effect for exercise performance through the reduction of oxidative stress and fatigue. These fruits are also very high in levels of vitamin C and vitamin E, which have been shown to improve immune function and the maintenance of red blood cell structure respectively [77,78].

Research also shows a psychological effect following anthocyanin consumption with one study reporting improved cognitive task performance and a reduction in monoamine oxidase-B (MAO-B) levels [79]. Inhibition of MAO-B prevents the breakdown of dopamine, a neurotransmitter responsible for stimulation of motor performance and a reduction in the energy cost of physical activity [79]. This biochemical effect could therefore be the mechanism behind the stimulation of the sympathetic nervous system, producing a psychophysiological effect that increases participant's motivation to perform, thus improving performance in time trial exercise disciplines.

2.5 NEW ZEALAND BLACKCURRANT EXTRACT

Blackcurrant (*Ribes nigrum* L.) has been studied extensively within dietary and supplement-based literature in recent years. Anthocyanin content in blackcurrant is greater in comparison to 22 other types of fruit including cranberries, strawberries and blueberries [80] at an estimated 250 mg per 100 grams of fresh fruit [81]. Four

major anthocyanin groups make up 90% of the polyphenol structure in blackcurrant (1) cyanidin-3-*O*-glucoside, [3-10%], (2) cyanidin-3-*O*-rutinoside [30-45%], (3) delphinidin-3-*O*-glucoside [5-20%] and (4) delphinidin-3-*O*-rutinoside [35-50%] [82,83]. Furthermore, both rutinoside groups make up 80% of the total anthocyanin structure in blackcurrants [84] (figure 4).

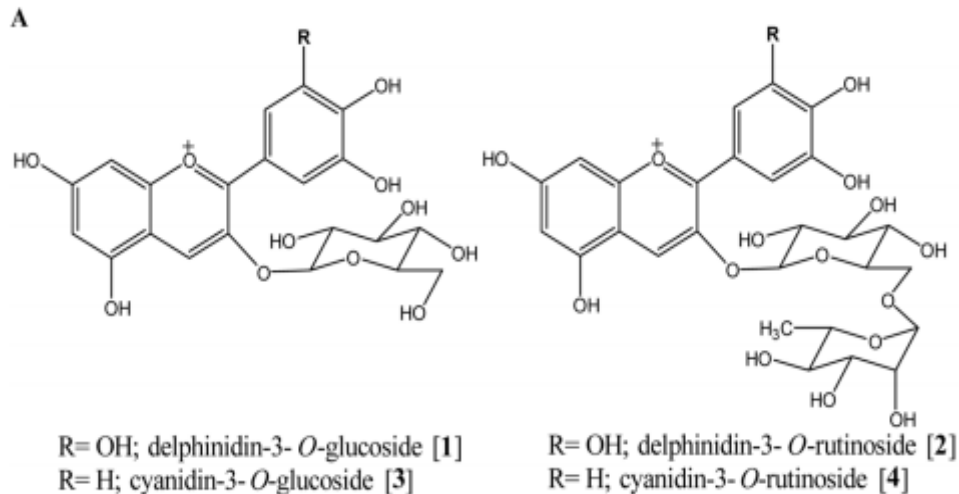


Figure 4. Chemical Structure of New Zealand Blackcurrant Anthocyanins⁸⁴

Upon ingestion, the primary phenolic metabolites, hippuric acid and ferulic acid, amongst other compounds appear in serum blood and urine. Serum phenolic metabolite concentration in plasma ranges between 10-2000 nanomoles per litre (nMol/L) and peaks between 2-30 hours post consumption with a half-life between 0.5-96 hours [85]. When focusing specifically on the chemical structure of NZBC, the four primary anthocyanins are immediately absorbed into the blood upon consumption in humans with maximal plasma concentration levels between 1.25-1.75 hours post intake [86].

The effects each group is unique in terms of both quantity and the physiological adaptations that take place following intake. The primary anthocyanin in blackcurrant is delphinidin-3-*O*-rutinoside whereas cyanidin-3-glucosylrutinoside is the primary anthocyanin in cherry [87], suggesting different potential effects on exercise performance, depending on the physiological pathway. Anthocyanins are also unique

in terms of their effective properties in the treatment of medical conditions. Delphinidin is associated with a reduction in physiological markers of CV and metabolic disease [88] whereas cyanidin does not produce the same effect [89].

This could provide evidence for suggesting potential benefits for exercise performance with both short- and long-term use. Research also links delphinidin-3-O-rutinoside intake to an increase in glucose tolerance [90] and cyanidin-3-O-glucoside to an increase in eNOS activity [91]. Blackcurrant intake might therefore prove useful in the prevention of type 2 diabetes [92] and CV disease [93], particularly when used in combination with a high intensity exercise programme.

While these findings demonstrate the benefits of blackcurrant intake for sedentary individuals, there is currently minimal research analysing the comparative effects against trained performers at rest. Anthocyanin concentration is approximate 1.5 times greater in NZBC with some new cultivars presenting concentrations as high as 850 milligrams per 100 grams of fresh fruit [94]. This, in combination with high levels of Oxygen Radical Absorbance Capacity (ORAC) and vitamin C [95] suggests possible improvements in immune function as well as exercise performance. ORAC was a method of measuring antioxidant capacities in fruits through the methods of fluorescent quenching [96].

Indeed a 10% solution of blackcurrant powder from New Zealand and Poland inhibited four different strains of the influenza virus as determined by Ikuta et al [97]. By reducing the rate of viral activity within cells, there is a reduction in the risk of contracting upper respiratory tract infections, the most prominent of all acute illnesses in athletes [98]. The quality of blackcurrant yields is determined by a mixture of cultivar properties and environmental conditions [99]. An analysis by Woznicki et al [100] using the primary markers of total polyphenolic compounds,

anthocyanins and ascorbic acid concentration determined growth of blackcurrant was greatest in cool summer conditions with adequate precipitation.

In research studies, NZBC is often taken in the form of 300 mg capsules, each containing 35% blackcurrant extract (105 mg), the nutritional equivalent of 85 blackcurrants [101]. This accounts for 35% of the nutritional structure in these fruits. The antioxidant properties of NZBC might explain the reduction in $\dot{V}O_2$ for the same exercise intensity, and [La] production during incremental endurance exercise following consumption [102].

2.5.1 SIMILARITIES TO BEETROOT JUICE

The properties and proposed exercise effects of NZBC are comparable to that of BRJ, a supplement that contains high levels of dietary nitrate [103]. Upon ingestion, this ion is broken down into nitrite in the mouth before being converted into NO via the digestive tract. From here the same CV, metabolic and musculoskeletal effects take place, leading to greater exercise efficiency, greater exercise performance and a reduction in fatigue (figure 5) [104].

Supplements taken in capsule form bypass breakdown in the oral cavity and take between 20-30 minutes to breakdown in the stomach. The delay of the physiological response to NZBC could influence the magnitude of any acute effect on endurance performance, even if such an effect exists. By increasing physiological levels of NO, the biochemical reactions are equivalent to those that take place following NZBC intake. Research suggests NO production following BRJ intake is immediate with effects seen 45 minutes following consumption [105]. The length of time for NO production following NZBC intake has yet to be studied. Studies analysing BRJ intake have identified improvements in exercise performance with both acute [106] and chronic intake protocols [107].

Common findings in the literature in this area includes a reduction in the oxygen cost of exercise [108] and improvements in mitochondrial efficiency [109]. BRJ contains high levels of polyphenols and antioxidant properties. A study by Wootton-Beard et al [110] analysing 23 different vegetable juices revealed total polyphenol and antioxidant levels were greatest in BRJ. A follow up study revealed a three-fold increase in total antioxidant capacity during the gastric phase and remaining high during the duodenal phase, even with a slight decrease, from a single 70 millilitre (ml) dose of BRJ [111].

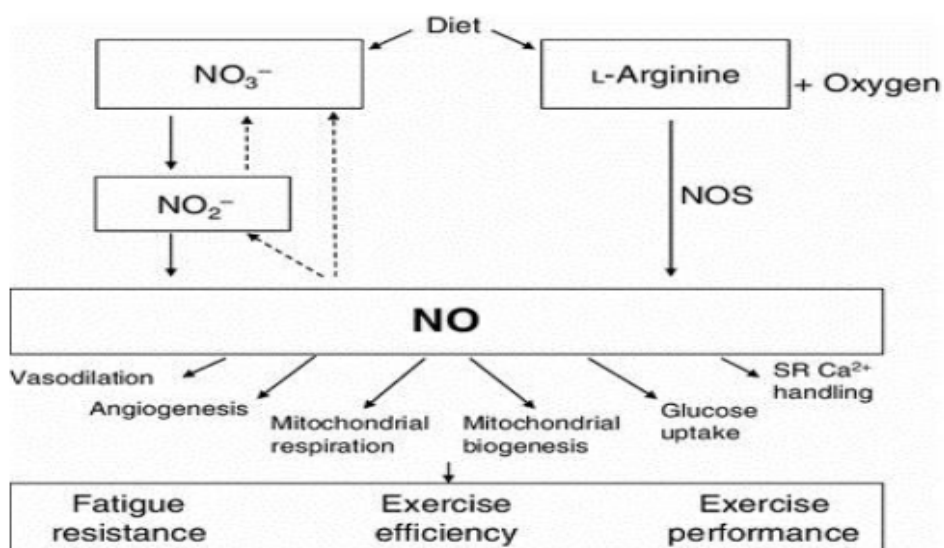


Figure 5. Cardiovascular, respiratory and musculoskeletal processes following Nitric Oxide (NO) production. Dietary Nitrate (NO₃⁻) intake acts as a production pathway for NO¹⁰⁴

2.6 EFFECTS OF NEW ZEALAND BLACKCURRANT EXTRACT ON EXERCISE PERFORMANCE

A large portion of studies analysing the effects of NZBC have primarily involved chronic supplementation with a dose of either 300 mg or 600 mg over a period of seven days from trained or elite male performers. The current optimal dosing strategy for NZBC is unknown with doses prescribed in line with manufacturers guidelines. Some studies have based their selected dose period on previous

research looking at the effects of berry intake over a multi-day period [102,128,137]. As of yet, no single study has analysed the effects of both acute and chronic intake of berries on endurance exercise performance. This makes it impossible to compare the differences in the magnitude of these effects following ingestion over different periods of time.

Although three studies have used female participants as part of the sample, there is currently minimal research concluding the effects of NZBC in untrained individuals or the general population. The first study to analyse NZBC intake in human participants was conducted by Willems et al. [102]. The participants, thirteen male triathletes, took either 6 grams (g) of Sujon NZBC powder or a placebo (PLA) for seven days prior to basic resting measurements of CV function. Despite no significant effects on HR and blood pressure, there was a 25% and 26% increase in CO and stroke volume (SV) respectively, accompanied by a 16% decrease in TPR. During two incremental cycling protocols, PO at OBLA was higher following NZBC in comparison to PLA during incremental exercise. This suggests that at the same exercise intensity, work can be produced for a greater amount of time due to a reduction of waste product accumulation in skeletal muscle tissue and increased peripheral blood flow.

Research following this study has employed either the same incremental protocol, where participants are required to exercise until volitional exhaustion, or a time trial (TT) protocol which involves participants being required to complete a set distance as quickly as possible. It is important to differentiate the validity and impact of the protocol on exercise results as effect sizes suggest that a 15% improvement in time to exhaustion protocols translates to a 1% improvement in time trial performance [112].

However, TT protocols represent a more accurate evaluation of performance due to greater external validity in the “real” athletic world [113]. Furthermore results from a laboratory based 10 km TT cycle revealed a significant correlation between average PO and already measured VO_2 max to field-based performance when normalised to body mass [114]. A large portion of studies measuring the effects of NZBC on TT performance have exercise protocols ranging from 25-30 minutes, with TT protocols having a set distance of 16.1km. A full summary of the literature of NZBC intake and exercise performance can be found below in table 1.

Table 1. Systematic Review of the Effects of NZBC on Exercise Performance

Author/s	Year	Subjects	Background/Intervention	Measures	Results	Summary
Cook et al.	2017	15 endurance trained male cyclists	300, 600 or 900 mg NZBC (CurraNZ™ 105, 210 or 315 mg ANT) for 7 days Alternatively PLA Final intake 2 hr prior to 2 hr cycle at 65% VO ₂ peak Randomised, counter balanced, Latin square study design 2 wk washout	VO ₂ VCO ₂ [La] RER CHO FO GLU	Significant dose effect of FO following NZBC consumption compared to PLA. No significant difference in [La], VO ₂ , VCO ₂ , GLU between conditions. Dose response effect on CHO but no significant differences between doses	7 day NZBC supplementation has a significant dose response effect on FO during 2 hr cycle in endurance trained male cyclists. A dose response effect on CHO, although insignificant, is present between conditions

Willems et al.	2015	13 trained triathletes	<p>6g Sujon NZBC powder (138.6 mg ANT) or PLA taken for 7 days</p> <p>Final intake 2 hr before INC cycle</p> <p>Double blind, PLA controlled, randomised, crossover study design</p> <p>4 wk washout</p>	<p>HR</p> <p>Plasma [La]</p> <p>BP</p> <p>SV</p> <p>CO</p> <p>MAP</p> <p>TPR</p> <p>VO₂</p> <p>PO</p> <p>OBLA</p>	<p>No differences in BP, MAP & HR at rest and during exercise</p> <p>Significant increase in SV, TPR and CO following NZBC consumption at rest but not during exercise</p> <p>No significant effect on maximal VO₂ or PO</p> <p>PO at OBLA significantly higher following NZBC compared to PLA</p>	<p>7 day NZBC supplementation has a significant effect on certain CV parameters at rest and attenuates the lactate response during incremental exercise in trained triathletes.</p>
Strauss et al.	2018	16 trained females	<p>600 mg NZBC (CurraNZ™, 210 mg ANT) for 7 days or PLA</p> <p>Randomised, double blind, crossover study design</p> <p>Taken 2 hr before 2 hr cycle at</p>	<p>FO</p> <p>CHO</p> <p>VO₂</p> <p>Plasma NEFA</p>	<p>FO significantly higher following NZBC consumption compared to PLA</p>	<p>7 day NZBC supplementation significantly increases fat oxidation during prolonged cycling which could be due</p>

			65% VO2 max	Glycerol RER	Plasma NEFA and glycerol significantly higher following NZBC intake at rest but not during exercise No significant effect of NZBC on CHO oxidation and RER	to an increase in resting NEFA and glycerol levels
Cook et al.	2015	14 healthy men	300 mg NZBC (CurraNZ™ 105 mg ANT) or PLA for 7 days Taken 2 hr prior to 16.1 km cycle Randomised, double blind, crossover study design 2 wk washout	TT FO Plasma [La] BP Blood Glucose PO RER	TT performance and FO significantly increased following NZBC consumption. Plasma [La] was significantly higher immediately following TT	7 day NZBC consumption significantly improves both TT performance and FO during a 16.1 km cycle
Willems et al.	2014	9 male endurance athletes	300 mg NZBC (CurraNZ™ 105 mg ANT) or PLA for 7 days 16.1 km TT cycle followed by [La] sampling for 20 min PE Randomised, double blind,	TT FO CHO oxidation [La]	TT performance significantly improved following NZBC consumption. [La] clearance significantly greater	7 day NZBC supplementation significantly improves 16.1 km TT cycling performance and [La] clearance

			<p>crossover study design</p> <p>2 wk washout</p>	<p>RER</p> <p>HR</p>	<p>following TT in NZBC than PLA.</p> <p>No significant difference on CHO oxidation, FO, HR & RER at any intensity between conditions</p>	<p>following endurance exercise.</p>
Lyall et al.	2009	10 healthy individuals (5 male, 5 female)	<p>Single bolus of 48 g NZBC (Vitec, ANT (240 mg) or PLA taken on day of trial</p> <p>120 mg taken immediately before 30 min row</p> <p>120 mg taken immediately following exercise</p> <p>Double blind, crossover study design</p> <p>1 wk washout</p>	<p>HR</p> <p>THP-1</p> <p>Plasma Carbonyl levels</p> <p>IL-6</p>	<p>Significant reduction in Plasma carbonyl levels cytokine secretion following BC ANT consumption.</p>	<p>BC ANT consumption reduces markers of oxidative stress and enhances immune system responses</p>
Perkins et al.	2015	13 active males	<p>300 mg NZBC (CurraNZ™, 105 mg ANT) or PLA for 7 days</p> <p>Final intake 3 hr prior to INT</p>	<p>[La]</p> <p>Running distance</p>	<p>Significant increase in running distance and sprint performance following NZBC in</p>	<p>7 day NZBC supplementation significantly improves</p>

			<p>running protocol</p> <p>Double blind, randomised, crossover study design</p> <p>2 wk washout</p>	<p>Sprint performance</p> <p>HR</p> <p>VO₂</p> <p>RPE</p>	<p>comparison to PLA.</p> <p>No significant effect of NZBC consumption on HR, RPE, VO₂ and [La] during exercise following NZBC</p> <p>[La] clearance significantly greater immediately following exercise in NZBC compared to PLA</p>	<p>performance in high intensity intermittent running.</p> <p>This is accompanied with a significant increase in [La] clearance following exercise, suggesting enhanced recovery benefits</p>
Braakhuis et al.	2014	23 trained female runners	<p>NZBC juice powder (300 mg ANT, 15 mg VC)</p> <p>VC (1g) or PLA taken for 3 week training block</p> <p>Randomised, three treatment, PLA controlled, crossover study design</p>	<p>Protein Carbonyl levels</p> <p>ASC acid</p> <p>Uric acid</p> <p>Running Speed</p> <p>5 km TT performance</p> <p>INC test</p>	<p>Significant increase following VC compared to NZBC juice and PLA in protein carbonyl levels and oxidative markers at rest</p> <p>Trend towards significant decrease in speed following VC consumption compared</p>	<p>Chronic use of VC significantly increases markers of oxidative stress which may impair running performance.</p> <p>Chronic intake of BC juice may</p>

