Individual and Additive Effects of New Zealand Blackcurrant Powder and Caffeine Intake on High-Intensity Intermittent Exercise Performance

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Sport and Exercise Science

Massey University, Auckland
New Zealand

Krutika Manoj Nanavati
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Krutika arrived in New Zealand in February 2020 after securing a Massey University Doctoral Scholarship based on her excellent GPA and high English language proficiency. She started her PhD on a topic entitled, "The Influence of a Protein-rich Dairy Beverage Fortified With Curcumin On Health And Wellness In Older Adults", in April 2020 and managed to write an excellent review article in a Q1 journal and won an award for her presentation skills. She also successfully completed her confirmation and two HDEC ethics applications and completed pilot testing for the acute and chronic trials. She also went through various challenges in finding participants for her studies.

Unfortunately, her time with her original research interest was chaotic. The on and off Covid-19-enforced lock downs, failure to procure the nutrition supplement for the study until November 2022, the moving of the sports and exercise science lab from its previous location into the new IC building, and the change in study protocol due to breakage of equipment needed for the study, caused a lot of turmoil in her first 2.5 years. It is when we failed to secure funding in 2023, and after liaising with my colleagues and the external sponsors, I suggested that we move her to the New Zealand blackcurrant and caffeine study. She was already part of the blackcurrant and caffeine study after helping with pilot testing (development of software and hardware of the m-LIST protocol, use of smart insoles, initial pilot of the procedures with some subjects, help with ethics application and clinical trials registry), since early 2022 as an RA and knew the study thoroughly.

Since joining her present study full-time, she successfully completed her data collection and data analysis for the performance measures by December 2023. She needed a few extensions in 2024 to complete the analyses of the plasma caffeine metabolites (using HPLC) and write up her thesis. The delay in analysing the caffeine metabolites was due to the following reasons:

1. The movement of the biochemistry lab from Oteha Rohe campus to the present location in the IC Building. The move disrupted the ongoing research and it took a while to get settled in the new place. As there were a lot of other ongoing projects, her study samples were not prioritised.

2. There was a period when the machine was not functional (due to some issues in the HPLC columns), and so she could not start the analyses until October 2023-December 2023.

3. Lastly, Assoc Prof Kay Rutherfurd-Markwick, her secondary supervisor who is the HPLC expert, took her voluntary retirement in December 2023 and hence the delays in finalising the HPLC assays.

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The rigours of a PhD means that students need to demonstrate adaptability, be flexible, and be extremely resilient when things go wrong. Krutika has demonstrated these attributes in abundance and is well versed in the research process, managing multiple tasks, and pivoting successfully from one study to another. Therefore, I have confidence that Krutika has developed all of the necessary skills to become an independent researcher in her own right; one that can help others on their own PhD journeys filled with obstacles and challenges-aplenty!

Signed, confirming this is a fair reflection of the impact of the pandemic, extreme weather events and/or university change processes on this research.

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Abstract

Background: Intake of New Zealand blackcurrant in various forms such as powdered extracted anthocyanins, juice concentrate, powdered juice concentrate, and powdered whole fruit (NZBC; containing 105-315 mg of anthocyanins) for 7 days has been shown to improve running and cycling performance and increase fat oxidation during exercise in trained athletes and recreationally active individuals. However, most studies provide the last dose during the 7-day intervention period 1-3 h before exercise, thus raising the question if the improvement in fat oxidation and performance was due to the 7-day loading phase or the acute intake. Caffeine, on the other hand, when consumed acutely, has been shown to improve repeated-sprint performance during a high-intensity intermittent running protocol. Thus, combining NZBC with caffeine may provide an additive or a synergistic effect to athletes to improve exercise performance. Similarly, evaluating the effect of a single dose of NZBC on fat oxidation may provide valuable insights on its potential to delay fatigue during exercise.

Aims: 1) To examine the acute effect of NZBC drink consumption (120 mg anthocyanins) on substrate oxidation and plasma free fatty acids (FFA) levels during mixed-intensity cycling in recreationally active males. 2) To examine the acute individual and additive effects of consumption of a NZBC drink (240 mg anthocyanins) with and without caffeine (240 mg) on sprint performance during high-intensity intermittent running in previously fatigued recreationally active males.

Methods: Fourteen recreationally active male participants were recruited for this double-blind, randomised controlled crossover trial and each participant took part in four main trials (>7-day washout between trials): placebo (PLA), placebo + caffeine (CAFF), NZBC drink, and NZBC + caffeine drink (NZBC-CAFF). Each main trial was divided into two visits across two days. On the afternoon of Day 1, participants consumed PLA or NZBC and after a 1-h wait period, completed a ~90-min cycling protocol at ~60% maximal power output. Breath-by-breath gas analysis was conducted during exercise (to measure substrate utilisation) and blood samples were taken before, during and immediately after the exercise (to measure FFA). Following cessation of exercise and post-exercise measures, participants were provided with a standardised dinner and reported to the laboratory the next morning after completing >8-h fast. On the morning of Day 2, participants consumed a standardised breakfast and one of the four experimental beverages. Following a 1-h wait period, participants undertook a 10-min standardised warm-up and then completed the modified Loughborough Intermittent Shuttle-Test (m-LIST) – a validated test designed to replicate the demands of intermittent team sports like football (soccer) and rugby. The m-LIST included 4 x 15-min "paced" intermittent exercise blocks (movement dictated by pre-determined audible "beeps"), followed by 2 x 15-min "prescribed" intermittent exercise blocks (movement at their own pace). Various performance metrics including sprint speed, maximum speed, and distance covered were measured during exercise using

sprint timing gates and insole-embedded inertial measurement units. Blood samples were taken before, during, and after the m-LIST to evaluate serum FFA concentration and blood lactate was measured every 15 min before, during and following the m-LIST.

Results: Consumption of the NZBC drink had no effect on substrate oxidation during the mixed-intensity cycling protocol. There were no differences in oxygen consumption, fat oxidation, carbohydrate oxidation, and serum FFA concentration with the intake of PLA and NZBC during the trial. There was an effect of treatment on average sprint speed ($p = 0.049$, $\eta^2 = 0.259$), with NZBC-CAFF and CAFF sustaining higher average sprint speeds from blocks 1 to 6 compared to other treatments during the m-LIST protocol. However, the sprint speed reduced significantly for all four treatments as the trial progressed from blocks 1 to 6 ($p = 0.032$, $\eta^2 = 0.341$). We also observed a higher peak deceleration with CAFF treatment compared to NZBC ($p = 0.038$) during the m-LIST protocol. Blood lactate concentrations were higher with NZBC-CAFF treatment compared to PLA ($p = 0.038$) and NZBC-CAFF was the only treatment in which serum FFA concentration kept increasing 1-h post exercise ($p = 0.042$ and $\eta^2 = 0.214$).

Conclusion: Ingestion of a single drink of reconstituted NZBC powder containing 120 mg anthocyanins had no effect on substrate oxidation and serum FFA concentration during mixed-intensity cycling in recreationally active males. Intake of caffeine with and without NZBC improved sprint performance when consumed, whereas consumption of the NZBC drink alone did not offer similar benefits. Previous studies indicated that the intake of NZBC for 7 days may enhance sprint and time to exhaustion performance, however, the current study's focus on acute effects did not show ergogenic properties on sprint and exercise performance during high-intensity exercise. The increase in serum FFA concentrations with the intake of NZBC-CAFF drink after high-intensity intermittent exercise highlights the need for investigating different markers such as glycerol and β-hydroxybutyrate before, during, and after exercise to provide further insights on fat metabolism. More research is needed to understand the mechanism of action and metabolic consequences of NZBC intake during moderate and high-intensity intermittent exercise.

Acknowledgements

Deciding to move to Auckland in February 2020 all alone to pursue my PhD was a brave one. I left my family behind and was greeted by COVID-19 before I could even settle down. Luckily, I was assured time and again that I am supported, loved, and not all alone.

I would like to thank my parents, Manoj and Nira Nanavati, and my sister, Karisma Nanavati (the greatest person alive) for being there for me, even if it was only possible virtually. I would also like to thank my dad specially for helping me financially when I didn't have the funds to pay my fees to continue studying and my mom who encouraged me to continue when I was at the brink of quitting and wanted to return home for good.

Thirdly, I would like to express my sincere gratitude to my supervisory board – Ajmol Ali, Kay Rutherfurd-Markwick, Kaio Vitzel, and Roger Hurst. You have been the best supervisors - from guiding me to write line-by-line, to saving me from failure, and being the light at the end of the tunnel, I couldn't have asked for more. So, thank you not only for your constant and timely support and for putting in countless hours to make this PhD better, but also for polishing me to the researcher I am today. A special thank you to Kaio, for guiding me a little every day, for the lunch conversations, for coming to the rescue when analysis was tricky, and lastly, the assurance that I have it in me to accomplish this and it will be good.

I would like to thank all the participants who volunteered their time and energy to make this research a success. Also, Max Dawson, Research Assistant who took over the administrative duties and was actively involved in the data collection. I would also like to thank Kyria, Ana, and Tristan, Research Interns who helped me with data collection and data management.

I would also like to acknowledge my two best friends and flatmates - Abilash Babu and Barath Kumar for taking over the house duties when I couldn't, for cooking for me when I was working late, for checking on me when I was incognito, and for being there for me when I needed them the most – which was every single day this year. So, thank you! I couldn't have done this without you two.

Finally, I would like to thank my partner, Nischal Chaubey for his patience and not giving up on us when times were hard. We have been apart for way too long, and completion of this doctorate will get us closer. I can't wait for us to start our first chapter together!

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AMPK	AMP-activated protein kinase
ARE	Antioxidant response element
BM	Body mass
BW	Body weight
CHO	Carbohydrate
EMG	Electromyography
eNOS	Endothelial nitric oxide synthase
FAS	Felt arousal scale
FFA	Free fatty acids
FRAP	Ferric reducing ability of plasma assay
FS	Feeling scale
GPS	Global positioning system
IMU	Inertial measurement unit
iMVC	Isometric maximal voluntary contractions
LIST	Loughborough Intermittent Shuttle-running Test
MeSH	Medical subject headings
m-LIST	Modified -Loughborough Intermittent Shuttle-running Test
NIR	Near infrared
NZBC	New Zealand blackcurrant products
NO	Nitric oxide
Nrf2	Nuclear redox factor2
PCr	Phosphocreatine
RAST	Running based anaerobic sprint test
RER	Respiratory exchange ratio
ROS	Reactive Oxygen species
RPE	Rate of perceived exertion
USG	Urine specific gravity test
WOS	Web of Science

Chapter 1
Introduction

1.1. Background

The use of berry-fruit anthocyanins to enhance sports performance and exercise recovery has gained popularity in the last decade due to their antioxidative, anti-inflammatory, and vasoactive properties. Anthocyanins are natural pigments responsible for the blue, purple, red and orange colours of many fruits and vegetables (de Pascual-Teresa et al., 2010). Juice, extracts, and powders from various berries such as cherries, blueberries, and blackcurrants have been investigated in both *in vitro* and *in vivo* studies to evaluate its effect on antioxidant capacity, fat metabolism, and exercise performance (Cook & Willems, 2019).

Evidence from recent studies have suggested that the health and well-being benefits of anthocyanins could be due to specific source-dependent anthocyanins. Firstly, different anthocyanins are present in different berries in varying quantities. For example, the main anthocyanin in cherries is cyanidin-3-glucosylrutinoside (Rothwell et al., 2013), in blueberries it is malvidin-3-monogalactoside (Kader et al., 1996), and in blackcurrant it is delphinidin-3-rutinoside (Benn et al., 2014). Per 100 g of fruit, cherries contain 2-450 mg, blueberries contain 62–300 mg, and blackcurrant contain 130-460 mg of total anthocyanins (de Pascual-Teresa et al., 2010). Secondly, delphinidin has been shown to improve metabolic and cardiovascular disease risk biomarkers in humans (Stull et al., 2010; Zhu et al., 2011), whereas cyanidin does not provide the same protective benefits (Curtis et al., 2009; Wright et al., 2013). It has also been reported that delphinidin has a higher antioxidant potency toward scavenging the superoxide radicals compared to cyanidin (Rahman et al., 2006). Similarly, blackcurrant anthocyanins have been shown to decrease inflammation and oxidative stress in mice with methionine and choline deficiency, while preventing the depletion of mitochondrial content and damage (Tang et al., 2015), and this has not been reported with blueberry anthocyanins to date. Thirdly, there is also a variation in anthocyanin content within the same fruit due to growing conditions. New Zealand blackcurrants have been shown to have higher concentrations of anthocyanins and other phytochemicals compared to those grown in other countries (Schrage et al., 2010). The anthocyanin content of non-New Zealand blackcurrants is between 170 to 310 mg/100 ml of juice, whereas the juice produced from New Zealand blackcurrants contains between 336 and 850 mg of anthocyanins per 100 ml (Schrage et al., 2010), thus, making it a superior source of anthocyanins.

Several studies have evaluated the bioavailability and the effect of New Zealand blackcurrant consumption on exercise performance (Hurst et al., 2019; Perkins et al., 2015; Willems et al., 2016). The New Zealand blackcurrant product provided in these studies varied and was in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC). A study by Hurst et al. (2019) assessed time- and dose-dependent

anthocyanin bioavailability in healthy participants over 6 hours following the ingestion of placebo or a NZBC anthocyanin-rich extract containing 0.8, 1.6, or 3.2 mg/kg total anthocyanins. There was an increase in plasma anthocyanins 30 min after consuming 3.2 mg/kg NZBC (14.6 ± 6.6 nM) which peaked at 2 h after consumption, with concentrations ranging from 11 to 1,059 nM.

A meta-analysis on the effect of NZBC intake on exercise performance concluded that NZBC, when consumed for 7 days with a final dose 1–2 h before exercise, mediates a small but significant improvement of 0.45% (95% CI 0.09–0.81, p = 0.01) on athletic performance (Braakhuis et al., 2020). The study by Braakhuis et al. (2014) was one of the first studies to investigate the effect of NZBC juice on sports performance. The study compared the intake of (i) 1 g of vitamin C, (ii) blackcurrant juice containing 300 mg anthocyanins, and (iii) placebo, on training progression, incremental running test, and 5-km time-trial performance, and reported that faster runners (defined by +1 standard deviation of mean speed on the incremental running test) of the cohort demonstrated a "*possible 1.9 ± 2.5% improvement*" on peak running speed with the intake of blackcurrant juice (Braakhuis et al., 2014).

Running performance, especially high-intensity intermittent running, is essential for success in field-based sports such as football, hockey, rugby, and basketball. High-intensity intermittent running consists of alternating periods of short sprints for maximum running intensity and moderate to low-intensity activities such as walking, running, and jogging for active recovery (Glaister, 2005). During football matches sprints are most frequent in the first 30 min and then from 75-90 min of the game, and thus, it is crucial for players to sustain high-intensity efforts at the start and at the end of the game (Oliva-Lozano et al., 2023). The match play intensity in European football has significantly increased in the recent years due to the greater high-speed running (19.8 – 25.1 km/h) and sprinting (>25.1 km/h) demands, which now account for approximately 7% to 11% and 1% to 3% of the total distance covered during a match, respectively (Chmura et al., 2018). Straight sprinting performed either by the scorer or the assisting player has been identified as the most frequent action just before goal situations (Faude et al., 2012; Martínez-Hernández et al., 2023). Additionally, there is evidence suggesting a positive association between high-speed running and sprint distance covered by wide-midfielders and strikers and the number of matches won by their team (Chmura et al., 2018). Thus, ergogenic aids that influence repeated sprint performance (speed and distance covered) during long duration high-intensity intermittent sports could have a direct impact on game result.