			3.7 wk washout	performance	to BC juice and PLA	improve performance in trained female runners.
Murphy et al.	2017	10 trained male cyclists	300 mg NZBC (CurraNZ™, 105 mg ANT) for 7 days Alternatively PLA Randomised, double-blind, crossover study design 1 wk washout	4 km TT HR [La]	Significant reduction in overall 4 km TT times following NZBC consumption compared to PLA No significant effect of NZBC consumption on HR or [La] at any point in the study	7 day NZBC supplementation significantly improves repeated short term TT cycling performance but has no significant effect on HR or [La] during or after exercise.
Godwin et al.	2017	24 males (15 recreationally active, 9 trained)	600 mg NZBC (CurraNZ™, 210 mg ANT) for 7 days Alternatively PLA Double blind, randomised, crossover study design 2 wk washout	Anaerobic sprint test performance FI	Sprint no. 5 significantly faster in trained individuals following NZBC consumption compared to PLA. No significance of NZBC consumption on sprint performance in recreationally active individuals in comparison	7 day NZBC supplementation significantly improves sprint based performance in trained males

					to PLA. Strong trend towards significantly lower FI following NZBC consumption in both trained and recreationally active individuals.	
Willems et al.	2016	13 healthy males	300 mg NZBC (CurraNZ™, 105 mg ANT) for 7 days Double blind, randomised, crossover study design 1 wk washout	Peak running speed HR Vertical jump power [La] Time to exhaustion	No significant effect of NZBC consumption on HR, jump power, [La] or time to exhaustion. Strong trend for greater maintenance of sprint times in running block 4 following NZBC compared to PLA. Significantly greater maintenance of sprint times in running block 5 following NZBC compared to PLA	7 day supplementation with NZBC may be beneficial in repeated short duration sprint based running performance
Willems et al	2018	17 healthy Thai males	600 mg NZBC (CurraNZ™, 210 mg ANT) or PLA for 7 days Randomised, double blind, PLA	VE VO ₂	No significant effect of NZBC consumption on VE, VO ₂ , BP, RER, FO,	7 day NZBC supplementation has no significant

			controlled, crossover study design 2 wk washout	BP RER FO CHO HR SV CO TPR	CHO, HR, SV, CO AND TPR at rest or during exercise Trend towards significant effect on SV and CO with a significant difference in TPR during 30 minute exercise following NZBC in comparison to PLA	effect on any cardiovascular, physiological or metabolic responses at rest in healthy Thai males. 7 day NZBC supplementation significantly affects TPR during exercise with a trend towards significantly higher SV and CO in healthy Thai males
Willems et al	2019	11 healthy male cyclists	600 mg NZBC (CurraNZTM 210 mg ANT) or PLA for 7 days Randomised, double-blind, crossover study design	Steady state cycle TT	No significant effect of NZBC consumption on TT performance, blood glucose & [La], HR, RER during steady state	7 day NZBC supplementation has no significant effect on steady state exercise-

				Blood Glucose [La] HR RER VO_2 VCO_2 CHO Oxidation Fat Oxidation	cycling and 16.1 km TT cycling performance	induced metabolic parameters and TT cycling performance in healthy male cyclists under normobaric hypoxia
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Abbreviations: ANT (anthocyanins), AIX (augmentation index), BC (blackcurrant), BP (blood pressure), CHO (carbohydrate oxidation), CJ (cranberry juice), CO (cardiac output), CV (cardiovascular), DVP (Digital Volume Pulse), FAD (femoral artery diameter), FI (fatigue index), FMD (flow mediated dilation), FO (fatty acid oxidation), g (grams), Hb (haemoglobin), hr (hour), HR (heart rate), INC (incremental), [La] (Blood Lactate), MAP (mean arterial pressure), mg (milligrams), ml (millilitres), MVC (maximal voluntary contraction), NZBC (New Zealand Blackcurrant Extract), PE (post exercise), PLA (placebo), PO (power output), POLPH (polyphenol), PW (pulse wave), PWA (Pulse Wave Analysis), PWV (Pulse Wave Velocity), RER (respiratory exchange ratio), RPE (rating of perceived exertion), SMO_2 (skeletal muscle oxygen saturation), SV (stroke volume), TPR (total peripheral resistance), TT (time trial), VE (ventilation), VO_2 (volume of oxygen consumption), VCO_2 (volume of carbon dioxide production), VC (vitamin C), wk (week)

2.6.1 EFFECTS ON CYCLING PERFORMANCE

Following NZBC intake, Willems et al. [115] found a significant improvement in 16.1 km TT performance accompanied by an increase in [La] clearance following exercise. These findings put forth the idea that there is an ergogenic effect on recovery time as well as performance following NZBC intake, providing a benefit to performers with busy training schedules. As the definition of recovery involves a multifaceted, restorative approach of physiological and psychological factors relative to time [116], researchers often look to use performance recovery measures, e.g. peak power in countermovement jump tasks and changes in submaximal HR during running tasks [117].

Cook et al. [118] found a 2.4% improvement in TT performance along with an increase in fat oxidation of 27% during a 2 hour steady state cycle at an intensity eliciting 65% VO_2 max in trained male cyclists following NZBC consumption (figure 6). Both of these differences were significant in comparison to PLA. When this change was identified in another study by Strauss et al. [119], using twice the dose of NZBC compared with other studies, an associated correlation was found with a reduction in carbohydrate oxidation in a sample of trained female subjects.

These changes were attributed to higher blood levels of both non-esterified fatty acid and glycerol pre-exercise. Performance improvements are not only limited to single exercise bouts, as evidenced by Murphy et al. [120] where trained male cyclists completed a double 4 km TT cycle 0.82% quicker following NZBC compared to PLA. Three of the aforementioned studies included a dose of 300 mg per day [115,118,120] with a 600 mg dose incorporated in the alternative study [119].

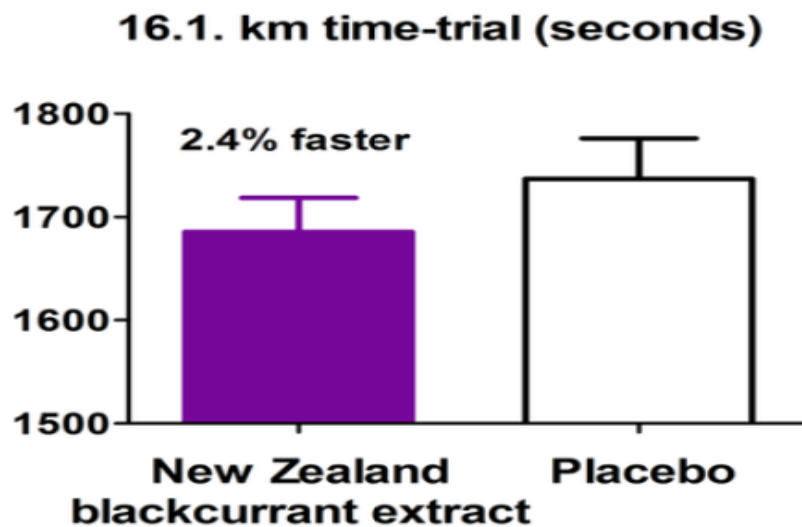


Figure 6. Mean 16.1 km TT performance times following NZBC or PLA intake¹¹⁸

2.6.2 EFFECTS ON RUNNING PERFORMANCE

Findings also show an ergogenic effect on alternative endurance capacity events. In a study by Perkins et al. [121], running distance covered during a multiple sprint protocol increased significantly by 10.6%. Distance covered during active recovery was also higher following NZBC consumption suggesting attenuation of fatigue during periods of low exercise intensity. There was also a trend towards greater [La] clearance following 15 minutes of passive recovery, in line with research from Willems et al [102], suggesting recovery benefits for multiple cardiorespiratory exercise disciplines.

However these findings are opposite to those of Cook et al. [122] who found no dose-response effect of NZBC on plasma [La] post-exercise in any condition following a 30 minute steady state cycle and 16.1km TT. This indicates that rate of physiological recovery might be affected by exercise modality, the intensity of exercise and the length of exercise protocols. In a study analysing sprint based running performance, Godwin et al. [123] found a reduction in fatigue index, demonstrated by a greater reduction in sprint speed in participants who were supplied a PLA. NZBC intake extends time to exhaustion during simulated team

sport activity, demonstrated by greater maintenance of running intensity during the Loughborough Intermittent Shuttle Run Test in trained males [124].

2.6.3 EFFECTS ON ROWING PERFORMANCE

Although no significant difference was found between groups for 2,000 metre rowing performance, a reduction in markers of superoxide dismutase and glutathione peroxidase, enzymes that act as oxidising agents [125], was present in individuals supplementing with 250 mg of a ground blackcurrant supplement in comparison to a PLA. These biochemical events were accompanied by an increase in total antioxidant capacity 24 hours following exercise, suggesting sustained enhancement of the immune response to physiological stressors. Ingestion of NZBC attenuated markers of oxidative stress immediately prior to and following a 30 min rowing TT [126], indicating that there is an acute effect on the level of certain biochemical parameters that define endurance performance following ingestion.

These findings are replicated by Braakhuis et al. [127] who compared the effects of blackcurrant juice against vitamin C supplementation. Interestingly, while an ergogenic effect was spotted for peak running speed following blackcurrant intake, chronic vitamin C intake resulted in a reduction in performance and potentially harmful effects on immune function. Researchers found an increase in antioxidant markers of catalase and protein carbonyl levels markers at rest and superoxide dismutase levels following exercise.

2.6.4 DOSE-RESPONSE EFFECTS OF NEW ZEALAND BLACKCURRANT EXTRACT

Currently only two studies have analysed the dose-response effects of NZBC on physiological adaptations following intake [122,128]. When analysing CV parameters during supine rest, no differences in blood pressure, HR and ejection time were identified between doses. However a linear response was seen between 600 and 900 mg for CO, SV and TPR [128]. During prolonged cycling however, a dose

response effect was found for decreased carbohydrate oxidation and increased fatty acid oxidation [122]. When compared to baseline data, individual percentage change in fat oxidation increased by 21.5% and 24.1% following 600 mg and 900 mg doses of NZBC respectively. As NZBC anthocyanins stimulate the release of NO through activation of eNOS, CV and respiratory parameters are stimulated in a manner that leads to an improvement in endurance performance.

Although the results of NZBC intake on CV markers at rest and during exercise is evident in previous literature [102,128], there is currently no research regarding the dose-response effects of NZBC on the relation of CV markers to peripheral muscle fatigue during exercise. Although no effects were found for rating of perceived exertion (RPE) and HRmax between conditions, Potter et al. [129] found an 8% increase in wall hang time, a 23% increase in climb duration and a reduction in mean HR values across three different climb disciplines following NZBC intake in comparison to PLA. Rock climbing involves sustained isometric contractions, muscle activity in the absence of joint movement, to hold the individual in position.

Evidence demonstrates increased arterial diameter and CO for up to 120 seconds during a voluntary isometric contraction [130] following NZBC intake. This reduces the rate of fatigue by increasing peripheral blood flow, an initial finding by Matsumoto et al. [75]. Therefore future research should analyse whether this remains the case during cycling, an exercise modality involving concentric muscular contractions, and whether changes in variables including [La], StO₂ and interleukin-6 are linear following higher doses of NZBC.

2.6.5 EFFECTS OF NZBC ON POST-EXERCISE RECOVERY

While literature on the effects of NZBC prior to and during exercise is evident, Hutchison et al. [131] produced research findings suggesting post exercise recovery benefits. Reductions in post exercise creatine-kinase and ORAC levels were evident

48 hours post exercise with lower interleukin-6 levels at 24 hours post exercise, suggesting reduced muscle damage, inflammation and oxidative stress. Another study found a reduction in post-exercise plasma oxidative activity and carbonyl levels in combination with maintained neutrophil function following intake of NZBC, suggesting attenuation of innate immunity damage [132].

Some research suggests endurance performance remains affected 48 hours following exercise induced muscle damage, with reduced VO_2 uptake and PO during a 15 min TT cycle as well as increased minute ventilation at the ventilatory threshold [133]. This suggests that optimal recovery following exercise is a minimum of two days. Future research should involve post-exercise ingestion of NZBC to determine whether recovery to a specified pre-exercise physiological state and resulting physical performance is affected with supplementation. A dose-response protocol should be implemented to determine if the rate of recovery is linear depending on the amount of NZBC ingested.

2.6.6 EFFECTS OF ETHNICITY ON RESPONSES TO NZBC

The level of physiological response to exercise and recovery following ingestion of NZBC may be different depending on gender or ethnicity of the research sample. A recent study [101] involving males from Thailand found no physiological effects during rest and only a greater reduction in TPR during a 30 min walking exercise. The authors concluded that ethnicity may act as quite a prominent factor in the physiological response to NZBC but as there is limited data at present, care should be taken with the sample chosen for research in this area.

2.7 EXERCISE PERFORMANCE IN HYPOXIA

Medically, hypoxia is a condition resulting from a partial or complete reduction in peripheral tissue oxygenation [134]. Although the terms are similar, hypoxia is not to be confused with hypoxemia, a condition where partial pressure of oxygen in the

blood is lower than normal. A commonly used method of chronically enhancing endurance performance is exercising in hypoxia. This is achieved most practically by going to an area of high altitude and training for a number of weeks in an area with low atmospheric levels of oxygen. This type of environment is termed hypobaric hypoxia (HH) and refers to an outdoor environment where lower atmospheric levels of oxygen result in a reduction in the fraction of inspired oxygen (FiO_2). However because of the financial and time bound constraints of altitude training for elite performers, many research studies adopt a normobaric hypoxia (NH) discipline and achieve this by reducing FiO_2 in an environment with regular atmospheric pressure [135].

Acute exposure to hypoxia lowers arterial oxygen pressure (SaO_2) and increases markers of oxidative stress including [La], plasma malondialdehyde, superoxide dismutase, lipid and protein oxidation, a reduction in glutathione levels and increased PCO_2 [136]. These are generated in response to the overproduction of ROS and free radicals including advanced oxidation protein products. This primary physiological response occurs at both rest and during exercise in hypoxia [137]. The mechanisms associated with ROS overproduction might include mitochondrial reductive stress [138], increased catecholamine production [139] and activation of the Xanthine oxidase pathway [140].

The detrimental effects of hypoxia are particularly prominent on the brain, an organ which makes up 2% of an average human's body mass but consumes 20% of oxygen supplied to the body [141]. The composition and properties of NZBC which initiate a number of CV adaptations could alleviate symptoms of significant oxidative stress, which in serious cases include a reduction in oxidative phosphorylation, decreased blood pH and creatine phosphate levels, a collapse of ion balance and a reduction in cellular sodium-potassium balance and ATPase from insufficient cytochrome c oxidase activity [142].

These changes lower exercise capacity in both recreational and elite performers and stimulate the mechanisms leading to increases in CO and minute ventilation to a greater extent than exercise in normoxia [143]. This has a significant effect on oxygen uptake kinetics at the start and end of exercise. At the start of exercise, performers spend 25-30% longer during the primary $\dot{V}O_2$ component phase [144], represented by a sudden, sharp increase in $\dot{V}O_2$ due to increased muscle oxygen demand [145]. This is accompanied by a greater reduction in the amplitude of the slow component phase following exercise of the same intensity [144].

Physiologically damaging effects from hypoxia are evidenced further through a greater number of strand breaks in deoxyribonucleic acid (DNA) molecules [146] during exhaustive exercise. The extent of this damage significantly increases following a bout of exhaustive exercise, suggesting that potential genetic defects could occur that lead to long term changes in cellular processes. Despite no acute effects on short duration sprint based performance, exposure to hypoxia leads to a reduction in overall endurance exercise performance of 17%, including a 16% reduction in TT performance [147]. Biochemical changes in mitochondrial and enzymatic activity increase the physiological response pathways to allow humans to adapt to this environment (figure 7) [148].

The magnitude of these changes is different between hypobaric and normobaric conditions. A meta-analysis conducted by Coppel et al. [149] determined that minute and alveolar ventilation were lower in HH than NH and this was accompanied by a significantly greater reduction in NO. An unexpected finding was that under HH, blood pH levels were higher above 4,500m when compared to NH despite a greater presence of acute mountain sickness.

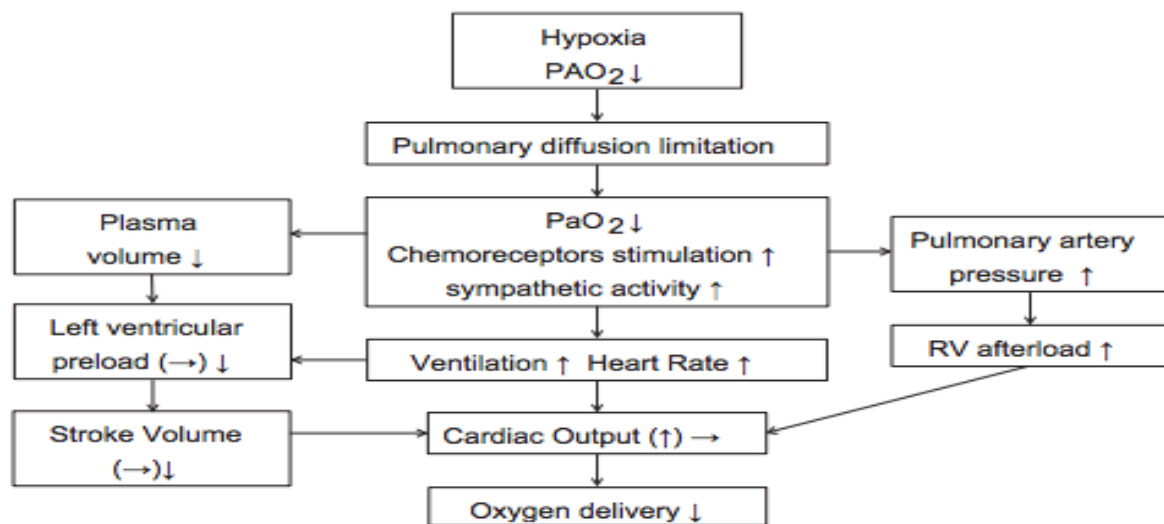


Figure 7. Physiological adaptations to exercise under hypobaric hypoxia at altitude or normobaric hypoxia (simulated altitude) via biochemical and enzymatic activity¹⁴⁸

When a prolonged reduction in SaO_2 occurs, the hypoxia-inducible factor-1 (HIF-1) pathway is activated, creating a cascade of cellular events that lead to homeostatic responses via the regulation and transcription of multiple genes (figure 8) [150]. These include the formation of new blood vessels (angiogenesis) or cells (proliferation), metabolism of glucose and production of erythropoietin (EPO), a hormone that is released from the kidneys that stimulates red bone marrow to produce more red blood cells (erythropoiesis). Knaupp et al. [151] found a 50% increase in EPO production just four hours following acute exposure to hypoxia.

Training status appears to heavily influence the exercise response to a reduction in VO_2 . One example from Faiss et al. [152] determined a greater decrement in VO_2 max in elite athletes in comparison to recreational performers during an incremental treadmill run. A correlation was also present between the VO_2 max decrement and SaO_2 levels in the elite performers only. There is also evidence that peak HR is decreased either due to lower oxygen tension or training status [153] with elite performers exhibiting lower SaO_2 and a reduction in HR_{max} in comparison to recreational performers.