Studies on high-intensity intermittent running have suggested that 300 mg of NZBC intake containing 105 mg of anthocyanins for 7 days may improve sprint performance (Perkins et al., 2015; Willems et al., 2016). Willems et al. (2016) reported that NZBC intake reduced slowing of the fastest maximal sprint speed during part A of the Loughborough Intermittent Shuttle Test (LIST) in recreationally active males. In addition, though there were no differences in time to exhaustion between placebo and NZBC

group during part B of the LIST, 8 out of 13 participants had improved their time to exhaustion by 15% (i.e., 2 min and 20 s longer) with NZBC consumption (Willems et al., 2016). Another study on recreationally active males showed that NZBC intake mediated an increase in the number of sprints (p = 0.02) during an incremental high-intensity intermittent running test protocol until exhaustion (adapted from the National Institute of Education Intermittent High-Intensity test) (Perkins et al., 2015). The increase in the number of sprints also contributed to a 10.6% increase in the total distance covered during the running protocol with NZBC intake compared to the placebo (p = 0.023) (Perkins et al., 2015). The authors from both studies speculated that the reduction in fatigue during high-intensity intermittent running was due to the effect of NZBC to increase peripheral blood flow and oxygen availability to the working muscles (Perkins et al., 2015; Willems et al., 2016). Perkins et al. (2015) also suggested that the fatigue during high-intensity exercise could be linked with the production of reactive oxygen species (ROS). As blackcurrant fruit is known to have a superior antioxidant activity (Bonarska-Kujawa et al., 2014), it is likely that NZBC products can also scavenge the ROS and decrease fatigue during exercise (Perkins et al., 2015).

Both studies by Willems et al. (2016) and Perkins et al. (2015) have explored the influence of NZBC intake on running performance using sprint speed and distance covered, however, the effect of NZBC consumption on maximum speed, acceleration, and deceleration remains unknown. Sprint performance testing when using the LIST and running-based anaerobic sprint tests can also incorporate accelerometry i.e., the measurement and use of acceleration data to quantify movement during testing (Mathie et al., 2004). Changes in acceleration and deceleration influence sprint speed (Oliva-Lozano et al., 2023), and since small margins in performance can determine a gold medal winner and a fourth-place holder (Christensen et al., 2017), there is a need for in-depth analysis for other sprint variables.

There is also growing interest in evaluating the effect of NZBC intake on substrate metabolism during prolonged exercise, such as while cycling. Fat oxidation is crucial for athletes due to its significant role in enhancing endurance performance and overall metabolic efficiency (San-Millán & Brooks, 2017). The ability to oxidise fat efficiently allows athletes to sustain prolonged periods of exercise by utilising fat as a primary energy source, thereby sparing glycogen stores for more intense efforts (Alghannam et al., 2021). Cook et al. (2015) evaluated the effect of 300 mg/day NZBC intake (105 mg of anthocyanin) for 7 days on substrate metabolism during cycling in recreationally active male participants. Participants in the study consumed the last two NZBC capsules 2 h before engaging in a continuous 30-min cycling protocol consisting of three 10-min stages at 45, 55, and 65% $\dot{V}O_{2max}$ (Cook et al., 2015). The NZBC group exhibited a trend for higher fat oxidation by 15% and 13% at exercise intensities of 45% (p = 0.077) and 55% $\dot{V}O_{2max}$ (p = 0.102), respectively, however, these rates were not

matched by a lower carbohydrate oxidation rate. At an exercise intensity of 65% $\dot{V}O_{2max}$, fat oxidation increased by 27% after the consumption of NZBC (p = 0.044) and it was consistent with a trend towards lower carbohydrate oxidation (p = 0.06). Correspondingly, there was a trend for the respiratory exchange ratio (RER) to be lower at 45 % $\dot{V}O_{2max}$ (p = 0.066) and 55 % $\dot{V}O_{2max}$ (p = 0.12), and RER was significantly lower at 65 % $\dot{V}O_{2max}$ (p = 0.043) with NZBC intake compared to placebo. As this was the first study to observe an improved fat oxidation during moderate intensity cycling following NZBC consumption, the authors speculated that the increase could have resulted from a combination of many pathways such as up-regulation of genes for proteins involved in fat oxidation, transport of fatty acids into mitochondria, improved nitric oxide availability and increased peripheral blood flow acting synergistically (Cook et al., 2015). Strauss et al. (2018) designed a randomised crossover trial with 16 endurance-trained female athletes to evaluate the effect of 600 mg of NZBC intake containing 210 mg anthocyanins on fat oxidation during prolonged cycling (120 min cycling at 65% $\dot{V}O_{2max}$). The consumption of NZBC increased mean fat oxidation by 27% during cycling compared to placebo (p = 0.042), and there was a trend for mean carbohydrate oxidation and RER to be lower with NZBC intake (p = 0.063, p = 0.063 respectively). Strauss et al. (2018) also reported that the pre-exercise plasma free fatty acids (FFA) and glycerol concentrations were 49% (p = 0.034) and 27% (p = 0.051) higher following NZBC intake compared to placebo, indicating that plasma FFA concentrations were moderately associated with mean rates of fat oxidation during exercise (r = 0.45, p = 0.016). It appears that most studies that have evaluated the effect of NZBC intake and exercise performance have utilised a 7-day NZBC loading regime and have also provided the last dose 2 h before the exercise, therefore making it unclear if the performance benefits are due to the 7-day intake or the final dose.

On the other hand, there is significant evidence supporting the ergogenic properties of caffeine during exercise (Christensen et al., 2017). Caffeine acts as a psychoactive drug and stimulates the central nervous system by binding to adenosine receptors and blocking their inhibitory neurophysiological action (Graham, 2001). Intake of caffeine has also shown to increase the concentration of FFA and glycerol 2-4 h after consumption in humans (Mougios et al., 2003), which can be beneficial for endurance performance (Zhu & Guo, 2024). The lipolytic effects of caffeine are mediated by its effect on (i) increasing the cyclic adenosine monophosphate levels that activate hormone-sensitive lipase enzymes for lipolysis and (ii) increasing the catecholamine levels by inhibiting adenosine receptor via the sympathetic nervous system to an increase in lipolysis in adipose tissue (Carrageta et al., 2018).

Recreationally active individuals and elite athletes, especially triathletes, cyclists, team-sport athletes, and weightlifters frequently consume caffeine to improve their performance (Del Coso et al., 2011). After the removal of caffeine as a banned substance from the World Anti-Doping Agency list in 2004, an evaluation of 20,686 urine samples of elite athletes reported that 73.8% of the samples contained

higher than 0.1 µg/ml of caffeine concentrations, indicating that three out of four elite athletes consumed caffeine before or during sports competition (Del Coso et al., 2011).

Studies on recreationally active males have also shown that a single dose of caffeine can improve sprint performance. For example, Glaister et al. (2008) showed that ingesting a single dose of 5 mg/kg body mass of caffeine improved the fastest sprint time during an indoor multiple sprint running trial by 0.06 s (1.4%). Another trial in recreationally active males reported that total sprint times were significantly faster when 6 mg/kg body mass of caffeine was consumed 60 min prior to repeated sprint running performance (Carr et al., 2008).

Individual consumption of both NZBC and caffeine has shown to improve sprint (Carr et al., 2008; Perkins et al., 2015) and cycling performance (Cook et al., 2015; Costill et al., 1978) in recreationally active males. Intake of caffeine has also shown to increase free fatty acid concentration at the end of an hour long steady-state exercise in recreationally active males and females (Mougios et al., 2003), whereas, intake of NZBC for 7 days has demonstrated an increase in pre-exercise FFA in endurance trained women (Strauss et al., 2018). This suggests that individual intake of NZBC and caffeine benefit recreationally active and trained adults and have the potential to be explored together for their synergistic and additive effects on exercise performance and performance.

Participants in the NZBC and caffeine intervention studies described above were asked to not engage in strenuous physical activity for 24 h prior to the testing day, hence participants were rested before the trial. However, training for field sports such as rugby often take place less than 48 h post-match and athletes are expected to train for two or more consecutive days during a week (Baker, 2001). Excessive training with inadequate recovery periods leads to accumulated fatigue, compromises neuromuscular performance (McLean et al., 2010; Pointon & Duffield, 2012; Webb et al., 2013), and causes under-performance on match-day (Johnston, Gabbett, et al., 2013; Johnston, Gibson, et al., 2013). Thus, exercise trials that test ergogenic aids on athletes in a fatigued state may provide a more ecologically valid understanding of their effect on performance.

1.2. Rationale for this PhD Study

At present, there is limited research available on the acute effect of NZBC and caffeine combinations on performance during high-intensity exercise. For example, Paton et al. (2022) found no significant individual and additive effects of consumption of a NZBC and caffeine drink on performance during repeated high-intensity cycling in trained male cyclists. There is no data available on the individual, additive, or potentially synergistic effects of acute consumption of NZBC and caffeine drink on high-intensity intermittent running performance as well as changes in serum free fatty acid concentration before, during, and after exercise. There is also no data available on effect of consumption of NZBC

and caffeine intake while exercising in a fatigued state. Lastly, there is limited information on the acute effect of NZBC on substrate metabolism during prolonged exercise. Therefore, the key areas of research for this study include (i) the acute individual, additive, and potentially synergistic effects of NZBC and caffeine intake while exercising in a fatigued state and (ii) the acute effect of a NZBC intake on substrate metabolism during cycling.

1.3. Overall Aims and Research Questions

The overall aim of this thesis was to examine the individual, combined, and possible synergistic effects of NZBC and caffeine intake on high-intensity intermittent running performance (speed and distance covered) in recreationally active males in a fatigued state, mimicking consecutive training sessions and/or inadequate recovery between games. The study also aimed to evaluate the effect of NZBC intake with and without caffeine on serum FFA concentration during and after exercise and the effect of a single dose of NZBC on substrate oxidation during mixed-intensity cycling. The study also focused on incorporating a smart insole-embedded inertial measurement unit (IMU) system to explore sprint performance variables such as peak sprint speed and acceleration during the high intensity intermittent running protocol.

1.3.1. Research Question 1

Can a consumption of NZBC drink containing 120 mg of anthocyanins influence substrate oxidation and serum free fatty acid (FFA) concentrations during cycling in recreationally active males?

A double blind, randomised, placebo-controlled, crossover study was designed to investigate the acute effect of NZBC intake on fat oxidation, carbohydrate oxidation, respiratory exchange ratio, energy expenditure, and plasma free fatty acid concentrations in recreationally active males while cycling at moderate-intensity for 90 min.

1.3.2. Research Question 2

What is the effect of consumption of New Zealand blackcurrant drink (NZBC) containing 240 mg of anthocyanins, with and without 240 mg of caffeine on high-intensity intermittent running performance in fatigued recreationally active males? Does NZBC and caffeine intake affect serum FFA concentration before and after exercise?

To investigate the above research questions, a double blind, randomised, placebo controlled, crossover study was designed. The study examined the effect of consumption of four different drinks, namely: i) placebo, ii) NZBC iii) caffeine, and iv) NZBC + caffeine on sprint speed, sprint time, distance covered, and reaction time using the modified Loughborough Intermittent Shuttle-running Test (m-LIST). The study also evaluated the changes in caffeine metabolites, free-fatty acids, and blood lactate

through blood samples during exercise. In order for participants to reach a fatigued state, a 90-min cycling protocol was implemented to reduce muscle glycogen content the day before the running trial (Vøllestad et al., 1992).

1.3.3. Research Question 3

Can we use a smart insole-embedded IMU system during the m-LIST to identify differences between biomechanical and kinematic sprint variables due to fatigue after the consumption of a single dose of NZBC with and without caffeine?

To answer this research question the study incorporated smart insole-embedded IMUs to assess maximum speed, average peak sprint speed, acceleration, deceleration, cadence, and other sprint performance markers during the m-LIST after the consumption of a single dose of NZBC with and without caffeine.

1.4. Thesis Structure

This PhD project follows the format of thesis by publication. An overview of the thesis chapters is presented below with identification of the chapters that are in preparation for publication.

Chapter 1 provides the background and rationale for this PhD study. It also introduces and describes the overall aims and research questions being addressed.

Chapter 2 is a narrative review and provides an overview of the potential of New Zealand blackcurrant intake to improve running and cycling exercise performance. This chapter also discusses mechanisms of action of New Zealand blackcurrant ingestion that play a role in exercise performance and recovery, such as blood flow, substrate oxidation, and oxidative stress. This chapter has been presented in a publication format and will be submitted to a journal.

Chapter 3 presents the first experimental chapter of this study and focuses on the effects of a single dose of New Zealand blackcurrant powder on substrate oxidation in recreationally active males while cycling and addresses research question 1 (Section 1.3.1). This chapter has been presented in a publication format and will be submitted to a journal.

Chapter 4 presents the second experimental chapter of this study that evaluates the individual and additive effects of blackcurrant powder and caffeine intake on high intensity intermittent exercise performance in recreationally active males and addresses research question 2 (Section 1.3.2). This chapter has been presented in a publication format and will be submitted to a journal.

Chapter 5 presents the findings of the individual and additive effects of NZBC and caffeine intake on various sprint variables assessed using the smart insole-embedded IMU system during the modified

Loughborough Intermittent Shuttle-running Test. This chapter has been presented in a publication format and will be submitted to a journal.

Chapter 6 discusses the main findings and highlights the main conclusions of the PhD project. It also presents the limitations of the research and the recommendations for future investigations into the performance and recovery benefits of New Zealand blackcurrants.

The Appendix section contains supplementary files and supporting documents for this thesis and includes research outputs associated with this project, including oral and poster presentations and awards (Appendix 1). It also includes the participant information sheets, consent forms, data collection sheets, and the randomisation protocol for the three experimental studies (Appendix 2), HPLC method (Appendix 3), supplementary graphs and tables for chapters 3 and 5 (Appendix 4), and DRC 16 forms for chapters written as manuscripts (Appendix 5). Appendix 6 comprises of a peer-reviewed journal publication, and two ethics approval letters from my previous PhD project on '*The Influence of a Protein-rich Dairy Beverage Fortified With Curcumin On Health And Wellness In Older Adults'*.

Chapter 2
The Potential of New Zealand Blackcurrant Products to Improve Cycling and Running Exercise Performance: A Review and Mechanistic Insights

Abstract

Anthocyanin-rich extracts derived from New Zealand grown blackcurrants have shown to have a small but significant effect on exercise performance with no known detrimental side effects. Improvement in running and cycling performance has been reported with the intake of New Zealand blackcurrants in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC) in recreationally active and trained athletes. For example, the acute intake of NZBC decreased completion time of a 5-km running time-trial, whereas the intake of NZBC for 7 days increased the number of sprints and time to exhaustion, and reduced slowing of sprints during high-intensity intermittent running protocol. For studies on cycling performance, consumption of NZBC for 7 days resulted in faster cycling times during time-trials and increased fat oxidation during long duration steady-state cycling. It is speculated that NZBC intake can benefit athletes by increasing muscle blood flow, as previously observed at rest and during sustained isometric contraction. Increase in blood flow can benefit athletes as it can attenuate the phosphocreatine degradation that causes fatigue, contribute to higher phosphocreatine resynthesis, improve removal of metabolites such as inorganic phosphate and adenosine diphosphate which would normally have a negative effect on force production, and thereby, improving exercise performance. Furthermore, i*n vitro* and animal studies have shown that anthocyanin-rich treatment increases AMPK activation in skeletal muscle and expression of genes involved in lipid metabolism, both of which could contribute to an increase in fat oxidation during exercise. More recently, NZBC has also shown to improve recovery from exercise-induced oxidative stress after 30-min steady-state rowing, potentially due to the activation of the Nrf2/ARE pathway and increasing the activity of cellular antioxidant capacity. Therefore, there is evidence that intake of NZBC for 7 days could act as an ergogenic aid for steady-state and high-intensity intermittent exercise performance and more research is needed to understand the mechanism of action behind the effect of NZBC on running and cycling performance and antioxidant capacity during recovery.