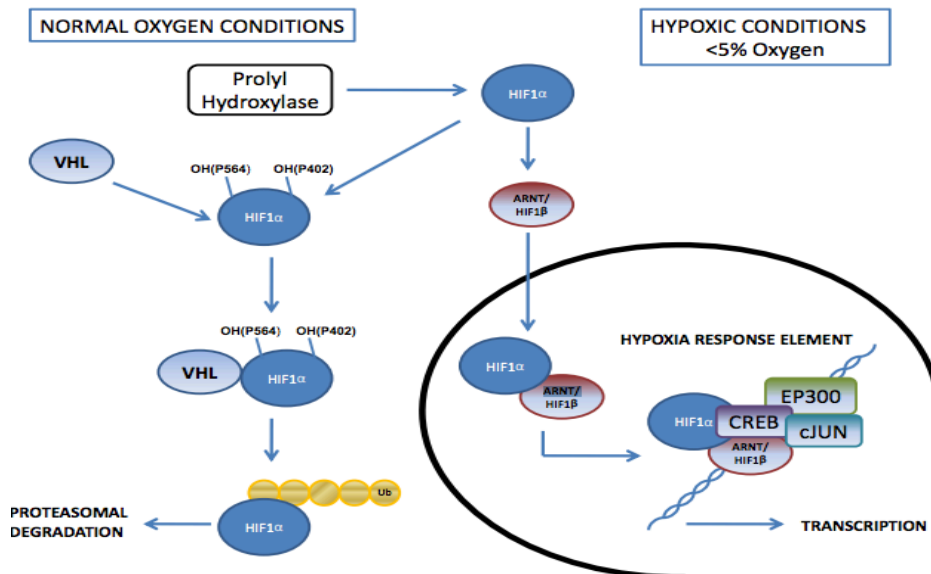


Figure 8. The Hypoxia Inducible Factor-1 (HIF-1) Pathway. In a state of cellular hypoxia, Prolyl Hydroxylase prevents HIF-1 α binding to VHL Protein, leading to the formation of the hypoxia response element and subsequent genetic transcription¹⁵⁰

2.8 POTENTIAL EFFECTS OF NEW ZEALAND BLACKCURRANT

EXTRACT IN NORMOBARIC HYPOXIA

Given the evidence that cardiorespiratory, biochemical and cellular alterations associated with chronic NZBC consumption under normoxic conditions ($FiO_2 = 21\%$) produce an ergogenic effect on exercise performance, there is reason to suggest that performance under hypoxia could be enhanced to a greater extent following NZBC intake. To date only one study has tested this hypothesis. Willems et al. [154] determined that both steady state and TT cycling performance at a simulated altitude of 2500m ($FiO_2 = 15\%$) were not affected following seven day intake of NZBC at a dose of 600 mg. Fat oxidation, carbohydrate oxidation and [La] during the steady state cycle were also the same between conditions. Although often perceived as detrimental, oxidative stress regulates ventilation responses during sudden exposure to hypoxia lowering sudden, negative physiological effects that are associated with a reduction in oxygen [155]. Supplementation with antioxidants may therefore be

detrimental as opposed to beneficial when it comes to acclimatising and exercising in hypoxia. Currently there have been no studies analysing the acute effects of NZBC on exercise in hypoxia, particularly at high doses.

2.8.1 ACUTE EFFECTS OF ERGOGENIC AIDS IN HYPOXIA

The findings of Willems et al. [154] correlate with those of Macleod et al. [156] who found the no effects on TT cycling performance in hypoxia and normoxia following BRJ consumption. A study by Muggeridge et al. [157] contradicts these findings when, following a single 70ml intake of BRJ, performance under simulated altitude improved by 2.2% in comparison to a PLA. This value similarly matches the improvement seen in cycling performance in Lansley et al. [106] and Cook et al. [118] for BRJ and NZBC respectively. Intake of BRJ regulates blood flow in skeletal muscle tissue as determined by Masschelein et al. [158]. Under severe hypoxia ($FiO_2 = 11\%$), no differences were found for changes in cerebral oxidation but an increase in peripheral (SpO_2) and muscle oxygenation was present. Potential factors influencing this could have included the dose of BRJ given, nitrate concentration or the time period between supplementation and the exercise protocol. Whether these differences are seen under moderate hypoxia ($FiO_2 = 15-16\%$) following acute NZBC consumption is yet to be proven.

All of the aforementioned studies included a sample of trained cyclists or athletes, indicating that the effects of BRJ intake on exercise performance in recreationally active individuals is limited. The size of the sample used in these studies were small, typically ranging from 8-13 individuals. Therefore it is likely that with a greater variance in differences between the experimental and control groups, the true effects of BRJ on cycling performance require greater scrutiny. Unlike the current research on NZBC, intake of BRJ prior to the exercise was mixed with some implementing an acute protocol with a single bolus and others involving chronic intake over a specified number of days prior to exercise.

Particular ergogenic aids may elicit greater physiological responses to acute hypoxic exposure in comparison to others. A single bolus of caffeine, a popularly used ergogenic aid taken by around 75% of endurance performance athletes [159], improves cycling efficiency during a time to exhaustion test by up to 12% under acute NH, despite no effects on neuromuscular or central fatigue [160]. The extent of these proposed effects on TT based cycling performance in hypoxia is yet to be analysed. A study by Mariacher et al. [161] found that a concentrated supplement of Alpha-Ketoglutaric acid and 5-hydroxymethylfurfural reduced performance deficits during an incremental cycling test to exhaustion when compared to a multi-antioxidant supplement, containing beta-carotene, ascorbic acid and riboflavin.

In all of the aforementioned studies, no psychological measurements were implemented. Indeed it has been demonstrated previously that blackcurrant intake stimulates an improvement in motor and cognitive performance, factors leading to an improvement in motivation and reaction times during the digit vigilance task, accompanied by reductions in fatigue [162]. To date, there is no research to suggest detrimental effects of hypoxia on performance in cognition tests [163] despite significant reductions in SaO₂, SpO₂ and cerebral oxygenation [164].

Certain compounds within NZBC may particularly influence in the physiological response. Infusion of cyanidin-3-O-glucoside into human endothelial cells reduced the presence of HIF-1 α protein in the blood [165]. This decreases the rate of angiogenesis and proliferation, events associated with mRNA transcription from the HIF-1 pathway.

The antioxidant compounds in NZBC may influence the breakdown of glucose for energy production. Ingestion of antioxidants increases the phosphorylation of pyruvate dehydrogenase (PDH) and reduces the rate of [La] elimination following sprint-based exercise [166]. PDH is an enzyme involved in the conversion of

pyruvate to acetyl-CoA in the presence of oxygen [167]. Acetyl-CoA is taken through the citric acid cycle where it releases carbon dioxide and converts ADP into ATP [168]. The increase in NO production and the higher antioxidant properties in comparison to other fruits [169] indicate the effects of NZBC intake on performance in hypoxia warrant further investigation.

2.9 THESIS RATIONALE

This review highlights the current gaps in the literature based on NZBC intake and endurance exercise performance. There is no study that has analysed the effects of a single bolus of NZBC taken 1-2 hours prior to exercise performance, the time after which blood plasma levels of NZBC anthocyanins peaks following intake. Currently only two studies have analysed the dose-response effects of NZBC with one study looking at CV effects at rest [128] and another looking at fat and carbohydrate oxidation during prolonged exercise [122]. Both studies used the same three doses of 300 mg, 600 mg and 900 mg. There is no study analysing the acute dose-response effects or resulting effects on TT cycling disciplines using the same doses of NZBC.

Currently there is just a single study looking at the effects of NZBC intake on TT performance under simulated altitude [154]. As with studies conducted under normoxic conditions, this study also implemented a chronic intake protocol over a period of 7 days. Therefore no acute intake analysis has been conducted for exercise participation in hypoxic conditions. Studies which included cycling as the exercise modality and TT as the exercise discipline have used a 16.1-km distance but to date no studies have yet analysed performance up to 20 minutes in duration. In recreational cyclists, this translates to a distance of ~10-km where commonly measured parameters such as mean PO are linked to peak PO and VO_2 max, indicating maximal performance effort over this time period.

The two research studies that formed the basis of the research in this thesis, cover previously unresearched areas in the field of NZBC in exercise performance. Following an analysis of existing studies, specifically the exercise procedures implemented, the dose ingested prior to performance and variables used to indicate subsequent endurance exercise performance, the protocols for the two studies were formed. A single bolus of NZBC was taken one hour prior to exercise to analyse whether acute intake of NZBC had any beneficial effects on TT cycling performance. Performance following three different doses was analysed to determine if any acute effects indicating improvements in performance were linear with higher doses of NZBC. The dose that had the biggest influence on TT cycling performance and endurance performance parameters in study 1 was used to analyse whether acute intake of NZBC would have a positive influence on TT cycling performance under simulated altitude, an environment with higher levels of oxidative stress. Mean $\dot{V}O_2$, HR, $\dot{V}O_2$, RER, RPE and [La] at rest and post-exercise were variables that were included to analyse the true influences on TT performance times.

2.9.1 AIMS AND HYPOTHESES

Three primary aims were proposed for this thesis, (i) to identify whether there are any acute effects on TT cycling performance or parameters of endurance performance including $\dot{V}O_2$, $\dot{V}O_2$ and HR following consumption of a single bolus of NZBC, (ii) to identify whether the proposed effects on these variables including resting values for HR, blood pressure, CO, TPR and [La] are greater following higher doses of NZBC pre-, during and post-exercise and (iii) to identify whether acute NZBC intake has any effects on TT cycling performance under moderate simulated altitude.

The research hypothesis for study 1 was that an acute dose-response effect for TT cycling performance and parameters of endurance performance pre-, during and post-exercise would be identified and that the magnitude of these effects would be

significantly greater following 900 mg in comparison to 300 and 600 mg NZBC. Following this, the research hypothesis for study 2 was that acute intake of NZBC would improve TT cycling performance and parameters of endurance performance to a greater extent under NH in comparison to a PLA under the same conditions.

3.0 DOSE-RESPONSE EFFECTS OF NEW ZEALAND BLACKCURRANT EXTRACT ON ENDURANCE CYCLING PERFORMANCE

Abstract

Purpose: To examine the acute dose-response effects of New Zealand Blackcurrant Extract (NZBC) intake on short duration time trial (TT) cycling performance & cardiometabolic parameters at rest and following exercise. **Methods:** Using a randomised, repeated measures, three-way cross-over design, eight physically active participants (5 males, 3 females; Mean \pm SD age 21.1 ± 3.7 years, stature 172.1 ± 6.2 cm, mass 72.3 ± 13.7 kg) completed three 10 km TT cycling protocols after ingesting one of three doses (CurraNZ™; 300, 600 or 900 mg) of NZBC. During testing, heart rate (HR), power output (PO), oxygen consumption (VO_2), and respiratory exchange ratio (RER) values were taken every km. Prior to exercise, cardiovascular (CV) function was measured through a number of different parameters (heart rate, systolic blood pressure, diastolic blood pressure, cardiac output and total peripheral resistance) to analyse changes pre- and post-intake (Portapres Model-2). A resting measurement of blood lactate [La] was taken 60 minutes following NZBC intake and four additional samples were collected every two minutes post exercise to analyse peak [La] values and recovery. Data were analysed using a selection of repeated measures ANOVA tests. **Results:** A strong trend towards significance was found for TT performance ($P = 0.053$, $\eta^2 = 0.41$) and mean PO ($P = 0.073$) following 900 mg NZBC. No significant effects were found for any CV markers or [La] between conditions at any of the respective time points. **Conclusion:** Acute intake of NZBC has no significant effects on TT-based performance or cardiometabolic parameters pre- and post-exercise.

3.1 INTRODUCTION

A dose-response relationship is defined as “an association between the dose of a stimulus and the incidence of a defined biological event in an exposed population” [170]. The most fundamental research based around this concept from an exercise perspective is the correlation between physical activity levels and improvements in physical and mental health markers [171,172] and all-cause mortality [173]. Studies examining this often implement a longitudinal protocol within a select section of the general population.

A large portion of research analysing the rate of improvement in race-based exercise performance, has included intake of performance enhancing ergogenic aids as the primary stimulus and trained athletes as the performance population. Many studies tend to include a specified dose and time period where the selected ergogenic aids are taken prior to exercise. Butterfield [174] stated that a dose-response relationship should be sought where appropriate when it comes to evaluating the true effects of ergogenic aids.

Literature examining the dose effects of polyphenol intake on various physiological markers during exercise is limited. Grosso et al. [175] observed reductions of 6% and 4% in all-cause and CV disease mortality, respectively, when intake of total flavonoids increased by 100 mg/day linearly over a period of several years. Rodriguez-Mateos et al. [45] found total cranberry polyphenol intake (up to 1238 mg) was associated with a dose-response effect on flow mediated dilation, peaking at 4 hours post intake. Following ingestion, sixty new metabolites were identified within plasma, twelve of which were linked to increased skeletal muscle blood flow. Intake of a specified type and dosage of polyphenols have potential to improve oxygen delivery to muscles [176]. This is linked with greater rates of oxidative phosphorylation resulting in higher levels of ATP production [177].

Research surrounding the use of NZBC prior to physical activity has used a mixture of different endurance exercise protocols based around cycling [102, 115,118,119,120, 122], running [121,123,124,127], rowing [126] and brisk walking [101]. The findings for each modality produced mixed conclusions. This research demonstrates that the cardiometabolic adaptations and performance contributions of NZBC are evident over a chronic intake protocol of at least seven days. Limited data show a dose-response effect of NZBC intake on numerous physiological parameters at rest and during exercise [122,128].

In the first study analysing whether increasing NZBC intake influences physiological variables in a correlative manner, Cook et al. [128] compared changes in numerous CV parameters following intake of either 300, 600 or 900 mg of NZBC for 7 days. During rest, CO and stroke volume (SV) increased significantly at 600 and 900 mg per day compared to baseline. Additionally, TPR decreased linearly indicating possible stress reductions on the heart during the diastolic phase. The linear effects on cardiorespiratory parameters were supported in a follow up study by Cook et al. [122] who found a dose-response effect on average fat oxidation up to 900 mg NZBC during 2 hours of steady state cycling at 65% VO_2 max.

At exercise intensities ranging between 55-74% VO_2 max, fat oxidation is the primary source of energy production [178]. During submaximal exercise of up to 2 hours in duration, it has been demonstrated that trained subjects rely on intramuscular triglyceride oxidation [179]. During short to medium term TT cycling performance, energy is generated through a combination of both aerobic and anaerobic systems [180] and the predominant physiological pathways are affected by pacing, which varies heavily at different points of race-based exercise [181]. Although Willems et al. [115] found an increase in [La] clearance following a 16.1-km time trial cycle compared to a PLA, there is no current research concluding if a dose-response effect exists for recovery parameters following exercise. Additionally, Cook et al. [122] concluded that a dose-response effect on TT based exercise protocols has yet to be carried out, despite evidence of fuel utilisation changes during steady state cycling of up to two hours in duration with higher doses of NZBC.

Taking everything into account, this study aims to describe the influence of a single bolus of NZBC on changes during and recovery following medium duration TT cycling performance. Changes in CV parameters & [La] were measured before and immediately following exercise at specified time points while changes in PO, VO_2 and respiratory exchange ratio (RER) were measured during exercise. The effects of

acute NZBC intake on exercise performance were analysed to a greater extent through the comparison of three different doses taken at the same time points prior to exercise to determine if physiological and exercise responses would appear linear.

A 10-km TT cycle was selected as the exercise mode based on the evidence that mean PO during cycling disciplines of up to 20 minutes in duration, significantly correlates with peak PO and VO_2 max [21]. A 10-km cycle will take around 20 minutes for recreational athletes to complete, indicating that a TT of this length will involve participants working at a high percentage of their maximal endurance capacity. This exercise modality has been selected as the exercise discipline in previous research listed in the literature review [107,114,156].

The research hypothesis posed for this study was that an acute dose-response effect for TT cycling performance and parameters of endurance performance pre-, during and post-exercise would be identified and that the magnitude of these effects would be significantly greater following 900 mg in comparison to 300 and 600 mg NZBC.

3.2 METHODOLOGY

3.2.1 SUBJECTS

The number of required participants was determined on G*Power, based on the test hypothesis, the statistical tests to be used, the alpha value required to determine significance, the smallest effect size for scientific interest and the selected power value. The specified number of participants required to determine a statistically significant difference was 32. However due to time constraints, this number was not met. Power was calculated based on the sample size and statistics provided in previous studies along with a specified effect size that had to be met to indicate a substantial difference in the independent and dependent variables in two or more groups.

Ten physically active sport and exercise science students (6 males, 4 females; Mean \pm SD Age 20.7 ± 3.9 yr, stature 171.9 ± 8.4 cm, mass 73.1 ± 16.1 kg), recruited from the University of Essex, volunteered to take part in this study. As part of the inclusion criteria, participants were required to (1) participate in moderate-high intensity sport or physical activity for a minimum of eight hours per week, (2) be free of any injuries or illnesses for a minimum of two weeks prior to the study and (3) be able to complete the 10 km TT cycle in < 25 minutes. Participants were excluded from the study if they were greater than 30 years of age, elite level performers, recreational smokers or if they were unable to complete the TT cycle in < 25 minutes during the familiarisation session.

Participants visited the laboratory on four occasions; one screening and familiarisation trial followed by three supplementation trials to test the acute effects of a single bolus of NZBC on cycling performance. All visits were separate by a minimum time period of 48 hours. Throughout all points in the study, participants were reminded of the experimental procedures, the benefits of participation and

associated risks of taking part in endurance exercise. During the process of data collection, two participants voluntarily withdrew from testing and as a result any associated data or results were excluded from the results section. Eight full sets of data were included for the final analysis.

For each visit, participants were instructed to arrive in an appropriate state for exercise which included having breakfast or lunch with at least 500ml of water 2 hours prior to exercise. Participants were advised to abstain from intense, vigorous exercise within 24 hours before testing, from consuming alcohol within 12 hours before testing and from taking caffeine in any form within 4 hours before testing. Participants did not receive any financial incentive or compensation.

3.2.2 ETHICAL APPROVAL

All study procedures received approval by the University of Essex Ethics Committee for scientific research involving human subjects. The study procedures, and the risks and benefits of participation were provided in an informed consent sheet during the familiarisation session. Participants were required to sign both this and a physical activity readiness questionnaire (PAR-Q) to be cleared to participate in the study. Throughout the duration of the study, participants were allowed to withdraw at any point without reason or question.

3.2.3 STUDY DESIGN

A randomised, counter balanced, single blinded, crossover study design was implemented to compare the effects of three different doses of NZBC on TT cycling performance. As this study was aiming to identify any acute effects from the different NZBC dosages, a prolonged washout period (> 2 days) was not required. Participants performed no more than two experimental procedures in a single week.

3.2.4 FAMILIARISATION TRIAL AND DATA COLLECTION PROCEDURES

The familiarisation session served to introduce participants to the testing protocol, the physiological measurements that would be taken and the methods with which data would be collected throughout their time on the study. For the first visit, stature (Wall Stadiometer: Seca 220, Birmingham, UK) and body mass (Scales: Seca 813, Birmingham, UK) were measured on three occasions to determine average values for both variables. Following this, resting CV parameters for HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), CO & TPR (termed CV parameters) were measured using a PortaPres Model-2 blood pressure monitor (Finapres Medical Systems, Amsterdam, Netherlands) on the right middle finger for a period of 90 seconds. A total of three measurements were taken to determine an average value. Coefficient of variation was calculated at 3.4% for HR and 4.8% for CO at rest based on limited data [182]. A sample of blood (20 μ g) was taken from the left ear lobe to get a resting measurement of [La].

Following exercise, the same CV parameters and [La] values were collected every 2 minutes to analyse the specific time point at which [La] concentration peaked. In line with these measurements, rate of physiological recovery was determined via the rate of [La] clearance over four difference time points every 2 minutes following the cessation of the TT. The latter was done on the basis of research suggesting peak [La] values are observed between 3-8 minutes post exercise [183].

A warm-up was performed for five minutes at a frequency of 70-80 revolutions per minute. Participants were instructed to reduce speed if heart rate was greater than 130 beats per minute. Participants then practised the 10 km time trial cycle on an initial air brake resistance of 3. This was done to reduce the risk of pacing error during the supplement trials and allowed participants an idea of the length of time it would take to complete the time trial.

During the TT, breath by breath values for VO_2 , VCO_2 and RER were measured using gas analysis software (Oxycon Masterscreen CPX, Vyaire Medical, Basingstoke, UK) with values taken at the time point of every completed km. Participants were informed of the distance travelled every 0.5 km throughout the cycle and upon discretion, exercise intensity was changed to suit the needs of participants.

All cycling protocols were performed on a Wattbike Pro Ergometer (Nottingham, UK). Throughout all exercise tests, HR was continuously monitored (Polar R300, Kimpele, Finland). VCO_2 measurements were only taken for the calculation of RER and no future data analysis was conducted for this variable as a single variable. Prior to every testing session, the breath-by-breath gas analyser was calibrated to known atmospheric gas concentrations. Calibration was achieved when concentration and time boundaries of oxygen and carbon dioxide were all < 0.5 . Measurements of each [La] sample were produced using a Biosen 5030 blood analyser (Biosen C-Line Clinic, EKF Diagnostic, Magdeburg Germany) with a calculated coefficient of variation between 1.3-3% [184]. Calibration of both analysers was essential according to manufacturer's guidelines to ensure measurements were valid and accurate.

3.2.5 SUPPLEMENTATION TRIALS

For the following visits, participants repeated the pre-exercise CVP measurements before ingesting one, two or three capsules of NZBC (one capsule – 300 mg active cassis, 105mg anthocyanins, 3-10% Cyanidin-3-O-Glucoside, 30-45% Cyanidin-3-O-Rutinoside, 5-20% Delphinidin-3-O-Glucoside, 35-50% Delphinidin-3-O-Rutinoside, CurraNZ™, Health Currency Ltd., Surrey, UK). 60 minutes following ingestion, based on research suggesting peak circulatory levels of anthocyanins at this time point [185], a second set of CV measurements were taken along with a single resting measurement of [La] immediately prior to the cycle.

Resting [La] was taken 60 minutes following blackcurrant ingestion. During the time period following ingestion, participants were strictly informed not to consume anything other than water. The 10 km TT cycle and post exercise CV and [La] measurements were repeated with participants encouraged to complete the exercise in the quickest time possible. A full schematic representation of the testing protocol can be found in Figure 9.

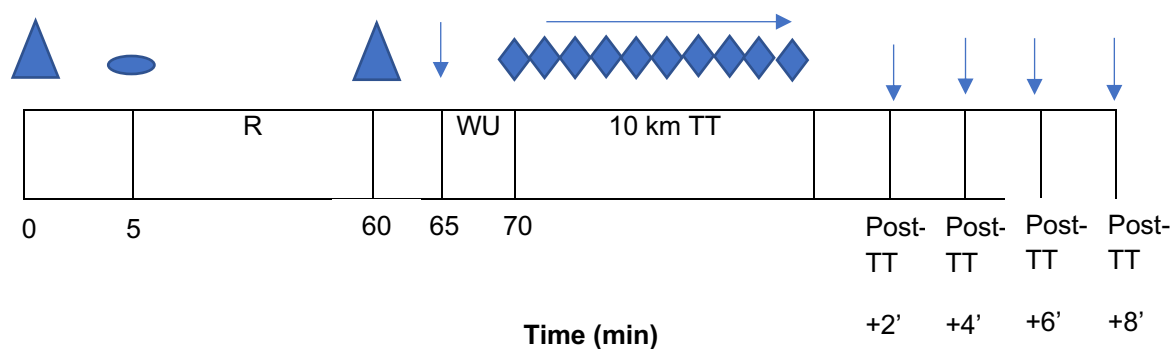


Figure 9. Schematic Representation of the TT Cycling Experimental Protocol. R = Rest, WU = Warm Up, TT = Time Trial

● NZBC Capsule = 300, 600, 900 mg

↓ Capillary Blood Sample

▲ PortaPres Cardiovascular Parameters

→ HR

◆ PO, VO₂, RER

3.2.6 DATA AND STATISTICAL ANALYSIS

Information on km completion times and PO were recorded on the Wattbike ergometer test file while statistics for gas data were recorded on the oxycon masterscreen software spreadsheet. Statistics for all variables were placed onto a data collection sheet, ready for analysis. All statistical analyses were completed using statistical package SPSS version 24.0 (SPSS Inc., Chicago, Illinois, USA). Due to the sample size, a Shapiro-Wilk's test [186] was run for all variables to determine

if data were normally distributed. This included an inspection of histograms, normal Q-Q plots, box plots and skewness / kurtosis indices [187]. Data normality values were analysed on the “tests of normality” output under the “Shapiro-Wilk” column. If normality was not indicated, based on an alpha value greater than 0.05, further tests were run to detect outliers and a Friedman test was run as a non-parametric alternative for any variables or groups. If normality was determined, a series of repeated-measures analysis of variance (ANOVA) tests were run to determine differences between groups for TT, HR, PO, VO_2 , RER and resting [La] between dose conditions with post hoc Bonferroni tests for significant condition effects. Significance and effect size values were analysed on the test of within-subjects effects output.

All values were reported as Mean \pm SD. A two-way, repeated-measures ANOVA was run to determine significant differences in post exercise CV variables and [La] over the respective time points. For all statistical tests, power ($1-\beta$) was based on a value of 0.8 and significance was based on an alpha value lower than 0.05. Effects sizes with partial eta squared (η_p^2) were used to determine the magnitude of effect between measurements and time points. For η_p^2 , effect sizes were deemed small at a value of 0.01, medium at 0.06 and large at 0.14. An alpha value greater than 0.05 but lower than 0.1 ($0.05 > P < 0.1$) was interpreted according to guidelines set by Curran-Everett and Benos [188].

3.3 RESULTS

3.3.1 TT CYCLING PERFORMANCE TIMES

There was a strong trend towards significance for quicker cycling performance times during the 10-km TT following 900 mg NZBC with a difference of 11.9 & 41.4 seconds between 300 mg and to 600 mg respectively ($F_{(2,14)} = 4.89$, $P = 0.053$, $\eta^2 = 0.41$).

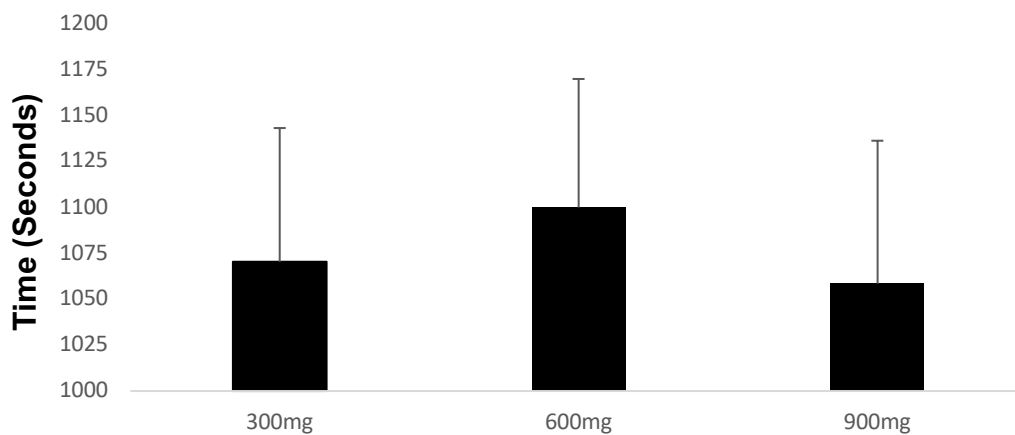


Figure 10. TT completion values (Mean \pm SD) for a 10 km time trial cycle following acute intake of NZBC

Analysis of pacing revealed mean km completion times were quickest from km 4 to km 10 following 900 mg of NZBC compared to 600 mg and 300 mg NZBC. In all conditions, mean km completion time was quickest during km 10.

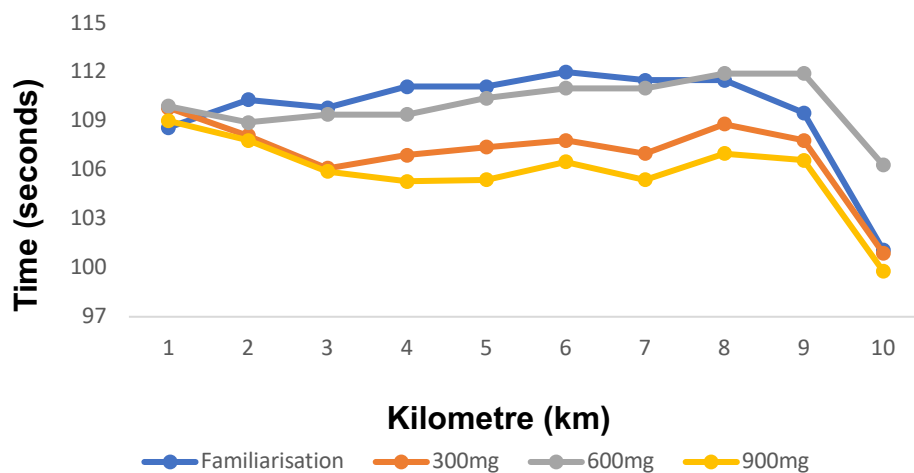


Figure 11. Mean km completion times for all TT cycling protocols

3.3.2 PO FROM TT

There was a trend towards significantly higher values for mean PO during the TT following 900 mg NZBC in comparison to 300 & 600 mg with percentage differences of 5.6% and 12.9% respectively ($F_{(2,14)} = 4.00$, $P = 0.073$, $\eta^2_p = 0.36$).

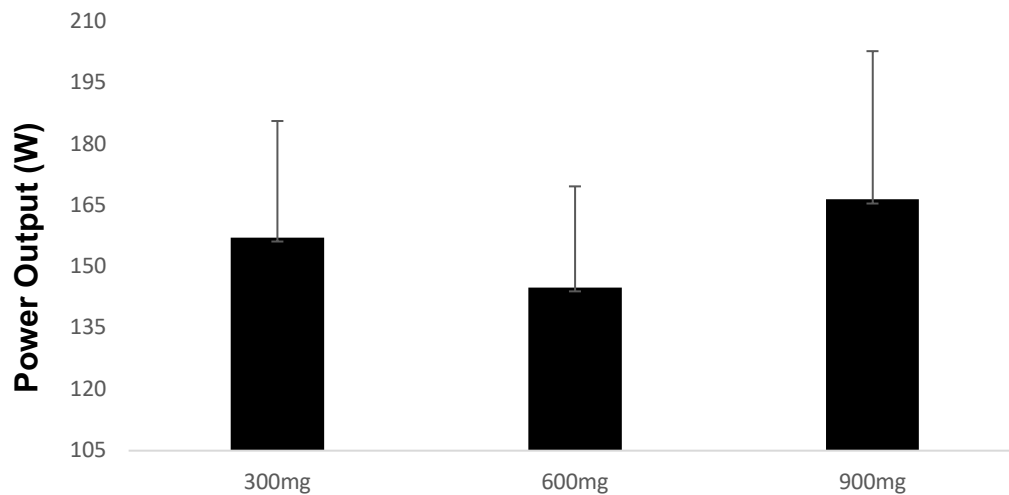


Figure 12. PO values (Mean \pm SD) during a 10 km TT cycle following acute intake of NZBC

An analysis of PO values per km revealed mean values were highest following 900 mg of NZBC between km 2 and km 10 during the TT when compared to 300 mg and 600 mg NZBC.

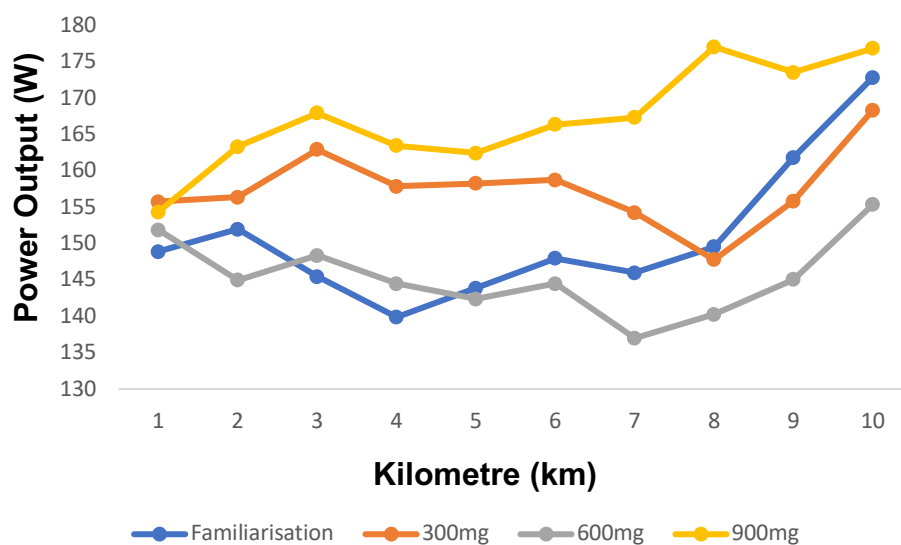


Figure 13. Mean PO values per km during a 10 km TT cycle

Peak PO values were significantly different between conditions (300 mg 178 ± 30.7 ; 600 mg 161.9 ± 28.1 ; 900 mg 193.8 ± 38.8 Watts, $F_{(2,14)} = 6.49$, $P < 0.05$, $\eta^2 = 0.48$). Post hoc tests using the Bonferroni post hoc test revealed that intake of 900 mg of NZBC elicited a slight increase in peak PO from 300 mg with a strong trend towards statistical significance ($P = 0.053$). However peak PO was significantly higher with 900 mg in comparison to 600 mg of NZBC ($P < 0.05$). A trend towards significance was also found between intake of 300 mg and 600 mg of NZBC ($P = 0.083$).

3.3.3 HR FROM TT

There was no significant effect of NZBC intake on mean HR with values highest following 900 mg NZBC at a difference of 4.4 and 5.7 BPM between 300 mg and 600 mg respectively ($F_{(2,14)} = 2.68$, $P > 0.05$, $\eta^2 = 0.28$).

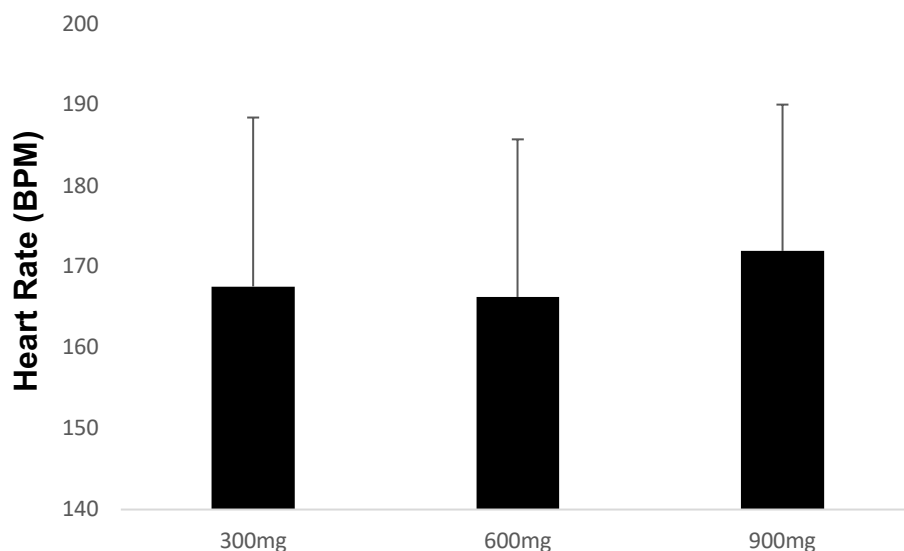


Figure 14. HR values (Mean \pm SD) during a 10 km TT cycle following acute intake of NZBC

3.3.4 VO_2 AND RER FROM TT

There was a trend towards lower VO_2 values during the TT following consumption of 600 mg NZBC during testing with a 10% difference between 300 mg and a 1.8% difference between 900 mg NZBC ($F_{(2,14)} = 2.89$, $P = 0.095$, $\eta^2 = 0.29$). Negligible differences between groups for RER were present with no statistical significance ($F_{(2,14)} = 0.85$, $P > 0.05$, $\eta^2 = 0.11$).

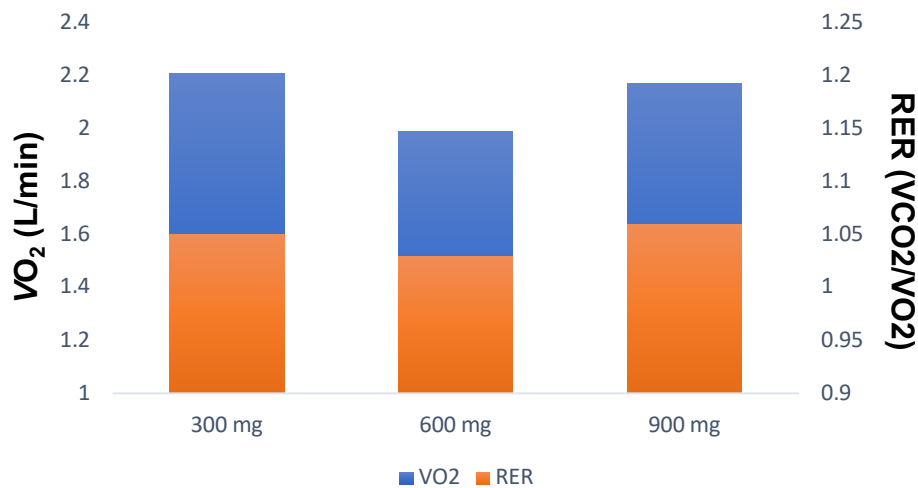


Figure 15. Gas exchange data values for (Mean \pm SD) during a 10 km TT cycle following acute intake of NZBC

3.3.5 CV PARAMETERS AT REST

Resting pre- and post-intake values of HR, SBP, DBP, PP, CO and TPR are presented in table 2. No significant interaction effects (dose x time) were found for any CV variables at rest ($P > 0.05$). When analysing main effects for dose and time in table 3, no significance was found for any CV variable ($P > 0.05$).