Keywords: Anthocyanins, substrate oxidation, lipid oxidation, and exercise performance

2.1. Introduction

Blackcurrants (*Ribes nigrum*) are fruit berries rich in anthocyanins, particularly, delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-glucoside that contribute to about 97-98% of total anthocyanin content (Gopalan et al., 2012). Blackcurrants grown in New Zealand are considered as a superior source of anthocyanins compared to the varieties grown in other countries due to their high anthocyanin content. For example, New Zealand blackcurrants contain 336 to 850 mg of anthocyanins per 100 ml of juice, whereas non-New Zealand blackcurrants contain anthocyanins ranging from 170 to 310 mg/100 ml of juice (Schrage et al., 2010).

Intake of New Zealand blackcurrant products in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC) has shown to influence exercise and sport performance, and therefore can be beneficial for trained athletes and recreationally active individuals. Consumption of 300 mg/day of NZBC for 7 days increased fat oxidation during moderate intensity cycling (Cook et al., 2015), improved time-trial performance by 2.4% (Cook et al., 2015), and increased the number of sprints during a repeated-sprint performance test (Perkins et al., 2015). Most studies that evaluated the effect of NZBC intake on sport and exercise performance have suggested multiple mechanisms for its influence on performance outcomes. First, intake of NZBC seems to contribute to an increase in blood flow (Matsumoto et al., 2005) and there is *in vitro* evidence for up-regulation of the endothelial nitric oxide synthase (Speciale et al., 2014) and promotion of vasodilation (Ziberna et al., 2013). Second, *in vitro* treatment of isolated rat adipocytes with cyanidin-3-glucoside has also shown to influence substrate oxidation by up-regulating the genes involved in lipid metabolism (Tsuda et al., 2005).

The studies on intake of NZBC and sports performance have not considered the literature on the pro-oxidant nature of blackcurrant anthocyanins which could be beneficial to training adaptation and post-exercise recovery (Hurst et al., 2019; Hurst et al., 2020). Intake of NZBC has been shown to increase pro-oxidants and oxidative stress biomarkers in the plasma when consumed 2 h before exercising. This increase in plasma oxidative stress has the potential to trigger activation of cellular redox-sensitive processes such as nuclear redox factor2 (Nrf2) transcription. When triggered, Nrf2 increases the expression of inherent cellular antioxidant enzymes which have the potential to protect against exercise-induced oxidative stress and improve exercise recovery (Hurst et al., 2019; Kropat et al., 2013; Yan et al., 2017). Thus, this review focuses on the potential of New Zealand blackcurrant products to improve cycling and running exercise performance. It also provides insights on the possible mechanisms of action of NZBC intake that affect blood flow and fat oxidation during exercise and pro-oxidant activity during exercise recovery.

2.2. Identification and screening for NZBC and exercise performance articles

The databases SCOPUS, Medline (PubMed), and Web of Science (WOS) were searched using a mix of Medical Subject Headings (MeSH) and free words for key concepts related to New Zealand blackcurrant, anthocyanins, aerobic exercise, running, cycling, substrate oxidation: ("New Zealand blackcurrant" OR "anthocyanins") AND ("exercise" OR "aerobic exercise" OR "running" OR "sprint" OR "cycling" OR "time-trial" OR "sports performance" OR "substrate oxidation" OR "fat oxidation") between May 2023 and March 2024. The search period was not restricted by year of publication (up to March 2024). The inclusion criteria was randomised controlled trials of original research containing quantitative information on running and cycling performance in healthy humans. The studies in which subjects were mentioned as healthy, recreationally active or trained, and of any sex and age were included. Exclusion criteria was defined as review articles, case studies, website articles, non-peer-reviewed papers, conference abstracts, prospective observational studies, non-human studies, and *in vitro* or animal studies. Only full-text articles written in English describing human trials were included in the analysis for this review. We identified 15 articles that fit the inclusion criteria and they have been included in our review.

2.3. Absorption of anthocyanins by humans

Anthocyanins in fruit are bound to a sugar molecule via a glycosidic bond and are called anthocyanin glycosides. Anthocyanins are mainly absorbed by the large intestine where they go through structural modifications such as deglycosylation, hydroxylation, and degradation (Kay et al., 2017). However, there are reports indicating that certain blackcurrant anthocyanins such as delphinidin-3-rutinoside and cyanidin-3-rutinoside are directly absorbed into the bloodstream (Matsumoto et al., 2001).

The most abundant New Zealand blackcurrant anthocyanins, cyanidin and delphinidin glycosides are degraded to protocatechuic acid and gallic acid respectively, and peonidin-3-O-rutinoside (identified as a minor compound in blackcurrant) is metabolised to vanillic acid (Costello et al., 2022). Consumption of 300 mg of NZBC containing 105 mg of anthocyanins showed a 320% increase in protocatechuic acid at 1.5 hours, 261% increase in gallic acid at 4 hours with no change in vanillic acid from baseline in healthy participants (Costello et al., 2022). Another study in healthy individuals assessed time- and dose-dependent blackcurrant anthocyanin glycoside bioavailability over 6 h after consuming NZBC containing 0.8, 1.6, or 3.2 mg/kg total anthocyanins (Hurst et al., 2019). All participants showed an increase in intact anthocyanin glycosides in plasma by 30 min (14.6 ± 6.6 nM) and this peaked at 2 h (217 ± 69 nM) with the intake of 3.2 mg/kg of NZBC extract (Hurst et al., 2019). Similarly, Matsumoto et al. (2005) reported that plasma total anthocyanin levels after the consumption of a New Zealand blackcurrant juice concentrate containing 17 mg/kg anthocyanins

peaked at 1-h post consumption in healthy individuals. The bioavailability profile of the plasma anthocyanins and their concentrations were similar to the study by Hurst et al described earlier (Hurst et al., 2019; Matsumoto et al., 2005). The slight differences in the time of the peak in plasma anthocyanin levels observed among the three studies could be due to a number of factors, such as different chemical anthocyanin compositions of the extracts, the methods used for plasma anthocyanin extraction and analyses, the amount of anthocyanins consumed by trial participants, and variation in the absorption kinetics by different participant cohorts (de Ferrars et al., 2014).

2.4. Effect of NZBC on cycling performance

New Zealand blackcurrant extract has also been studied for its potential to increase fat oxidation during cycling and improve time-trial cycling performance (Cook et al., 2015; Cook, Myers, Gault, Edwards, et al., 2017; Murphy et al., 2017). Fat oxidation is particularly beneficial for endurance athletes, as higher rates of fat oxidation are associated with improved performance in activities such as triathlons, cross-country skiing, and marathons (Knechtle et al., 2004). Oxidising fat efficiently allows athletes to sustain prolonged periods of exercise by using fat as a primary energy source, thereby sparing glycogen stores for more intense efforts (Murray & Rosenbloom, 2018). Studies have shown that peak fat oxidation is positively correlated with endurance performance metrics, such as time-trial performance and citrate synthase activity in skeletal muscle, which is indicative of mitochondrial density and oxidative capacity (Maunder et al., 2018).

This section discusses the effect of NZBC intake on cycling time-trial performance, and exercise intensity, blood lactate concentration, and substrate metabolism.

2.4.1. Time trial performance

A 2.7 to 8.7% reduction (mean reduction 2.4 ± 3.7%) in time taken to complete 16.1-km cycling time-trial was observed with NZBC intake in trained male cyclists (Cook et al., 2015). Since the smallest worthwhile change for road time-trial cyclists is around 0.6% (Paton & Hopkins, 2006), the mean decrease of 2.4% in time-trial represents a significant practical advantage to the participants, especially because there were no changes in the training or diet before the time-trial (Cook et al., 2015). In contrast, NZBC intake failed to decrease time taken to complete 16.1 km cycling time-trial in normobaric hypoxia conditions (Willems et al., 2019) and during a home-based online training simulator trial (Montanari et al., 2023). The study by Montanari et al. (2023) also reported that NZBC intake benefitted slower athletes with higher power output and speed at the 12-km mark (quartile analysis) and faster time-trial completion time compared to the placebo with no effect on heart rate and cadence (Montanari et al., 2023).

Montanari et al. (2020) also evaluated the effect of 300 mg/day and 600 mg/day NZBC intake on time taken to complete 16.1-km time-trials carried out on three occasions over 7 days, whilst in a fed state (day 1, day 4, and day 7) in trained cyclists. A decrease in time taken to complete the time-trial was observed between day 1 and day 4 with an increase in average speed for 600 mg/day NZBC intake. However, there were no difference between the other days and between the two doses of NZBC (Montanari et al., 2020). The authors speculated that the differences in fitness level of the participants ($\dot{V}O_{2max}$ ranged from 45 to 71 $ml \cdot kg^{-1} \cdot min^{-1}$) could have potentially masked the beneficial effects of consuming 600 mg/day of NZBC over a week.

Lastly, the total time taken to complete 2 x 4-km cycling trials was 0.82% faster with NZBC consumption (7 out of 10 participants having faster total times) (Murphy et al., 2017). As NZBC extract has previously been shown to improve blood flow in the forearm following venous occlusion (Matsumoto et al., 2005), the authors speculated that the NZBC extract enhanced blood flow during cycling and in the recovery phase between repeated high intensity cycling bouts. They believed that the increase in blood flow may have enhanced the overall performance time of repeated 4-km time trials and reduced the contribution of anaerobic energy production with reduced production of metabolic by-products causing fatigue (Murphy et al., 2017).

Overall, it appears that NZBC intake may improve time-trial performance, with three out of five studies showing results in favour of NZBC intake. However, performance during time-trial also depends on training status of individuals and slower athletes may benefit more from NZBC intake. Furthermore, NZBC intake failed to decrease the time taken to complete 16.1 km in normobaric hypoxia conditions likely due to hypoxia-induced compensatory vasodilation, indicating that it is not effective at improving performance in a reduced oxygen environment.

2.4.2. Blood lactate

Short-term NZBC intake has demonstrated a combined downward and rightward shift of the lactate curve during an incremental cycling protocol in trained triathletes, indicating that NZBC intake led to lower plasma lactate levels (Willems et al., 2015). The study also reported that there was a trend for cycling intensity to be higher by 6% at 4.0 mmol/l of blood lactate accumulation with NZBC intake (11 out of 12 participants showed an increase, p = 0.007) (Willems et al., 2015). The authors proposed that anthocyanin intake may have potentially increased fat oxidation and removal of lactate by increasing the blood flow (Willems et al., 2015).

In contrast, higher absolute lactate values were obtained during the 20-min passive recovery following a 16.1 km time-trial with NZBC consumption (Cook et al., 2015). According to the authors, the increase

in lactate during recovery could have been due to alterations in production or removal of lactate through blood flow or changes in membrane lactate transport mechanisms (Cook et al., 2015).

Four other studies identified in this review reported no effect of NZBC intake on lactate levels and clearance. The studies used protocols such as long duration moderate-intensity cycling (Cook, Myers, Gault, Edwards, et al., 2017), 2 x 4-km time-trials (Murphy et al., 2017), and a mix of incremental/steady-state exercise followed by a time-trial (Montanari et al., 2020; Willems et al., 2019). The inconsistent response obtained from these studies indicate that the effect of NZBC extract intake on lactate may depend on the intensity and duration of the cycling protocol, and hence, warrants further investigation.

2.4.3. Exercise intensity

Willems et al. (2015) observed a 4% and 6% increase in exercise intensity at 1 mmol/L and at 4 mmol/L lactate accumulation during an incremental cycling protocol with NZBC intake. In another study relative exercise intensity increased over time while cycling at 65% $\dot{V}O_{2max}$ 120-min following a 7-day intake of 300, 600, and 900 mg of NZBC extract with no difference between doses (Cook, Myers, Gault, Edwards, et al., 2017). Furthermore, Murphy et al. (2017) reported a trend for mean power to be 7 Watts higher with an NZBC extract (p = 0.095) over 2 x 4-km cycling trials with the consumption of NZBC extract. It is possible intake of NZBC enhanced blood flow during cycling possibly decreasing the contribution of anaerobic energy production, reducing the production of metabolic by-products that cause fatigue, and thus, contributing to an increase in exercise intensity (Murphy et al., 2017).

2.4.4. Substrate metabolism

Consumption of NZBC for 7 days has been shown to increase fat oxidation by 15% (p = 0.077), 13% (p = 0.102), and 27% (p = 0.043) at 45%, 55%, and 65% $\dot{V}O_{2max}$, respectively, during a 30 min incremental cycling protocol (Cook et al., 2015). Correspondingly, the respiratory exchange ratio (RER) was significantly lower with NZBC intake at moderate exercise intensity (see Table 2.2). Participants in the study consumed one slice of bread along with the last two NZBC extract capsules 2 h before the trial (Cook et al., 2015). As this was the first study to observe an improved fat oxidation during moderate intensity cycling following NZBC intake, the authors speculated that the increase could have resulted from a combination of many pathways such as up-regulation of genes for proteins involved in fat oxidation, transport of fatty acids into mitochondria, improved nitric oxide availability and/or increased peripheral blood flow acting synergistically (Cook et al., 2015). However, when a similar study was conducted in normobaric hypoxia conditions (simulated altitude of ~2500 m, ~15% of O_2), NZBC intake failed to increase fat oxidation in trained athletes (Willems et al., 2019). It is possible that NZBC could not influence the compensatory vasodilation or the increase in carbohydrate metabolism

due to lack of available oxygen (Casey & Joyner, 2012; Dinenno, 2016; Morishima et al., 2014) to increase the fat oxidation (Willems et al., 2019).

A study on endurance trained female athletes reported a 27% increase in fat oxidation was observed during 2 h of steady-state moderate intensity cycling with NZBC intake for 7 days (Strauss et al., 2018). The study also reported that pre-exercise plasma FFA and glycerol concentrations were 49% and 27% higher following NZBC consumption (Strauss et al., 2018). The increase in FFA concentration was moderately associated with mean rates of fat oxidation during exercise ($r = 0.45$, $p = 0.016$), which indicated that a higher rate lipolysis could increase the availability of plasma free-fatty acids (FFA) available for oxidation during exercise (Strauss et al., 2018).

Furthermore, a dose- and time-dependent effect on fat oxidation has been observed with the intake of 300, 600, and 900 mg of NZBC for 7 days during 2 h of steady-state moderate intensity cycling (Cook, Myers, Gault, Edwards, et al., 2017). A 21.5% and 24.1% increase in fat oxidation was observed after 60 min of cycling with 600 and 900 mg/day NZBC intake compared to 0 mg/day. In contrast, intake of 300 and 600 mg/day of NZBC for 7 days had no effect on fat oxidation, carbohydrate oxidation, and RER (Montanari et al., 2020). The study by Montanari et al. (2020) opted for a 10-min cycling protocol at 65% of $\dot{V}O_{2max,}$ and thus, it is possible that the duration of the protocol was too short to observe any difference in substrate oxidation between different doses of NZBC as compared to the study by Cook, Myers, Gault, Edwards, et al. (2017).