Table 2. Resting values for cardiovascular parameters pre- and post-intake of NZBC

	Time Point	300 mg	600 mg	900 mg	F	P	η^2
HR (BPM)	Pre Intake	72.0 ± 13.4	75.4 ± 3.0	78.6 ± 11.0	2.38	0.155	0.37
	Post Intake	77.4 ± 7.6	83.4 ± 14.9	74.6 ± 14.7			
SBP (mmHg)	Pre Intake	127.0 ± 5.1	118.6 ± 10.1	114.6 ± 6.6	2.47	0.147	0.38
	Post Intake	121.8 ± 9.4	124.8 ± 18.1	128.2 ± 18.3			
DBP (mmHg)	Pre Intake	69.8 ± 3.8	70.8 ± 7.7	60 ± 6.0	2.68	0.128	0.40
	Post Intake	67 ± 10.0	77.4 ± 10.0	73.2 ± 15.9			
PP (mmHg)	Pre Intake	57.2 ± 2.9	47.8 ± 7.8	54.6 ± 10.9	0.03	0.968	0.008
	Post Intake	54.8 ± 14.8	47.4 ± 16.0	53.0 ± 9.0			
CO (L.min ⁻¹)	Pre Intake	5.6 ± 1.9	5.7 ± 1.2	5.8 ± 1.3	1.05	0.395	0.21
	Post Intake	6.1 ± 1.3	6.8 ± 2.5	5.9 ± 1.9			
TPR (mmHg)	Pre Intake	0.98 ± 0.25	1.04 ± 0.28	0.90 ± 0.16	1.15	0.363	0.22
	Post Intake	0.90 ± 0.16	0.94 ± 0.23	1.04 ± 0.54			

Values are Mean ± SD, pre-intake, post-intake,

HR = Heart Rate; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; PP = Pulse Pressure; CO = Cardiac Output; TPR = Total peripheral resistance; BPM = Beats per minute; mmHg = millimetres of mercury; L.min⁻¹ = Litres per minute; η^2 = partial eta squared effect size

Table 3. Main Effects for Dose and Time in Resting CV Parameters Following NZBC Intake

Variable	Main Effect	F	P	η^2
HR (BPM)	Time Point	0.506	0.516	0.11
	Dose	0.341	0.721	0.08
SBP (mmHg)	Time Point	1.373	0.306	0.26
	Dose	0.28	0.765	0.07
DBP (mmHg)	Time Point	3.66	0.128	0.48
	Dose	3.12	0.099	0.44
PP (mmHg)	Time Point	0.294	0.616	0.7
	Dose	1.76	0.233	0.31
CO (L.min ⁻¹)	Time Point	0.781	0.427	0.16
	Dose	0.333	0.726	0.08
TPR (mmHg)	Time Point	0.018	0.901	0.004
	Dose	0.119	0.889	0.03

3.3.6 CV PARAMETERS POST EXERCISE

Post-exercise values for SBP, DBP, CO, PP & TPR are presented in table 4. There were no significant interaction effects (dose x time) for any CV variables following exercise ($P > 0.05$). When analysing main effects for dose and time in table 5, a post-hoc Bonferroni analysis revealed a significant main effect in the time point for TPR ($P < 0.05$, $\eta^2 = 0.34$) with a significant difference in TPR values between 2-min and 8-min post-exercise ($P = 0.03$).

Table 4. Post exercise values for cardiovascular parameters following acute intake of NZBC

	Time Point	300 mg	600 mg	900 mg	F	P	ηp^2
SBP (mmHg)	2 min	104.4 ± 15.7	116.3 ± 20.1	103.0 ± 14.5	1.12	0.368	0.14
	4 min	119.4 ± 20.1	126.4 ± 28.0	115.3 ± 14.8			
	6 min	115.1 ± 16.3	124.6 ± 25.5	113.9 ± 25.0			
	8 min	113.5 ± 20.0	120.0 ± 21.1	123.4 ± 21.3			
DBP (mmHg)	2 min	60.5 ± 10.0	73.6 ± 12.0	62.8 ± 6.2	1.79	0.125	0.20
	4 min	68.5 ± 9.7	73.5 ± 14.2	69.3 ± 8.5			
	6 min	65 ± 9.9	73.6 ± 14.3	69.1 ± 10.1			
	8 min	66.3 ± 10.1	72.4 ± 11.3	73.6 ± 8.7			
PP (mmHg)	2 min	43.9 ± 8.6	42.6 ± 10.9	40.3 ± 10.2	0.89	0.508	0.11
	4 min	50.9 ± 12.1	52.9 ± 15.6	46.0 ± 10.0			
	6 min	46.5 ± 10.2	51.3 ± 14.1	44.8 ± 17.4			
	8 min	47.3 ± 11.1	47.6 ± 16.0	49.8 ± 14.9			
CO (L.min ⁻¹)	2 min	7.7 ± 2.2	6.5 ± 1.8	7.3 ± 2.5	1.28	0.286	0.16
	4 min	7.8 ± 2.0	7.3 ± 2.3	7.3 ± 1.9			

	6 min	7.0 ± 2.2	7.0 ± 2.2	6.9 ± 2.0			
	8 min	6.7 ± 1.7	6.6 ± 1.7	7.3 ± 3.1			
TPR (mmHg)	2 min	0.61 ± 0.21	0.85 ± 0.31	0.68 ± 0.23	1.14	0.354	0.14
	4 min	0.68 ± 0.23	0.78 ± 0.29	0.72 ± 0.21			
	6 min	0.71 ± 0.21	0.81 ± 0.30	0.77 ± 0.22			
	8 min	0.74 ± 0.18	0.83 ± 0.21	0.81 ± 0.29			

Values are Mean \pm SD, 2 min, 4 min, 6 min, 8 min

Table 5. Main Effects for Dose and Time in Post-Exercise CV Parameters Following Acute Intake of NZBC

Variable	Effect	F	P	η^2
SBP (mmHg)	Time Point	2.426	0.094	0.26
	Dose	1.372	0.286	0.16
DBP (mmHg)	Time Point	1.96	0.151	0.22
	Dose	2.444	0.123	0.26
PP (mmHg)	Time Point	2.409	0.096	0.26
	Dose	0.374	0.695	0.05
CO	Time Point	1.723	0.227	0.19
	Dose	0.692	0.539	0.09
TPR (mmHg)	Time Point	3.614	0.03	0.34
	Dose	2.597	0.110	0.27

3.3.7 BLOOD LACTATE AT REST

Following NZBC ingestion, there were no significant differences in blood [La] values at rest between conditions with values 14.2% & 18.5% lower following 900 mg NZBC in comparison to 300 mg and 600 mg respectively ($F_{(2,14)} = 0.65$, $P > 0.05$).

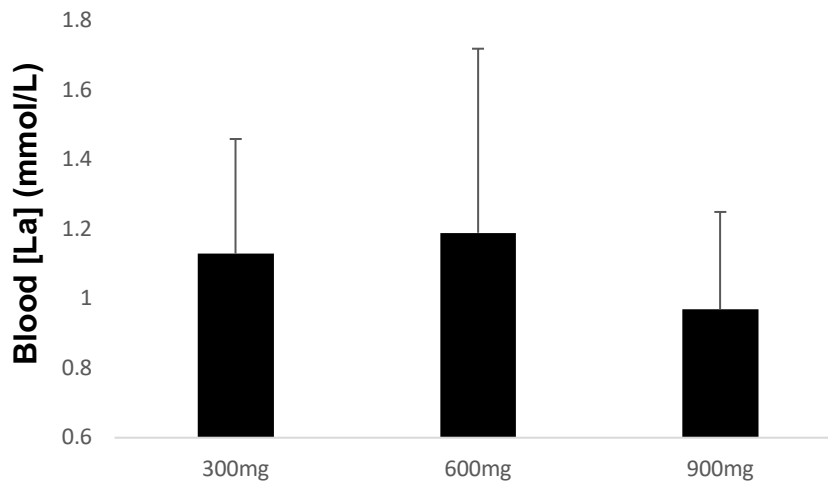


Figure 16. Resting [La] Values (Mean \pm SD) Following Acute Intake of NZBC

3.3.8 BLOOD LACTATE POST EXERCISE

Post exercise values for [La] are presented in table 6. There was no significant interaction effect (dose x time) for [La] at any time point following exercise ($P > 0.05$). When analysing main effects for dose and time in table 7, a bonferroni post hoc test determined a significant main effect was found in [La] values for the time point for [La] ($P = 0.011$, $\eta^2 = 0.52$).

Table 6. Post Exercise [La] Values Following Acute Intake of NZBC

	Time Point	300 mg	600 mg	900 mg	F	P	η^2
[La] (mmol/L)	2 min	7.77 ± 3.89	6.82 ± 3.20	7.73 ± 2.91	0.88	0.524	0.15
	4 min	7.22 ± 4.17	6.49 ± 2.88	7.90 ± 1.86			
	6 min	6.83 ± 3.85	5.91 ± 2.75	7.02 ± 1.99			
	8 min	6.67 ± 3.91	5.48 ± 2.61	6.03 ± 1.36			

Values are Mean ± SD, 2 min, 4 min, 6 min, 8 min

[La] = Blood Lactate, mmol/L = Millimoles per litre, mg = milligrams, min = minutes

Table 7. Main Effects for Dose and Time in Post-Exercise [La] Values Following Acute Intake of NZBC

Variable	Effect	F	P	η^2
[La] (mmol/L)	Time Point	5.331	0.011	0.52
	Dose	0.693	0.523	0.12

3.4 DISCUSSION

The dose-response relationship relates to the magnitude of change in physiological and psychological adaptations following an increase in the dose of an ergogenic aid. This is the first study to analyse acute, dose-response changes in physiological performance markers during exercise and recovery markers post exercise following a single bolus of NZBC in physically active participants. For this study, we hypothesised that acute intake of NZBC would induce physiological changes, leading to enhanced TT cycling performance. Additionally, we stated that a dose-response adaptation would occur following NZBC intake with the magnitude of these effects significantly greater following 900 mg in comparison to 300 and 600 mg NZBC.

The research hypothesis for this study was not supported by the findings but differences were present between the 300 mg, 600 mg and 900 mg intake protocols. The main finding was that a strong trend was present for significant improvements in TT completion times following 900 mg NZBC. When comparing overall differences between the mean values of each dose group, the TT protocol was completed 2.45% quicker following 900 mg in comparison to 600 mg and 300 mg NZBC. This figure matches the range of change found in performance in studies looking at the effects of BRJ [106] and NZBC [118].

The difference between these two studies however is the duration of intake prior to exercise with Lansley et al. [106] comparing results to a PLA following a single bolus of BRJ while consumption of NZBC at a daily dose of 300 mg took place over a seven day period. Interestingly, the percentage differences determined by Lansley et al. [106] for 4 km and 16 km TT cycling protocols was 2.8% and 2.7% respectively following intake of BRJ. These are larger when compared with a 2.4% difference seen in Cook et al. [118] for a 16.1 km TT following intake of NZBC. This indicates

that although the physiological effects induced by these supplements are similar to each other, the length of time taken to experience beneficial adaptations to endurance performance is different for underlying reasons.

The improvements in TT performance times were illustrated by an increase in mean PO values and significantly greater peak PO values during the TT following 900 mg of NZBC. When calculating overall differences between the mean values of each dose group, PO was 9.3% higher following 900 mg NZBC intake when compared to 300 mg and 600 mg of NZBC. The significant increase in peak PO might suggest possible stimulation of anaerobic energy pathways, leading to greater exercise capacity. When taking pacing into account, mean km completion times were quicker at the end of the fourth km until the cessation of the TT following 900 mg of NZBC. This was accompanied by higher values for mean PO for all but one km during the TT when compared to both 300 mg and 600 mg of NZBC.

It is difficult to suggest whether acute NZBC intake has any effect on muscle recovery post-exercise as there was no statistically significant differences in post-exercise [La] values. However greater [La] removal was indicated at 6 min and 8 min time points following 900 mg NZBC. Although there was no statistical significance with regards to differences between groups based on the dose x time interaction, there is a significant main effect for time point with values significantly lower at 8 mins post-exercise in comparison to 2 mins post-exercise following 300 mg and 900 mg NZBC ($P < 0.05$, $\eta^2 = 0.52$). It appears that there is no influence on the amount of NZBC ingested and physiological recovery following exercise. Post exercise, [La] was greatest at 2 minutes post exercise following ingestion of 300 mg and 600 mg NZBC whereas [La] peaked at 4 minutes post exercise following 900 mg NZBC. Because no post-exercise performance measure was included in the present study, further research is needed to assess whether muscular performance is enhanced

following this, as well as the magnitude of these changes and the time points at which muscular performance peaks when exercise is repeated.

In this study, the effects of NZBC on CV function were quantified by comparing HR, SBP, DBP, CO and TPR prior to and following exercise as well as HR during exercise. No significant dose- or time-dependent responses were found for any variable, indicating NZBC had no short-term effect on CV physiology. This matches the findings of Willems et al. [102] who found no significant differences in HR, SBP and DBP at rest and any CV variables during exercise with an intensity range between 40-80% intensity following intake of Sujon NZBC blackcurrant powder. Their study also found a significant increase in CO and SV alongside a reduction in TPR.

These observations were not made in the current study but are present in previous research implementing a dose-response analysis with the same doses of NZBC on resting CV variables [122,128]. For example, Cook et al. [128] reported 15% and 28% increases in CO with 7% and 18% increases in SV between a control group and 600 mg and 900 mg per day respectively. A 20% linear decrease in TPR was also observed with a dose of 600 mg and 900 mg doses per day. In both dose-response studies by Cook et al. [122,128], the percentage differences indicate a linear response in effects based on dose with higher doses having a greater effect on the aforementioned variables with smaller doses seen with smaller doses.

In this study, mean results for overall TT performance, PO, HR, VO_2 and [La] were curvilinear, indicated by the dip in these parameters following 600 mg NZBC. This was not expected as values following 600 mg NZBC were predicted to indicate a moderate change in performance and these factors between 300 mg and 900 mg. Reasons for this could have included preparatory factors including poor sleep, nutritional intake prior to the trial or the time of day that the scheduled session took

place. Details of these factors should be compared against those of the other supplement trials to identify what might have been different on the days participants ingested 600 mg NZBC prior to exercise.

Motivational factors might have reduced performance on the day the 600 mg trial took place, if for example, there were still sessions to undertake following completion of the 600 mg trial. With the prescription of a different number of capsules to match the stated dose, participants could have recognised the potential for a performance plateau with two capsules as opposed to three and might not have worked as hard as they could have done as a result. There is evidence to suggest that a lack of actual effect from NZBC itself might have caused no change in performance from baseline for this particular trial. Further research is warranted to identify whether a control group would determine if this was the case not only for 600 mg NZBC but for other doses as well.

A comparison of the same CV variables post-exercise revealed no dose x time interaction for any variable. There is a significant main effect for time point in TPR with values higher at 8 mins post-exercise in comparison to 2 mins post-exercise following 300 mg and 900 mg NZBC ($P < 0.05$, $\eta^2 = 0.34$). The duration of the intake protocol would have likely affected the physiological responses to NZBC. Both research studies from Cook et al. [122,128] involved a chronic intake period of the specified doses of NZBC over a period of 7 days whereas the current study involved a single bolus prior to exercise. Along with this, the absence of a notable response in any CV or physiological variable, especially at 900 mg, is based on the suggestion that higher intakes of NZBC limit the physiological ability of various mechanisms in the breakdown and absorption of anthocyanins [189].

The measurements taken for CV variables may have been affected due to large variability between readings for each participant. At present data is limited regarding

coefficient of variation for SBP, DBP & TPR with only a single study analysing HR and CO values using a similar Finapres monitoring device [182]. Evidence also suggests that this method is inaccurate for the assessment of CO in the absence of invasive calibration [190], indicating that multiple devices are likely needed to accurately assess the level of CV stimulation in individuals, irrespective of whether this is at rest or during exercise.

Evidence suggests that plasma concentration levels of anthocyanins peak between 1.25-1.75 hours following blackcurrant consumption [86], possibly demonstrated by the increase in CO in all conditions and a reduction in resting HR following 900 mg NZBC. However the delay between the point at which plasma concentration of a supplement or its ingredients reach their highest levels and stimulation of the desired physiological mechanisms needs to be taken into account.

The findings of Willems et al. [102] might explain the increase in mid-exercise PO during the current study. In the aforementioned study, exercise intensity was 6% higher at OBLA in comparison to a PLA with no effects on HR or $\dot{V}O_2$ following Sujon NZBC powder intake, demonstrating an improved physiological ability to buffer [La]. In the current study, negligible differences were seen between mean HR and $\dot{V}O_2$ values but PO values during the TT were higher following 900 mg of NZBC. A reduction in resting [La] following NZBC intake might have improved exercise tolerance at the [La] threshold and OBLA. Although neither of these markers were not determined in the study, the increase in [La] removal following exercise provides evidence that the ability of the muscles to buffer [La] build up during exercise was greater following a higher intake of NZBC.

Despite no significant differences found between any variables from a statistical sense, the effects of acute NZBC intake on improving TT performance by several seconds following 900 mg in comparison to 300 mg and 600 mg, presents a lot of

significance from a field-based perspective for recreational athletes. Mean time to complete the 10-km TT was quicker by more than 10 seconds following 900 mg NZBC and this was indicated by higher PO values per km throughout the TT from the second km of the protocol. Athletes participating in medium duration TT exercise disciplines might look to improve their personal best or previous performance by 2-3%. With an improvement of 2.45% seen following 900 mg NZBC, a single bolus of 900 mg NZBC appears suitable for improving performance in a suitable manner from baseline. As it appears that [La] removal increases following 900 mg NZBC, athletes undertaking multi-day exercise events or training sessions over several consecutive days might consider using this to improve physiological recovery. This will reduce the likelihood of experiencing post-exercise soreness and improve both physical resting state and exercise efficiency on subsequent days.

For η^2 effect sizes, values were deemed small at 0.01, medium at 0.06 and large at 0.14. The effect size for TT performance as 0.41 indicating a large effect of 900 mg of NZBC when compared to 300 mg and 600 mg. The largest effect size of 0.48 was found for peak PO, the only variable with a statistically significant difference between conditions. With regards to CV parameters, effect size values post-exercise were smaller in comparison to resting values with the exception of PP. indicating NZBC intake had more of an effect on participants in a resting state and thus might have health benefits in individuals who do not undertake regular physical activity.

In this study, a control condition was not included, failing to fully replicate similar studies by Cook et al. [122,128]. If a control was included, participants would have undertaken the testing and exercise procedures without ingesting any form of NZBC with data from the same listed variables included in the final data analysis. This would have assisted the research by identifying whether NZBC was having any true effects or whether the experimental procedures were having the desired effects. It is therefore more difficult to draw a full conclusion on the true influence of acute NZBC

intake on endurance exercise performance because of having a control group would have therefore allowed discrimination of the participant outcome from an outcome caused by external or psychological factors. Time and PO values per km from the familiarisation trial were included in the data analysis, providing some perspective on how the results might have looked if a control group was implemented for all variables.

A greater number of participants and study costs would have been incurred as a result of having a control group. As participants had already completed a familiarisation session as part of the first testing session, including a control group where they would repeat the exact same protocol with no supplement ingestion, could have presented issues with regards to motivation to perform and return for subsequent sessions. This could have potentially lead to a greater dropout rate and power not being met. One of the aims of this study was to provide athletes already using ergogenic aids information regarding whether dose of NZBC automatically affects TT performance and endurance performance parameters post ingestion. From the perspective of these individuals, Including a control group would have had no significant value from a practical perspective if participants felt these improved their performance both physically and mentally.

PRACTICAL APPLICATIONS

Because mean TT completion times, mid-exercise PO, and [La] removal following exercise are greatest following 900 mg NZBC, it may be preferential for physically active individuals to use this ergogenic aid at the specified dose prior to training sessions or race based performance. By minimising fatigue during the latter stages of endurance-based exercise and increasing the rate of recovery post exercise, optimal physiological adaptations can be achieved without negative impacts on training ability.

Future studies should identify the magnitude of change in both TT based performance and physiological adaptations associated with a dose-response effect for chronic supplementation protocols. Additionally further research is required to determine the underlying mechanisms responsible for the production of eNOS and CV responses to NZBC and whether these responses are significantly greater following higher intakes of NZBC or differ depending on athletic status.

LIMITATIONS

Prior to the start of exercise and throughout the duration of the participants time on the study, diet was not directly controlled, possibly leading to aspects of performance being affected by alternative ingredients or nutrients than those present in NZBC. The use of capsules as opposed to fluid are likely to have delayed some physiological responses to the supplement, evidenced by the absence of linear responses to HR, TPR, SBP & DBP pre- and post-intake at rest. The consumption of supplements in capsule form bypasses potentially degrading properties of saliva in the oral cavity, and the breakdown of anthocyanins into the bloodstream is delayed [191]. A different number of capsules were prescribed to individuals corresponding to the specified doses. This provided participants with a firm idea of the dose they were being given on the day which might have influenced them to work harder. Future changes to this aspect would include giving participants a selection of gelatin capsules with a certain number of these containing NZBC powder with others filled with a placebo powder. This will be done to ensure participants remain unaware of the dose of NZBC they received at that moment.