To conclude, NZBC intake has been shown to influence substrate metabolism while cycling in recreationally active and trained athletes by increasing the fat oxidation. However, the effect on fat oxidation likely depends on the exercise duration and intensity. The effect on NZBC intake on time trials, changes in blood lactate levels, and exercise intensity has reported inconsistent results and warrants further investigation.

Table 2.1 Summary of studies investigating the effect of New Zealand blackcurrant on cycling performance.

Author and Year	Participants	Study Design	Study Duration	Study Groups	Blackcurrant Dose	Timing of last dose before exercise	Exercise Protocol	Key Findings (NZBC compared to placebo)
Cook et al. (2015)	14 males 38 ± 13 years trained	double-blind, randomised, crossover design	7 days	placebo and NZBC extract	300 mg/day (105 mg anthocyanins)	2 h before with one slice of buttered toast or bread	at rest	† DBP, SBP, blood lactate and glucose at rest
							30-min of cycling (3 × 10 min at 45, 55 and 65 % $\dot{V}O_{2max}$)	† $\dot{V}O_2$, $\dot{V}CO_2$, heart rate, cycling economy, absolute power, blood plasma lactate, blood glucose, energy expenditure
								At 45% $\dot{V}O_{2max}$: ↑* 15% FATox (p = 0.077), † CHox, ↓* RER (p = 0.066)
								At 55% $\dot{V}O_{2max}$: ↑* 13% FATox (p = 0.102), † CHox, ↓* RER (p = 0.120)
								At 65% $\dot{V}O_{2max}$: ↑↑ 27% FATox (p = 0.044), ↓* CHox (p = 0.06), ↓↓ RER (p = 0.043)
							16.1-km time-trial	↓↓ 16.1 km completion time (p = 0.027)
								↑* Power across time trial (p = 0.155)
								↑↑ Blood lactate during recovery (p = 0.003)

Willems et al. (2015)	8 males and 5 females 38 ± 8 years trained	double-blind, randomised, crossover design	7 days	placebo and NZBC powder	6 g/day (138.6 mg anthocyanins)	2 h before with a light breakfast of toast and water	at rest	† DBP, SBP, mean arterial pressure, and HR
								↑↑ 25% SV (p = 0.006), ↑↑ 26% cardiac output (p = 0.015) ↓↓16% lower total peripheral resistance (p = 0.05)
							incremental cycling protocol	† DBP, SBP, SV, mean arterial pressure, cardiac output, HR, Total peripheral resistance
								↑↑ 4% Intensity at 1 mmol/L lactate level (p = 0.02)
								↑↑ 6% Intensity at 4 mmol/L lactate level (p = 0.007)
								↑↑ 27% (p = 0.001), 22% (p = 0.002), 17% (p = 0.001) and 13% (p = 0.004) plasma lactate levels at 40%, 50%, 60% and 70% of maximum power
							maximum oxygen uptake protocol	maximum oxygen uptake was obtained with 14% lower lactate values (p = 0.02)
								† maximal oxygen uptake and power across the trial
Cook, Myers, Gault, Edwards, et al. (2017)	15 males 38 ± 12 years endurance trained	double-blind, randomised, crossover design	7 days	placebo and 3 doses of NZBC extract	300, 600, and 900 mg/day of NZBC extract (105, 210, and 315 mg of anthocyanins)	2 h before with one slice of buttered bread or toast	120-min of cycling at 65% $\dot{V}O_{2max}$	† plasma lactate and glucose
								† heart rate, energy expenditure, relative intensity
								300 mg/day: ↑* 17.5% FATox (p = 0.124), † CHox, † RER
								600 mg/day: ↑↑ 21.5% FATox (p < 0.05), † CHox, ↓↓ RER (p < 0.05)
								900 mg/day: ↑↑ 24.1% FATox (p < 0.05), † CHox, ↓↓ RER (p < 0.05)

Murphy et al. (2017)	10 males 30 ± 12 years trained cyclist	double-blind, randomized, crossover design	7 days	placebo and NZBC extract	300 mg/day (105 mg anthocyanins)	2 h before exercise*	2 × 4 km cycling time-trial	† HR and lactate
								two cycling trials were 0.82% faster with NZBC intake (p = 0.034)
								† time to complete 2 x 4 km of cycling
								↑* mean power by 7 Watts (p = 0.095)
Strauss et al. (2018)	16 females 28 ± 8 years endurance trained	double-blind, randomized, crossover design	7 days	placebo and NZBC extract	600 mg/day (210 mg anthocyanins)	2 h before with standardised breakfast providing 1 g/kg body mass of carbohydrate	120-min cycling at 65% $\dot{V}O_{2max}$	† HR, $\dot{V}O_2$, plasma glucose, and energy expenditure
								↑↑ 27% FATox (p = 0.042), ↓* CHox (p = 0.063), ↓* RER (p = 0.058)
								↑↑ 49% FFA (p = 0.034) and ↑* 27% glycerol concentrations (p = 0.051) pre-exercise
								Pre-exercise plasma FFA concentrations were moderately associated with mean rates of fat oxidation during exercise (r = 0.45, p = 0.016)
(Willems et al., 2019)	11 males 38 ± 11 years trained cyclists and triathletes	double-blind, randomized, crossover design	7 days	placebo and NZBC extract	600 mg/day (210 mg anthocyanins)	2-h before with one slice of bread and a glass of water	at rest	† SBP, DBP, arterial oxygen saturation
							30-min cycling (3 × 10 min at 45, 55% and 65% $\dot{V}O_{2max}$)	† $\dot{V}O_2$, $\dot{V}CO_2$, cycling economy, glucose, lactate, FATox, CHox, RER and heart rate intensities
							16.1-km time-trial at ~2500 m (stimulated)	† time to complete 16.1 km cycling

							incremental intensity cycling	† blood lactate
Montanari et al. (2020)	13 males 39 ± 10 years endurance trained cyclists	double-blind, randomized, crossover design	7 days	placebo and 2 doses of NZBC extract	300 and 600 mg/day of NZBC extract (105 and 210 mg of anthocyanins)	2 h before with one slice of bread and a glass of water	10-min cycling at 65% $\dot{V}O_{2max}$	† $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, and HR
								† FATox, † CHox, † Average RER
							16.1-km time-trial	Time trial ↓↓ between day 1 and day 4 after consuming 600 mg (p = 0.05) with an ↑↑ speed (p = 0.04)
								† No difference was observed within condition for placebo and 300 mg NZBC intake
Montanari et al. (2021)	26 males and 8 females 38 ± 7 years endurance trained cyclists	double-blind, randomized, crossover design	1 day	placebo and NZBC extract	900 mg/day of NZBC extract (315 mg of anthocyanins)	1.5 h before warm-up**	16.1-km time-trial (home-based)	All cyclists: † time-trial, power, speed, HR, cadence, RPE
								Slow cyclists: time trial was 20 s faster with NZBC with a small effect size, higher cycling power and speed were observed with NZBC but without an effect on HR and cadence
								Fast cyclists: There were no difference in cycling time, power, speed, heart rate, cadence.

↑↑, significant increase; ↓↓, significant decrease; †, no significant difference; ↑*, trend to be higher; ↓* trend to be lower; NZBC, New Zealand blackcurrant; DBP, diastolic blood pressure; SBP, systolic blood pressure; $\dot{V}O_2$, volume of oxygen consumed; $\dot{V}CO_2$, volume of carbon dioxide produced; FATox, fat oxidation; CHox, carbohydrate oxidation; RER, respiratory exchange ratio; HR, heart rate; SV, stroke volume; FFA, free fatty acids; $\dot{V}E$, minute ventilation, *Breakfast not specified; **breakfast consumed 3 h before warm-up

2.5. Effect of NZBC on running

Many field sports such as football (soccer), rugby and field hockey consist of alternating periods of short maximal intensity running in the form of sprints and moderate to low-intensity activities such as walking and jogging marked as active recovery (Glaister, 2005). A match analysis of a 90-min football game in young football players reported that 3.3% of the total distance in both halves was covered by high-intensity sprints (Aslan et al., 2012). The last two decades of research on European football has also witnessed an association between sprint performance and outcomes of the game such as (i) sprints performed by the attacking players has been identified as the most frequent action preceding goal situations (Faude et al., 2012; Martínez-Hernández et al., 2023), (ii) positive association between sprint distance covered by wide-midfielders and strikers and the number of matches won by their team (Chmura et al., 2018), and (iii) high number of goals in the last 15 min of the second half when the players are likely experiencing physical fatigue (Reilly & Williams, 2003).