In an ideal scenario, blood samples would be taken from a skeletal muscle in either the arm or leg to measure the concentration of anthocyanins in the bloodstream prior to exercise. This would be done over multiple time points to determine a suitable value that has to be reached prior to when exercise can take place. The

measurements taken for CV variables may have been affected due to large variability between readings for each participant.

At present data is limited regarding the coefficient of variation for the portapres model 2 for measurements of SBP, DBP & TPR and only a single study has calculated values for HR and CO using a similar Finapres monitoring device [182]. In an ideal study, a method of assessing blood flow invasively alongside a non-invasive technique such as a Portapres model system, will need to be incorporated together to accurately assess the level of CV stimulation in individuals, irrespective of whether this is at rest or during exercise.

Although participants underwent welfare checks for injuries or any barriers that might have prevented them from undertaking exercise, participants were not asked whether they were taking any other ergogenic aids prior to testing. If ingestion of any form of performance enhancing supplement was taken for an extended period of time prior to the study, the effects present as a result of NZBC intake could have been overshadowed by the effects directly from these other ergogenic aids. One of the stipulations prior to exercise was to refrain from consuming alcohol within 12 hours of testing.

This presented potential issues with regards to the time of sessions on a scheduled day. Under these circumstances, participants with an afternoon or evening session would have been aloud to consume alcohol late into the previous evening, thus increasing the risk of exercise performance being impaired due to presence of alcohol in the blood and surrounding issues with cardiovascular or muscular function. Next time, a time boundary of 24 hours will be implemented to reduce the risk of these events.

Prior to the study, a power analysis was conducted to determine the number of participants required to get a significance value of less than 0.05. With only 8

participants, the study was massively underpowered, leading to no statistically significant differences found in any variable and the true effects of NZBC unknown. In this study, male and female participants were involved and included altogether in one sample group. The absence of a comparison group analysing differences between sexes could have lead to noticeable differences in certain physiological variables going unnoticed.

There is evidence to suggest that the menstrual cycle might affect performance negatively. One of the stipulations implemented in this study was that sessions for each individual had to be separated over a minimum period of 48 hours. Because of this, participants appeared for testing sessions over a period of 2-3 weeks. Because of this, it is likely female participants were in the middle of the menstrual cycle for at least one if not multiple sessions, which may have reduced their performance capacity depending on the phase they were in. Future analysis should involve a sex based comparison to fully determine if any particular trends or patterns in performance can be identified based on sex.

CONCLUSION

In conclusion, the findings in the present study provide important evidence for the use of NZBC as an ergogenic aid for recreational athletes, in terms of the dose-response effects on TT cycling performance. The form that NZBC was taken, the dose of NZBC that was ingested, the duration of NZBC intake and the length of time between the bolus and exercise contributed to a strong trend in significant differences in mean TT performance and improved peak PO values seen following 900 mg NZBC. At the same time, no significant differences were seen in mid exercise HR, VO_2 and RER along with resting and post exercise [La] remaining unchanged between conditions. This work provides new understandings regarding the use of NZBC on endurance cycling related disciplines and may assist

recreational athletes in deciding whether to use NZBC prior to race based exercise performance.

4.0 ACUTE EFFECTS OF NEW ZEALAND BLACKCURRANT EXTRACT ON ENDURANCE CYCLING PERFORMANCE IN NORMOBARIC HYPOXIA

ABSTRACT

Purpose: To examine the acute effects of New Zealand blackcurrant Extract (NZBC) on short duration time trial (TT) cycling performance in simulated altitude and cardiometabolic parameters pre- and post-exercise. **Methods:** Using a randomised, crossover design, 14 physically active participants (8 males, 6 female; age 22.3 ± 3.4 years, stature 173.8 ± 7.2 cm, mass 74.8 ± 13.4 kg) completed a 10 km TT cycle under normobaric hypoxia (NH; FiO_2 : 15-16%) 60 minutes following ingestion of NZBC (CurraNZ™; 900 mg; ANT 315 mg) or a placebo (PLA; Three identical gelatin capsules; 900 mg). Resting measurements of blood pressure, heart rate (HR) and blood lactate [La] were taken 45 minutes following supplementation. During testing, measurements of power output (PO), heart rate (HR) and rating of perceived exertion (RPE) were taken at the end of every km. Tissue oxygen saturation (StO_2) values were recorded every 0.5 km. Four additional [La] samples were taken every 2.5 mins post-exercise to analyse peak values and removal rates. Throughout the TT, acute mountain sickness symptoms were checked using the Lake Louise scale. Data were analysed through a series of paired samples t tests. **Results:** No improvements in 10 km TT performance were observed between NZBC and PLA (NZBC: 1090.3 ± 109.7 , PLA: 1085 ± 101.2 seconds, $d = 0.05$, $P > 0.05$). Additionally, no changes found between conditions for HR, RPE, StO_2 and [La] at rest and following exercise between conditions. **Conclusion:** acute intake of NZBC does not significantly improve 10 km TT cycling performance in NH.

4.1 INTRODUCTION

Hypoxia is defined as “any combination of reduced barometric pressure and/or a reduced inspired fraction of oxygen (FiO_2), which ultimately results in an inspired partial pressure oxygen (PiO_2) less than 150mmHg” [192]. Multiple training protocols based in and around hypoxic or high-altitude environments have been developed with the live-high, train-low method providing the greatest performance benefits in submaximal endurance exercise [193]. Performance effects during or following exposure to hypoxia are measured in one of two primary forms: HH, an environment

in which an individual is exposed to high altitude with lower barometric pressure in comparison to sea level ($\text{FiO}_2 = 20.9\%$; barometric pressure < 760 mmHg) and NH, an environment in which an individual is exposed to a reduction in FiO_2 with no change in barometric pressure (NH: $\text{FiO}_2 < 20.9\%$; barometric pressure = 760 mmHg) [194]. Oxygen manipulation has emerged as a popular ergogenic aid itself [195] and NH has developed into a popular method of determining performance in elite athletes under simulated altitude due to a reduction in the severity of acute mountain sickness (AMS) [196] and oxidative stress [197] when compared to HH.

Acute exposure to hypoxia induces greater muscle sympathetic activity and heart rate responses through chemoreceptor stimulation [198] while endothelium derived NO production increases systemic vasodilation while reducing pulmonary vasoconstriction [199]. The effects of blackcurrant extract in activating NO production are well documented [65] with higher anthocyanin intake associated with reduced arterial stiffness and blood pressure, suggesting CV benefits [200]. Research into the acute effects of ergogenic aids on performance in hypoxia is limited with one study showing a 15% improvement in 6 km cycling performance and greater CV function following sildenafil supplementation [201]. Sildenafil, a phosphodiesterase-5 inhibitor, has been shown to stimulate pulmonary vasodilation [202] and is now a recognised treatment for primary and secondary pulmonary hypertension [203]. These effects are replicated under hypoxic conditions with the conversion of eNOS to NO optimised following a single bolus of sildenafil [204]. Following a single serving of BRJ, Muggeridge et al. [157] found an improvement in 10-km TT cycling performance under NH in trained cyclists, indicating short term CV stimulation through the breakdown of dietary nitrate [104].

Currently only one study has analysed the effects of NZBC on endurance performance in NH with Willems et al. [154] finding no change in any physiological parameters during steady state cycling at 45%, 55% and 65% VO_2 max or TT cycling

performance following NZBC supplementation over 7 days at a dose of 600 mg in healthy male cyclists. This does not fall in line with previous research replicating the same supplementation and exercise protocols with NZBC [119] under normoxic conditions. Chronic antioxidant supplementation over a period of three weeks improved PO at the ventilatory threshold during acute exposure to high altitude [205], contrasting findings to those of Mariacher et al. [161]. However, a large portion of evidence suggests there is no effect of antioxidants on markers of oxidative stress [206] or exercise induced muscle damage [207] with suggestions that amino acid based ergogenic aids are more effective at reducing performance detriments in hypoxia [161].

Taking everything into account, the current study aimed to describe the influence of a single bolus of NZBC on physiological and psychological changes during exercise performance and recovery following short duration TT cycling performance under simulated altitude. As study 1 was conducted under normoxic conditions, a hypoxia based study was selected as the follow up to analyse the acute effects of NZBC under simulated altitude. When compared to sea level conditions, oxygen intake will be significantly reduced to simulate exercising at altitude. As such, oxidative stress will increase and allow us to identify whether the proposed physiological adaptations following NZBC intake improve exercise under these conditions when compared to taking a PLA. Changes in [La] were measured before and immediately following exercise at specified time points while changes in PO, RPE and HR were measured during exercise. Based on the results of the previous study, a prescribed dose of 900 mg of NZBC was selected. The research hypothesis for study 2 was that a single bolus of NZBC would improve TT cycling performance and parameters of endurance performance to a greater extent in comparison to a PLA.

4.2 METHODOLOGY

4.2.1 SUBJECTS

The number of required participants was determined on G*Power, based on the test hypothesis, the statistical tests to be used, the alpha value required to determine significance, the smallest effect size for scientific interest and the selected power value. The specified number of participants required to determine a statistically significant difference was 46. However due to time constraints, this number was not met. Power was calculated based on the sample size and statistics provided in previous studies along with a specified effect size that had to be met to indicate a substantial difference in the independent and dependent variables in two or more groups.

Fifteen physically active individuals (9 males, 6 females; Mean \pm SD age 22.1 ± 3.3 yr, stature 173.6 ± 7.0 cm, mass 74.5 ± 13.0 kg), recruited from the University of Essex volunteered to take part in this study. As part of the inclusion criteria, participants were required to (1) participate in moderate-high intensity sport or physical activity for a minimum of eight hours per week, (2) be free of any injuries or illnesses for a minimum of two weeks prior to the study and (3) be able to complete the 10 km TT cycle in < 25 minutes. Although not essential, previous experience of training or exercise at real or simulated altitude was advantageous. Participants were excluded from the study if they were greater than 30 years of age, elite level performers, recreational smokers or if they were unable to complete the TT cycle in < 25 minutes during the familiarisation session.

Given the results from the previous study, a trained cyclist cohort was preferred, however due to difficulty in recruiting cyclists at this time, recreational or novice cyclists were approached. Participants visited the laboratory on three occasions, one screening and familiarisation trial followed by two supplementation trials to test the

acute effects of NZBC on cycling performance. All visits were separated by a minimum of 48 hours. Throughout all points in the study, participants were reminded of the experimental procedures, the benefits of participation and associated risks of taking part in endurance exercise. During the process of data collection, one participant voluntarily withdrew from testing and as a result any associated data or results were excluded from the results section. Fourteen full sets of data were included for the final analysis.

Prior to each visit, participants were instructed to arrive in an appropriate physical state for exercise. This included having lunch or breakfast with at least 500ml of water 2 hours prior to the allocated testing session. Participants were advised to abstain from intense, vigorous exercise within 24 hours before testing, from consuming alcohol within 12 hours of testing and from taking caffeine in any form within 4 hours of testing. Participants did not receive any financial incentive or compensation.

4.2.2 ETHICAL APPROVAL

All study procedures were approved by the University of Essex Ethics Committee for scientific research involving human subjects. All participants were provided with an informed consent sheet detailing the study procedures, risks and benefits. Participants were also required to complete a Physical Activity Readiness Questionnaire (PAR-Q) to be cleared to participate in the study. Throughout the duration of the study, participants were allowed to withdraw at any point without reason or question.

4.2.3 STUDY DESIGN

A counterbalanced, single blinded, crossover study was implemented to compare the effects of 900 mg of NZBC on exercise performance in simulated altitude. As this study was aiming to identify any acute effects from NZBC intake, a prolonged

washout period (> 2 days) was not required. Participants performed no more than two experimental procedures in a single week.

4.2.4 FAMILIARISATION TRIAL AND DATA COLLECTION PROCEDURES

As with study 1, prior to the supplementation trials, a familiarisation session was implemented to introduce participants to the testing protocol, the physiological measurements that would be taken and the methods through which data would be collected throughout their time on the study. For the first visit, stature (Wall Stadiometer: Seca 220, Birmingham, UK) and body mass (Scales: Seca 813, Birmingham, UK) were measured on three occasions to determine an average value. Following this, resting measurements of SBP, DBP, HR, [La] and hydration were taken. BP and HR were measured using an upper arm cuff connected to an electronic monitor (Omron M2, Kyoto, Japan). A total of three measurements were taken to determine an average value.

A sample of blood (20 μ g) was taken from the left ear lobe and analysed for a resting measurement of [La]. Following exercise, [La] was measured every 2.5 minutes to analyse the point of peak [La] concentration. Rate of recovery was measured via the rate of [La] clearance over 4 different time points following cessation of the TT. Hydration was measured following the provision of a urine sample (90 ml) by participants using a portable analysis unit (Osmocheck-CRANLEA; Vitech Scientific, West Sussex, UK). Tests were repeated following additional hydration if a reading greater than 900 milliosmoles per kg body mass (mOSM/kg) was produced. Following all resting measurements, participants underwent a 15 min familiarisation breathing in hypoxic air generated from a Cloud9 simulated altitude generator (15-16% FiO₂; Sporting Edge Ltd., Basingstoke, UK).

A warm-up was performed for five minutes at a frequency of 70-80 revolutions per minute. Participants were instructed to reduce speed if HR was greater than 130

beats per minute. Participants then undertook the 10 km time trial cycle on an initial air brake resistance of 3. This was done to reduce the risk of pacing error during the supplement trials and allowed participants an idea of the length of time it would take to complete the time trial. During the TT, PO, HR and RPE were collected every km. Participants were informed of the distance travelled every 0.5 km throughout the cycle and upon discretion, exercise intensity was changed to suit the needs of participants.

As with study 1, all cycling protocols were conducted on a Wattbike Pro ergometer (Nottingham, UK). Throughout all exercise tests, heart rate was continuously monitored using a short-range telemetry monitor (Polar R300, Kimpele, Finland). Tissue oxygen saturation (StO_2) was measured continuously using a pulse oximeter connected to the Cloud9 generator with measurements collected every 0.5 km during the test. Values were displayed on a screen along with HR values with changes seen every 10 seconds. Exercise tests were terminated with immediate effect if StO_2 dropped below 75% for longer than 15 seconds.

Prior to exercise, participants were familiarised with the Lake Louise acute mountain sickness (AMS) scale [208] for detrimental symptoms related to hypoxia exposure and the 6-20 RPE Borg scale [209] for subjective feelings of work rate. The AMS scale was only used for screening and participant welfare purposes and no future data analysis was conducted for this variable. Following exercise, [La] samples were collected on four occasions at separate 2.5 min time points. This was done on the basis of research suggesting that peak [La] values are observed between 3-8 minutes post exercise [183] and was measured for the purpose of determining both the specific time point at which [La] peaked and the rate of recovery via [La] clearance. Measurements of each [La] sample were produced using a Biosen 5030 blood analyser (Biosen C-Line Clinic, EKF Diagnostic, Magdeburg, Germany) with a calculated coefficient of variation between 1.3-3% [184], according to manufacturers

guidelines. Calibration of the analyser was essential according to manufacturer's guidelines to ensure measurements were valid and accurate.

4.2.5 SUPPLEMENTATION

For the following visits, participants repeated the pre-exercise BP, HR, hydration and [La] measurements before ingesting either 900 mg NZBC (Three capsules - 900 mg active cassis, 315 mg anthocyanins, 3-10% Cyanidin-3-O-Glucoside, 30-45% Cyanidin-3-O-Rutinoside, 5-20% Delphinidin-3-O-Glucoside, 35-50% Delphinidin-3-O-Rutinoside, CurraNZ™, Health Currency Ltd., Surrey, UK) or a placebo (3 similar looking opaque gelatine capsules, Cornflour 900mg; PLA). 45 minutes following ingestion, resting BP, HR and [La] were taken prior to a 15 minute air familiarisation. 60 minutes post ingestion, based on research suggesting peak circulatory levels of anthocyanins at this time point, participants repeated the 10 km TT cycle and post exercise [La] measurements. A full schematic representation of the study is presented in figure 17.

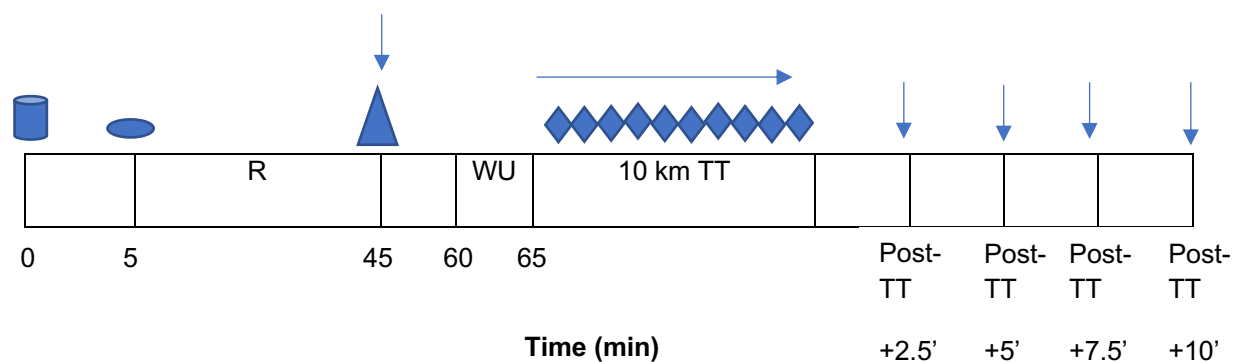








Figure 17. Schematic Representation of the TT Cycling Protocol. R = Rest, WU = Warm up, TT = Time Trial

-  Urine Sample Provision
-  NZBC/PLA Capsules
-  SBP, DBP & HR
-  PO, HR, RPE
-  StO₂
-  Capillary Blood Sample

4.2.6 STATISTICAL ANALYSIS

Information on km completion times and PO were recorded on the Wattbike ergometer test file while statistics for oxygen output data were indicated on a sensor connected to the Cloud9 altitude generator. [La] values were analysed following. Statistics for all variables were placed onto a data collection sheet, ready for analysis. As with study 1, all statistical analyses were completed using SPSS version 24.0 (SPSS Inc., Chicago, Illinois, USA). A series of paired samples t-tests were run to determine differences between treatments for TT, HR, PO, RPE and resting [La]. Data normality was checked using a Shapiro-Wilks test [186] and included an inspection of z score values, histograms, normal Q-Q plots, box plots and skewness / kurtosis indices [187]. Data normality values were analysed on the “tests of normality” output under the “Shapiro-Wilk” column. If normality was not detected, based on an alpha value greater than 0.05, a Wilcoxon signed-ranked test was used as a non-parametric alternative to any variables or groups. Effects sizes with Cohens d (d) were used to determine the magnitude of effect between measurements and time points.

For d , effect sizes were deemed small at a value of 0.2, medium at 0.5 and large at 0.8. A two-way repeated-measures ANOVA test was run to determine significant differences in post exercise [La] over respective time points. For all statistical tests, power ($\beta-1$) was based on a value of 0.8 and significance was based on an alpha value lower than 0.05. Significance values were analysed on the paired samples test output. D was calculated by subtracting the mean difference identified in the control group (PLA) from the supplement group (NZBC) and dividing this value by the pooled standard deviation. An alpha value greater than 0.05 but less than 0.1 ($0.05 > P < 0.1$) was interpreted according to guidelines set by Curran-Everett & Benos [188].

4.3 RESULTS

4.3.1 TT CYCLING PERFORMANCE TIMES

There was a difference of 5.3 seconds between conditions with no significant statistical difference in the time to complete the 10 km cycle under NH ($t(13) = 0.66$, $d = 0.05$, $P > 0.05$) (figure 18). Pacing values remained consistent for each km between conditions throughout the TT with a notable increase in speed during km 10 (figure 19).

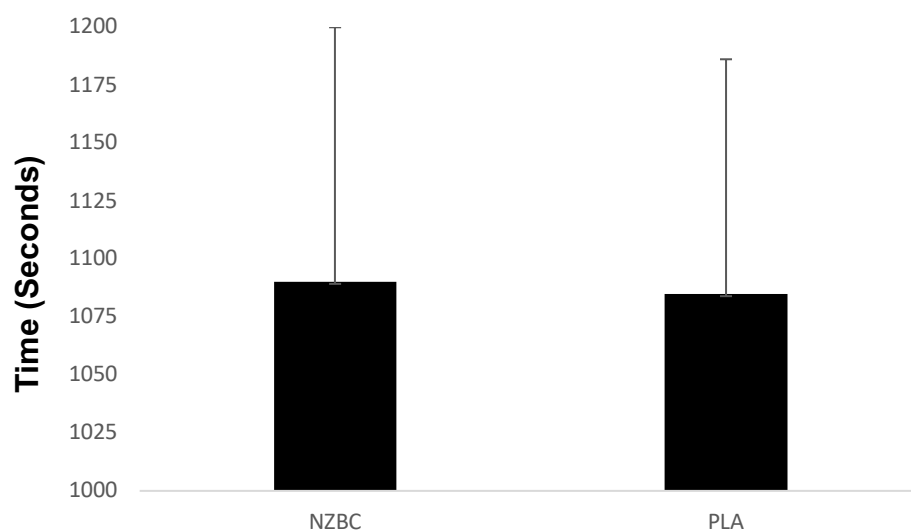


Figure 18. Time to completion values (Mean \pm SD) for a 10 km time trial cycle under NH following acute intake of NZBC or PLA

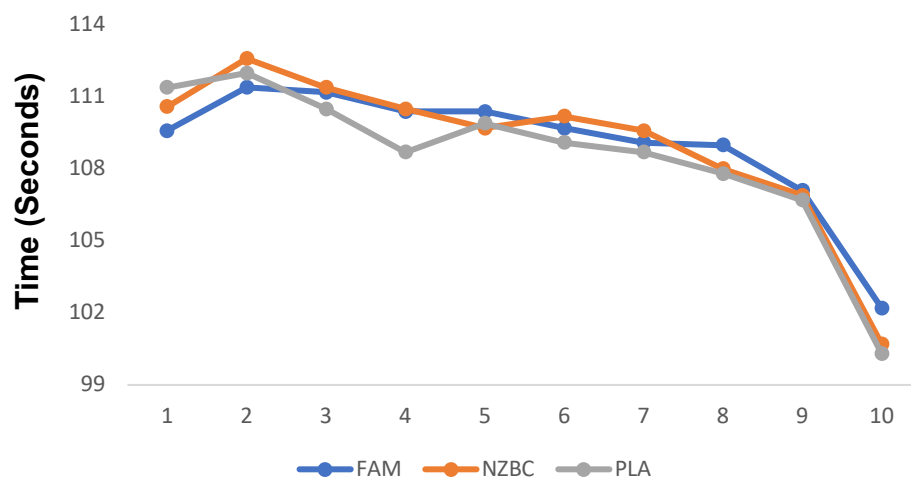


Figure 19. Mean km completion times following intake of NZBC or PLA under NH

4.3.2 PO FROM TT

There was a negligible difference of 0.01% between conditions for peak PO (NZBC: 203.9 ± 55.3 ; PLA: 201.6 ± 56.2 watts, $t(13) = 0.41$, $d = 0.04$, $P > 0.05$) and 0.02% between conditions for mean PO during the 10-km time trial cycle ($t(13) = -0.99$, $d = -0.07$, $P > 0.05$) (figure 20). PO values per km remained consistent between conditions at most time points with a linear increase in values between km 8 and km 10 (figure 21).

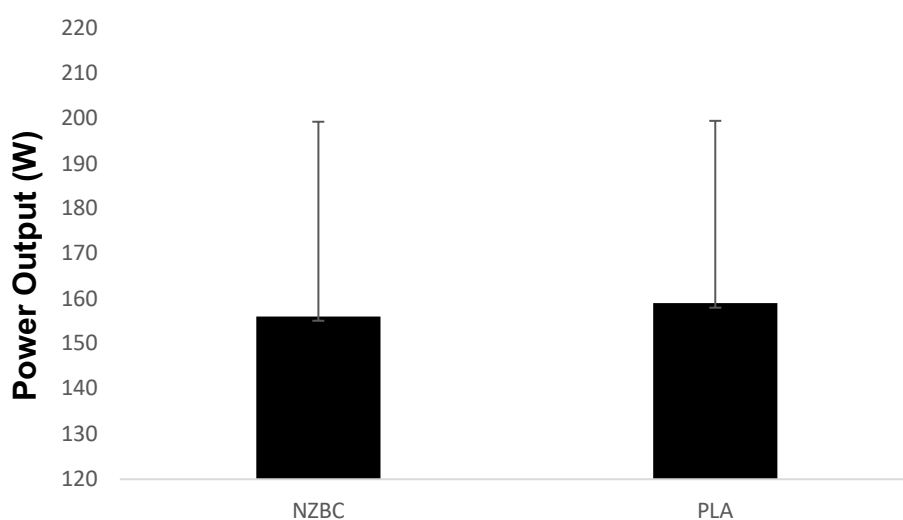


Figure 20. Power output values (Mean \pm SD) during a 10 km TT cycle under NH following acute intake of NZBC or PLA

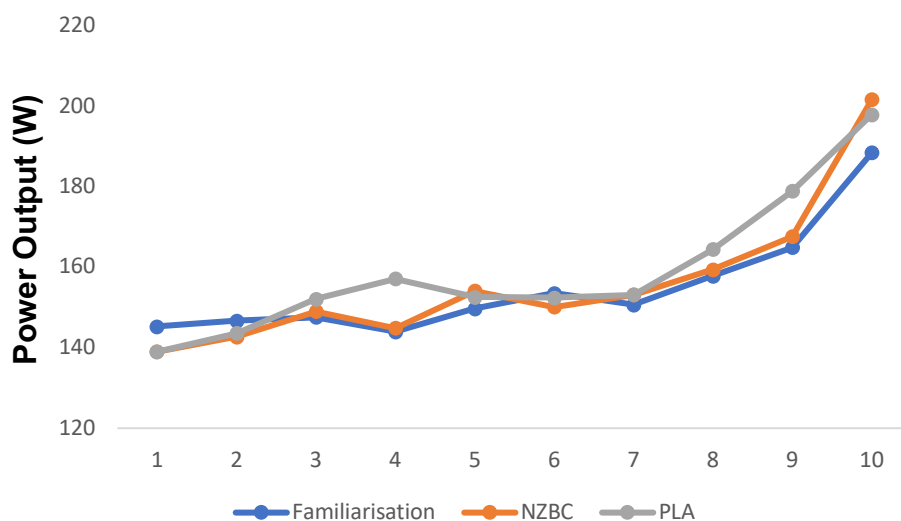


Figure 21. Mean PO values per km during a 10 km TT cycle under NH

4.3.3 HR FROM TT

Both mean and peak HR during the 10 km TT cycle remained unchanged between conditions with a difference of 0.2 ($t(13) = 0.08$, $d = 0.02$, $P > 0.05$) and 0.7 BPM respectively ($t(13) = 0.48$, $d = 0.05$, $P > 0.05$).

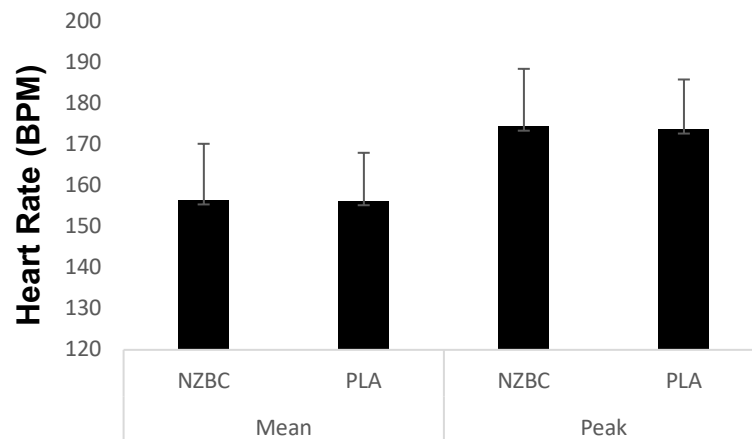


Figure 22. Mid exercise and peak HR values (Mean \pm SD) during a 10 km TT cycle under NH following acute intake of NZBC or PLA

4.3.4 RPE FROM TT

Mean RPE scores during exercise remained unchanged between conditions (NZBC: 12.7 ± 2.4 ; PLA 12.4 ± 2.5 , $t(13) = 1.05$, $d = 0.12$, $P > 0.05$). For all conditions, there was a linear increase in values per km between conditions.

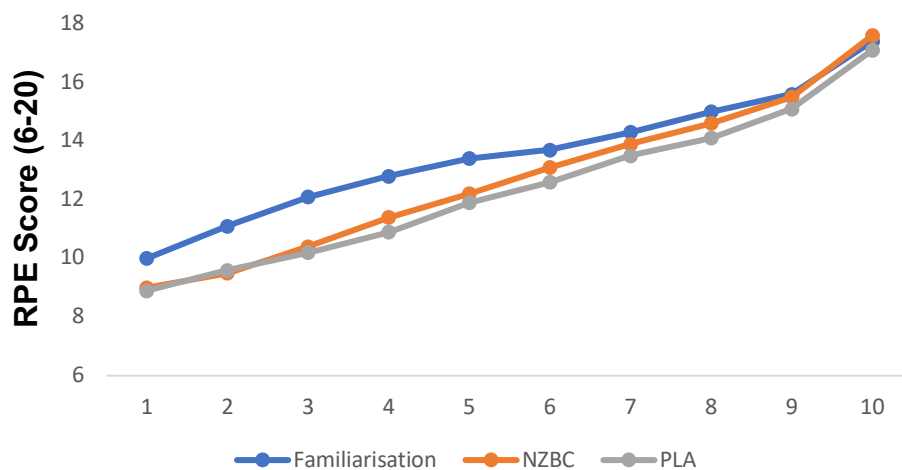


Figure 23. Mean RPE values per km during a 10 km TT cycle under NH for all conditions

4.3.5 TISSUE O₂ SATURATION FROM TT

During the TT, average FiO₂% values were 15.2 ± 1.2 for NZBC and 15.0 ± 0.9 for PLA, simulating an altitude of approximately 2,700 metres. There was no significant difference in mean StO₂ values across the TT between conditions (NZBC: 91.2 ± 3.3 ; PLA: 92.4 ± 3.8 StO₂, $t(13) = -1.39$, $d = -0.34$, $P > 0.05$).

4.3.6 HEART RATE & BLOOD PRESSURE DURING REST

A difference of 1.8% was seen between conditions for resting HR with no statistical significance (NZBC: 61.1 ± 9.6 ; PLA: 60.0 ± 7.7 BPM, $t(13) = 0.59$, $d = 0.13$, $P > 0.05$). A 3% difference was observed between conditions for SBP ($t(13) = 1.43$, $d = 0.37$, $P > 0.05$) while a difference of 4.5% was observed between conditions for DBP ($t(13) = 1.40$, $d = 0.35$, $P > 0.05$).

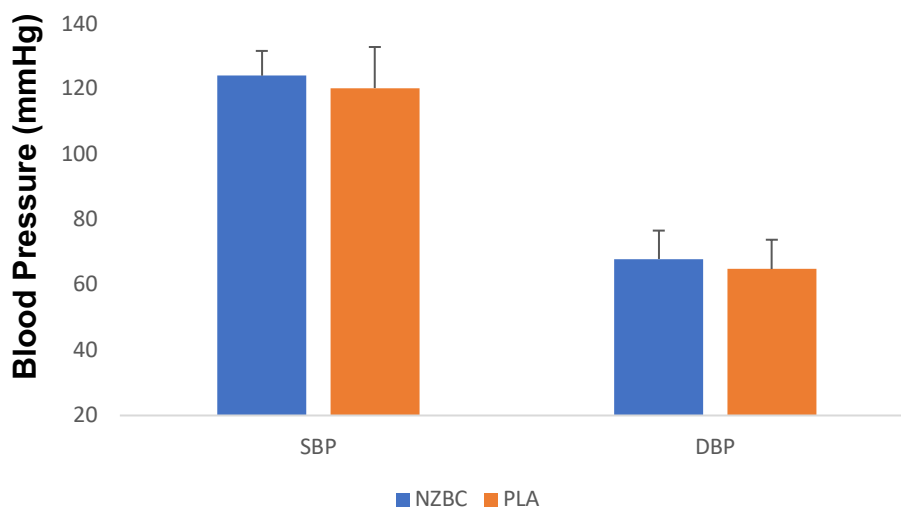


Figure 24. Resting values for systolic and diastolic blood pressure (Mean \pm SD) following acute intake of NZBC or PLA

4.3.7 BLOOD LACTATE AT REST

There was no significant difference in [La] values at rest between conditions with values 9.1% lower following NZBC in comparison to PLA ($t(13) = -0.91$, $d = -0.30$, $P > 0.05$).

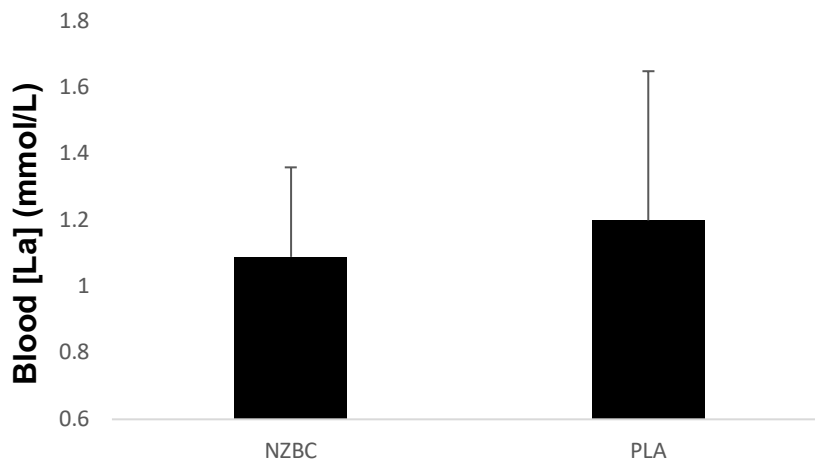


Figure 25. Resting [La] Values (Mean \pm SD) Following Acute Intake of NZBC or PLA

4.3.8 BLOOD LACTATE POST EXERCISE

Post-exercise values for [La] are presented in table 8. There was no significant interaction effect (Condition \times time) for [La] at any time point following exercise ($P > 0.05$). When analysing main effects for dose and time in table 9, a bonferroni post hoc test determined a significant main effect was found in [La] values for the time point ($P = 0.00$, $\eta^2 = 0.42$).

Table 8. Post Exercise [La] Values Following Exercise under NH

	Time Point	NZBC	PLA	F	P	$\eta\rho^2$
[La] (mmol/L)	2.5 min	6.45 \pm 3.13	6.70 \pm 3.64	0.95	0.426	0.07
	5 min	6.73 \pm 3.28	6.60 \pm 3.34			
	7.5 min	6.15 \pm 2.92	6.63 \pm 3.37			
	10 min	5.78 \pm 2.99	5.60 \pm 3.09			

Values are Mean \pm SD, 2.5 min, 5 min, 7.5 min, 10 min

[La] = blood lactate, NZBC = New Zealand Blackcurrant Extract, PLA = Placebo, min = minutes

Table 9. Main Effects for Condition and Time in Post-Exercise [La] Values Following Acute Intake of NZBC or PLA

Variable	Effect	F	P	η^2
[La] (mmol/L)	Condition	0.006	0.941	0.000
	Time	9.483	0.000	0.422

4.4 DISCUSSION

This is the first study to characterise TT endurance performance under NH following acute consumption of NZBC in recreational athletes. For this study, we hypothesised that acute intake of NZBC would induce physiological changes, leading to enhanced TT cycling performance under NH and that the changes would be significantly greater in comparison to a PLA. The results showed no significant improvement in TT performance between NZBC and PLA. Differences in values for mean PO, StO₂ and RPE during the TT, resting values of SBP, DBP and HR and post-exercise values for [La] were negligible between conditions matching the findings of Willems et al. [154]. Intake duration prior to performance was the primary difference between these two studies with a single bolus of 900 mg NZBC incorporated in this study while intake of 600 mg NZBC over a period of 7 days was used in Willems et al. [154]. Therefore the research hypothesis for this study was not supported by the findings. The sample used in the current study included 14 recreationally active participants, similar to the size and training status used in Willems et al. [154] who included 11 healthy cyclists.

The sample used in this study were recreationally active individuals, with limited experience of exercise either at altitude or hypoxia. Faiss et al. [152] concluded that the physiological responses to simulated altitude are heavily affected by training status, demonstrated by a greater detriment in SpO₂ and VO₂ max in elite athletes when compared to recreational athletes. Because of the limited experience the study sample had with prior simulated altitude exercise, the effects of sudden exposure to hypoxia could have generated a bigger effect on performance than NZBC itself. Objective measurements of anthocyanin concentration in the bloodstream alongside additional markers of the physiological responses to hypoxia could have provided

greater insight into the true effects of NZBC based on the level of inter-individual variability.

With regards to ergogenic aid supplementation in trained athletes, previous research has demonstrated a mixture of conclusions with one study reporting no significant effects of BRJ intake on 10-km TT cycling performance [210]. It was concluded that the physiological and performance responses of BRJ consumption are likely reduced in trained athletes possibly due to previous attainment of CV and metabolic adaptations, similar to those induced by BRJ. In contrast, Cermak et al. [107] demonstrated greater performance following BRJ consumption in trained cyclists. The fundamental difference between the two studies was the duration of the intake protocol with Cermak et al. [107] analysing the effects of BRJ over a consumption period of six days in comparison to a single bolus implemented by Wilkerson et al [210]. Certainly for elite performers, this indicates that a duration-dependent relationship exists with the magnitude of effects correlating to the duration of consumption prior to exercise.

Intake of NZBC has been proven to increase eNOS production, leading to greater concentration of NO in skeletal muscle. Training status has a significant impact with trained athletes exhibiting higher levels of eNOS compared to their untrained counterparts [211]. Similar to BRJ, the influence of NZBC intake is likely going to be reduced in more elite athletes due to previously acquired physiological adaptations not present in recreational performers. The selection of recreational participants in this study was due to the fact that the CV responses to NZBC are similar to those seen following BRJ under NH and likely to be more pronounced in these individuals. This would not only affect the responses of HR but also the rate of change in StO_2 especially towards the end of the TT where pace increased. Mean differences in StO_2 values per km were not analysed in the current study and the absence of elite

athletes made a comparison between individuals of different athletic levels impossible.

In the current study, overall mean StO_2 remained unchanged between conditions, matching the findings of Muggeridge et al. [157] and Macleod et al. [156] following BRJ intake, whose studies involved a single bolus of BRJ prior to exercise. This suggests that in the current study, alternative underlying performance mechanisms were present but not identified. These results do not replicate those of Masschelein et al. [158] who found significantly higher SpO_2 values at rest following BRJ intake. Interestingly, the effects of BRJ on TT performance under simulated altitude are mixed. Macleod et al. [156] found no difference in TT cycling performance in both normoxia and hypoxia, matching the findings of this research. However, Muggeridge et al. [157] found enhanced TT performance of 2.2% following acute NZBC intake, matching similar improvements on cycling performance seen by Lansley et al. for acute BRJ intake [106] and Cook et al [118] for chronic NZBC under normoxia.

Although no differences were found in any resting CV parameter between conditions, previous research has found that increasing anthocyanin intake is associated with reductions in mean arterial stiffness and blood pressure [200]. These changes are especially prominent in blackcurrant consumption with Delphinidin intake associated with a reduction in the risk of CV and metabolic diseases [88], via a combination of improvements in glucose tolerance and eNOS activity [90,91]. Chronic intake of NZBC could prove effective for the sedentary population to use for the improvement of physical health [93]. Further research should focus on the longitudinal effects of NZBC on physical health markers with a dose-response protocol used to determine the ideal dose that individuals within clinical populations should use.

No significant differences found between any variables from a statistical sense and numerical differences between variables were miniscule. Mean time to complete the

10-km TT was slower by 5.3 seconds following NZBC and this was indicated by a minor difference in mean PO values per km throughout the TT. Athletes participating in medium duration TT exercise disciplines might look to improve their personal best or previous performance by 2-3%. With no improvements seen following NZBC, a single bolus of 900 mg NZBC appears unsuitable for improving performance under simulated altitude, in an environment where oxygen intake is heavily reduced in comparison to conditions at sea level. As it appears that [La] removal is greater following PLA, it appears NZBC intake has no beneficial effects for athletes undertaking multi-day exercise events or training sessions under hypoxia over several consecutive days. The metabolic adaptations following exposure to hypoxia and how NZBC intake might effect these in a manner that causes no beneficial effects on performance in an environment with greater oxidation stress for the same exercise intensity.

For *d*, effect sizes, values were deemed small at 0.2, medium at 0.5 and large at 0.8. The effect size for TT performance as 0.05 indicating a very small effect of NZBC when compared to PLA. The largest effect sizes of 0.35 and 0.37 was found for resting SBP and DBP, indicated by differences of 3% and 4.5% respectively. This indicates that acute NZBC intake had a small to moderate effect on these variables, indicating a possible link to improvement of CV health markers at rest. Although there was no significant differences between groups on the condition x time interaction between supplement groups, a significant main effect for [La] following exercise at respective time points was present with values significantly lower with PLA at 10 min post-exercise.

In this study, the placebo group acted as the control with participants being given capsules containing no active ingredients. This replicates previous research involving a specified dose of NZBC and a PLA [101,115,118,119,121,124,125] and an analysis of the resulting effects on endurance exercise performance following

chronic intake. This was the first study to include a single bolus of NZBC on a TT exercise protocol and having a PLA would have helped to conclude if NZBC was having a true effect on performance. Time and PO values per km from the familiarisation trial were similar to values in the PLA trial when both were included in the data analysis. This demonstrates that there were likely no improvements in exercise that were present from a psychological perspective as a result of the PLA effect.

PRACTICAL APPLICATIONS

Because mean TT completion times, mid-exercise PO, mid-exercise and peak HR values and RPE values per km were unchanged following NZBC intake, it may not be a preferred ergogenic aid for recreational athletes to use prior to endurance exercise at altitude. [La] removal following exercise was greatest following PLA, suggesting the absence of physiological pathways that aid in preventing and lowering oxidative stress. Additionally further research is required to determine whether the underlying mechanisms responsible for the production of eNOS and CV responses to NZBC are influenced by a reduction in FiO_2 . and whether these responses are significantly greater following higher intakes of NZBC or differ depending on athletic status.

LIMITATIONS

Prior to the start of exercise and throughout the duration of the participants time on the study, diet was not directly controlled, possibly leading to aspects of performance being affected by alternative ingredients or nutrients than those present in NZBC. The use of capsules as opposed to fluid are likely to have delayed some physiological responses to the supplement, evidenced by the absence of changes in HR, RPE and PO between NZBC and PLA.

During the TT, consistency of StO₂ readings between participants was variable. The consumption of supplements in capsule form bypasses potentially degrading properties of saliva in the oral cavity, and the breakdown of anthocyanins into the bloodstream is delayed [191]. In an ideal scenario, blood samples would be taken from a skeletal muscle in either the arm or leg to measure the concentration of anthocyanins in the bloodstream prior to exercise. This would be done over multiple time points to determine a suitable value that has to be reached prior to when exercise can take place.

Although participants underwent welfare checks for injuries or any barriers that might have prevented them from undertaking exercise, participants were not asked whether they were taking any other ergogenic aids prior to testing. If ingestion of any form of performance enhancing supplement was taken for an extended period of time prior to the study, the effects present as a result of NZBC intake could have been overshadowed by the effects directly from these other ergogenic aids. This is especially important with regards to supplements that affect performance more under hypoxia in comparison to normoxia the need to buffer oxidative stress is greater.

One of the stipulations prior to exercise was to refrain from consuming alcohol within 12 hours of testing. This presented potential issues with regards to the time of sessions on a scheduled day. Under these circumstances, participants with an afternoon or evening session would have been allowed to consume alcohol late into the previous evening, thus increasing the risk of exercise performance being impaired due to presence of alcohol in the blood and surrounding issues with cardiovascular or muscular function. As alcohol intake is likely already affecting oxygen carrying capacity in normoxia, the effects seen under hypoxia would likely have been amplified, leading to oxidative stress increasing further and performance intensity having to reduce. Next time, a time boundary of 24 hours will be implemented to reduce the risk of these events.

Prior to the study, a power analysis was conducted to determine the number of participants required to get a significance value of less than 0.05. With only 14 participants, the study was massively underpowered, leading to no statistically significant differences found in any variable and the true effects of NZBC unknown. In this study, male and female participants were involved and included altogether in one sample group. The absence of a comparison group analysing differences between sexes could have lead to noticeable differences in certain physiological variables going unnoticed.

There is evidence to suggest that the menstrual cycle might affect performance negatively. One of the stipulations implemented in this study was that sessions for each individual had to be separated over a minimum period of 48 hours. Over the course of this study, participants appeared for testing sessions over a period of 2-3 weeks. Because of this, it is likely female participants were in the middle of the menstrual cycle for at least one if not multiple sessions, which may have reduced their performance capacity depending on the phase they were in. The physiological processes involved in the menstrual cycle would have reduced oxygen carrying capacity significantly when compared to male participants.

Because it is more likely that male physiology was more likely to remain consistent over the specified study period in comparison to females, the percentage difference in performance caused directly by the menstrual cycle should be factored into the equation when looking at performance differences between sexes. To ensure this, additional methods of measuring StO_2 are needed and values should be collected more often to ensure female participants still have the physiological capabilities to exercise to the best of their abilities. Future analysis should involve a sex based comparison to fully determine if any particular trends or patterns in performance can be identified based on sex.

CONCLUSION

In conclusion, this study expands on the research surrounding the use of NZBC on endurance performance under simulated altitude. No significant differences in any physiological variables (StO₂, HR, PO) and psychological variables (RPE) during exercise contributed to 10-km TT performance remaining unchanged between conditions. This may provide important information to recreational athletes with regards to the effectiveness of ergogenic aids prior to and following exercise under simulated altitude, the time taken for identifiable effects on performance to be seen and the form of supplements that should be taken.

5.0 OVERALL SUMMARY & RECOMMENDATIONS

5.1 THESIS SUMMARY

Athletes use ergogenic aids to improve their physiological and psychological preparation prior to exercise, increase efficiency during exercise and reduce recovery time following exercise [27]. NZBC is an ergogenic aid that has been shown to improve endurance performance through anthocyanin induced vasodilation and vasorelaxation [76], via the release of NO via phosphorylation of eNOS [71]. The primary body of research suggests that intake of NZBC is most effective following intake over a period of 7 days at a dose between 300 mg and 600 mg. However the optimal dosing strategy of NZBC and subsequent performance has not been defined and dosing in studies is determined based on manufacturers guidelines. No data is currently available analysing the effects of a single bolus of NZBC on TT cycling performance. An important aspect of identifying the true effects of an ergogenic aid is to explore effects in exercise disciplines and intake protocols with minimal or no previous coverage. These findings can then be used to identify whether the magnitude of changes seen post-intake matches those in previous research.

Therefore the aim of this thesis was to identify the influence and potential implications of acute NZBC intake on 10-km TT cycling performance. Physiological and psychological parameters measured during exercise including $\dot{V}O_2$, HR, RER and RPE and physiological recovery post exercise via [La] were measured based on the methods used from existing human and exercise research. These were used to conduct two novel studies analysing the effects of NZBC following intake of three different doses as well as performance under simulated altitude in recreational athletes. Thus, this thesis expands greatly on the knowledge provided to researchers and athletes regarding the implementation of NZBC.

Section 3 presents a novel study that sought to examine the acute effects of NZBC intake on 10-km TT cycling performance and physiological parameters in

recreationally active individuals. This study also examined the influence of dose modification of NZBC on variables including HR, PO, VO_2 and [La] by comparing a dose of 300 mg with a dose of 600 mg and a third dose of 900 mg. In study 1, we hypothesised that acute intake of NZBC would induce physiological changes, leading to enhanced TT cycling performance. Additionally, we stated that a dose-response adaption would occur following NZBC intake with the magnitude of these effects significantly greater following 900 mg in comparison to 300 and 600 mg NZBC.

We failed to support that hypothesis but did show a strong trend towards a significant improvement in TT performance following 900 mg of NZBC at an overall difference of 2.45% faster when compared to 600 mg and 300 mg NZBC. Additionally, Peak PO was significantly higher when compared to 600 mg of NZBC and had a strong trend towards a significance difference when compared to 300 mg NZBC. Following an 8 minute recovery period, no significant difference was found in rate of [La] removal following 900 mg of NZBC. There was a significant main effect of NZBC on time point following exercise with [La] values lower at 8 min post-exercise compared to 2 min post-exercise in all three dose conditions. However this is an indication that greater intake of NZBC has no influence on physiological recovery following exercise compared with smaller doses.

Interestingly, no changes in any CV parameters were found between any time point at rest and following exercise, suggesting that responses of the CV system to NZBC may be time-dependent rather than dose-dependent. In this study, the relationship between ingested dose of NZBC and all of the aforementioned parameters was curvilinear, with mean values indicating reduced performance following 600 mg NZBC. The absence of a plasma anthocyanin check immediately prior to exercise accompanied with pre-exercise preparatory stipulations that lacked strong conviction, likely contributed to the absence of effects on exercise as a result of NZBC intake and resulted in lowered performance as a result.

This does not support the findings of studies from Cook et al. [122,128] both of which included the same doses of NZBC as used in the present study. However the intake protocols differed from the current study with participants taking NZBC over a 7 day period in the aforementioned studies while the present research involved a single bolus of NZBC, 60 mins prior to exercise. Factors including the activity status of participants in the sample, dietary control and supplement use prior to NZBC intake and gender might have influenced the results of this study by having a more profound effect on performance than the effects seen by NZBC. Between group comparisons should be studied in future research to determine where the magnitude of these effects is greatest to ensure the ideal intake and experimental protocols can be implemented.

Section 4 presents a follow up novel study that employed the 10-km TT cycling protocol, measures of endurance performance and intake of NZBC at a dose of 900 mg. This study included a bout of exercise under simulated altitude, to determine the magnitude of effects seen in conditions eliciting a reduction in FiO_2 . Exercising under NH or HH is commonly used by athletes to induce hormonal changes in the body leading to CV and metabolic adaptations that significantly enhance performance at sea level. In study 2, we hypothesised that acute intake of NZBC would induce physiological changes, leading to enhanced TT cycling performance under NH and that the changes would be significantly greater in comparison to a PLA.

The main finding of this study was that no significant, acute changes were found between conditions for TT cycling performance. Difference in values for mean PO and RPE during the TT, resting values of SBP, DBP, & HR and post-exercise values for [La] were negligible between conditions, matching the findings of Willems et al. [154]. A significant main effect was found for [La] values with values significantly lower at 10 min post exercise compared to 2.5 min following PLA. This might indicate that acute NZBC intake directly prior to exposure of participants to hypoxia provides

no impact against post-exercise muscle damage or a buffering of waste products under simulated altitude. Therefore we failed to support the research hypothesis for this study. However, as intake of 600 mg of NZBC took place over a seven day period in Willems et al. [154], it is impossible to determine if the responses seen in the current study occurred due to the same underlying physiological mechanisms.

In similar studies involving a single bolus of BRJ, Macleod et al. [156] found no differences in TT cycling performance whereas the difference of 2.2% found by Muggerridge et al. [157] matches similar values in studies by Lansley et al. for acute BRJ intake [106] and Cook et al. for chronic NZBC intake [118]. Therefore the physiological adaptations that occur are likely influenced by the form that BRJ and NZBC supplements are taken and the duration of time that they are taken prior to performance. Faiss et al. [152] concluded that training status of individuals in a study sample is likely going to be a major factor in the physiological responses to simulated altitude. Whether NZBC intake amplifies or reduces these adaptations in different training population groups is something that requires careful consideration in future studies.

5.2 PRACTICAL APPLICATIONS

Sport and exercise scientists and nutritionists should look to the following guidelines to identify recommendations for athletes preparing for and undertaking short duration race-based endurance exercise:

1. Improvements in TT cycling performance and post-exercise recovery might be expected following acute intake of 900 mg NZBC. It is important to note that this is based on a sample size of 8 recreationally active individuals and changes were not linear depending on dose with a lower magnitude of difference seen with 300 mg compared to 600 mg NZBC

2. When conducting TT based cycling exercise, a dose-response comparison study over both short- and long-term intake protocols will likely be required to observe the timeline of physiological events following consumption of NZBC
3. Acute intake of NZBC appears insufficient for enhancement of TT cycling performance and post-exercise physiological recovery under short-term exposure to normobaric hypoxia. Recreationally active participants were used as the study sample, suggesting there might possibly be a difference depending on athletic status.
4. Acute intake of NZBC has no effects on various CV parameters including HR and blood pressure at rest on healthy individuals

In both studies forming the basis of this thesis, a selection of recreationally active individuals were selected to participate, limiting the ability to suggest whether the magnitude of the effects of NZBC are different in elite athletes. We were unable to meet the required number of participants, thus reducing the ability to determine the true effects of NZBC and finding a difference of statistical significance. Prior to suggesting concrete guidelines, further research is warranted to conclude the presence and magnitude of the effects of NZBC in individuals from all specified athletic populations. Studies should include different exercise modalities performed for different durations to determine whether physiological adaptations are better suited to a particular form of exercise and whether this form of performance improves up to a particular time point.

5.3 RECOMMENDATIONS FOR FUTURE RESEARCH

The findings presented in this thesis will develop further understanding on the influence of NZBC on locomotor-based endurance exercise performance and physiological recovery post-exercise. This research puts forward multiple questions

that are yet to be answered regarding how NZBC intake can prepare athletes for race-based cycling performance. The following areas require further research:

1. The influence of 900 mg NZBC on medium duration TT cycling performance times and post-exercise recovery markers are not truly understood. Linear improvements in TT performance and endurance markers between the three different dose groups were non-existent with lower performance values following 600 mg as opposed to 300 mg. The sample size was also too small to indicate whether performance was truly affected by NZBC. Further knowledge is required to establish whether these effects are different in a study including a chronic, dose-response intake protocol, performance bouts > 1 hour in duration and with a sample size matching the calculated power requirements of the study.
2. In physically active individuals, NZBC intake induces cardiometabolic changes with the biggest variations seen in resting HR, SBP, DBP, post-exercise DBP in study 1 and [La] values following exercise in study 2. These values were not statistically significant matching the findings of Cook et al. [128], Willems et al. [102] and Cook et al [122]. Further research should be designed to address whether elite athletes, undertaking chronic endurance exercise training, experience the same physiological changes prior to and following short-term TT cycling performance.
3. The underlying physiological adaptations that occur following NZBC intake under normal conditions are absent during exercise under simulated altitude at moderate hypoxia (FiO_2 15%). Therefore more work is needed to determine whether this remains the same under severe hypoxia or with a chronic, dose-response supplementation prior to the same exercise protocol.
4. In recreationally active individuals, NZBC appears to have no acute, dose-response effects on any CV parameters including HR, CO and TPR. Future

research with larger study sample sizes and a combination of invasive and non-invasive CV measurements will help to determine the magnitude of stimulation following NZBC intake at rest and during exercise. A comparison between sexes should be measured to determine whether CV adaptations vary due to physiological differences between males and females following intake. Health status may influence the physiological response to supplements that induce changes in CV function. Research should be carried out to address whether NZBC intake in individuals with health conditions including hypertension or coronary heart disease, has a positive effect on resting CV health and whether the magnitude of this interaction occurs in a dose-response manner.

LIMITATIONS

Despite some evidence of physiological recovery following NZBC intake, the absence of post-exercise performance measurements in both studies following TT cycling performance makes it impossible to truly analyse the effects of NZBC intake on the rate of performance recovery. During the TT, readings of CV markers, VO_2 , StO_2 and $[La]$ varied heavily between participants, despite equipment calibration. The Portapres Model 2 monitoring system has limited data regarding coefficient of variation. This study used capsules to provide the supplement and the absence of fluid-based NZBC made it impossible to compare how the effects of the ergogenic aid might have differed depending on the form that it was taken.

In both studies, the quantity of NZBC consumed by participants was the only indicator of anthocyanin intake in participants. Prior to exercise, no biochemical tests were carried out to determine the physiological presence of anthocyanins. Invasive measurements such as a blood assay were ideally required to determine whether plasma levels of anthocyanin were at a level indicative of changes in CV function.

This would also be carried out following exercise to determine if physiological recovery markers including [La] were influenced by the amount of NZBC anthocyanins remaining in plasma.

CONCLUSION

Acute NZBC intake has no statistically significant dose-response effects on medium duration TT cycling performance although a strong trend towards significance was present following 900 mg of NZBC in comparison to doses of 300 mg and 600 mg. A 900 mg dose of NZBC had no influence on any physiological variable and pacing during a TT cycle under NH, suggesting a reduction in FiO_2 presents underlying physiological effects not affected by NZBC intake. Despite expanding on the existing knowledge regarding intake of NZBC on medium duration TT cycling performance, further research is required to determine if these findings would be replicated if a chronic, dose-response intake protocol of NZBC is implemented for exercise under both normoxic and hypoxic conditions.

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