Inadequate oxygen availability to replenish phosphocreatine (Haseler et al., 1999), accumulation of metabolites such as hydrogen (Glaister, 2005), and/or due to oxidative stress caused by the production of free radicals (Morales-Alamo & Calbet, 2014) may contribute to fatigue while running and decrease performance. A recent meta-analysis on the effect of NZBC on exercise performance concluded that intake of NZBC for 7 days (105–210 mg anthocyanins) with a final dose 1–2 h before exercise mediates a small, significant improvement on performance (Braakhuis et al., 2020). Therefore, combating fatigue using ergogenic aids such as NZBC may allow players to maintain their repeated maximal sprint performance, especially later in the game, and benefit the players in accomplishing a favourable outcome of the game.

2.5.1. Repeated-sprint performance

Intake of NZBC for 7 days increased the number of sprints and total running distance during a high-intensity intermittent running test protocol in recreationally active males (Perkins et al., 2015). Similarly, Willems et al. (2016) observed less slowing of the fastest maximal sprint during block 5 of the Loughborough Intermittent Shuttle-running Test (LIST) with NZBC intake. The study also reported that 8 out of 13 participants improved their time to exhaustion by 15% while having a higher heart rate. In contrast, a study on trained rugby players showed no differences in the sprint speed in a series of 6 x 35-m sprints (running based anaerobic sprint test; RAST) with NZBC intake (Burnett & Willems, 2022). However, observed moderate effect sizes for the slowing of sprint 2 and sprint 6 compared to sprint 1 (out of 6 sprints) and reported a faster mean sprint time (trivial effect) with the intake of NZBC extract. It is possible that NZBC intake influenced repeated-sprint performance by increasing peripheral blood flow that led to higher phosphocreatine (PCr) resynthesis, reduced metabolite

accumulation (Perkins et al., 2015), increased oxygen availability, and reduced the oxidative stress (Willems et al., 2016).

One study also evaluated the effect of NZBC consumption on sprint performance using the RAST in both trained youth (English professional club) and recreationally active football players (university players) and reported that NZBC intake seems to benefit repeated-sprint performance only in trained football players (Godwin et al., 2017). In trained youth players, NZBC intake reduced slowing of the sprint 5 time and there was a trend for an effect of NZBC intake on the slowing of the sprint 1 time of the RAST. However, in recreationally active athletes, NZBC intake had no effect on the change in sprint times of sprints 2 to 6 compared to the fastest sprint time of sprint 1 (Godwin et al., 2017). It is speculated that this difference in response could have been caused by trained youth football players having better re-oxygenation rates with NZBC intake. The lack of consistent results in this study could have been due to its limitations. Firstly, there was no restriction on dietary polyphenol intake during the study which could have affected anthocyanin levels in the blood independent of NZBC intake. Secondly, the study applied a sprint threshold criterion to avoid pacing, however, some participants did manage to run faster in the sixth sprint compared to the fifth sprint. This performance could be explained by the central governor model which demonstrates that participants tend to have the ability to increase their speed for the final 10% of an exercise session regardless of their previous level of exertion (Noakes, 2012) or by the rating of perceived exertion template for pacing (Schallig et al., 2018). Lastly, the sprint performance for both groups were tested on different artificial surfaces which could significantly affect the absolute sprint times (Godwin et al., 2017).

2.5.2. Running performance

Acute intake of NZBC has been shown to improve running performance by 3% during a 5-km time trial in trained runners (Moss et al., 2023), however, did not change critical speed and distance covered during incremental running performance in recreationally active males (Pastellidou et al., 2021). Furthermore, effect size calculation in 60% of participants in the study by Pastellidou et al. (2021) reported a weak and a non-significant trend towards a 10–40% increase in distance covered, suggesting that certain cohorts may possibly gain a small benefit from NZBC intake.

The faster completion time of the 5-km run (Moss et al., 2023) can be speculated by the increase in arterial dilation and the subsequent increase in blood flow to the working muscle with NZBC intake (900 mg/day) as previously seen in the typing study by Matsumoto et al. (2005). The lack of results reported in the study by Pastellidou et al. (2021) could have been due to the low dose of NZBC (300 mg/day for 2 days) and the absence of loading phase before the exercise trial as seen in other studies (Godwin et al., 2017; Willems et al., 2015). Thus, concluding that the acute intake of 900 mg/day of

NZBC can improve time to complete 5-km time-trial, however, does not improve critical speed and distance covered during an incremental running protocol.

2.5.3. Blood lactate

Blood lactate serves as an indicator of anaerobic metabolism, which is prominent during high-intensity exercise when the oxygen delivery to the muscles is insufficient. Glycolysis under anaerobic conditions reduces pyruvate to lactate catalysed by lactate dehydrogenase, thus, monitoring blood lactate levels helps assess the intensity and effectiveness of training and exercise performance (Lee, 2021). Willems et al. (2016) reported no significant differences for blood lactate with NZBC intake during part A and part B of the Loughborough Intermittent Shuttle Test (LIST) in recreationally active athletes. In contrast, Perkins et al. (2015) reported a trend for blood lactate to be higher in the NZBC group by 15% in 9 out of 13 participants after participating in a high-intensity intermittent running test protocol. Furthermore, there was a trend for a larger decrease in blood lactate levels in the NZBC group at 1, 2, 3, 4, 10, 15, and 30-min with NZBC intake. The authors suggested that the larger decrease in blood lactate during recovery with NZBC could potentially be due to higher lactate values at the start of the recovery which led to faster lactate clearance (mass action effect) (Perkins et al., 2015).

Pastellidou et al. (2021) assessed the effect of NZBC intake on lactate threshold during a stepwise ramped incremental exercise test and reported that blood lactate accumulation did not significantly change with NZBC intake. In addition, lactate threshold assessed by oxygen consumption, heart rate, and running economy also showed no significant differences with NZBC intake (Pastellidou et al., 2021). The lack of effect of NZBC on lactate levels in this study is consistent with findings by Willems et al. (2016). Thus, from the limited available evidence, it would be reasonable to conclude that NZBC has no effect on blood lactate levels irrespective of the duration of the supplementation period.

2.5.4. Substrate metabolism

Intake of 600 mg/day of NZBC for 7 days demonstrated an increased fat oxidation during 60-min of fasted running at 65% $\dot{V}O_{2max}$ in hot ambient conditions (Hiles et al., 2020). Additionally, both carbohydrate oxidation and mean exercise respiratory exchange ratio (RER) were lower throughout exercise with NZBC consumption (Hiles et al., 2020).

In contrast, Pastellidou et al. (2021) and Moss et al. (2023) found NZBC intake did not influence substrate utilisation and fat oxidation during a steady-state run at lactate threshold. The participants from both studies were in a postprandial state as they consumed their last meal along with the last dose of NZBC 2 h prior to exercise. Hence, it is possible that the participants had elevated insulin levels at the start of the exercise which inhibited lipolysis, and thus, reduced availability of fatty acids available for oxidation during exercise (King et al., 2018). Furthermore, both studies evaluated the

effect of the acute intake of NZBC, and thus, it is possible that a 7-day loading phase is necessary to see a difference in substrate metabolism.

2.5.5. Fatigue index and ratings of perceived exertion

In the study by Godwin et al. (2017) there was a strong trend for the fatigue index to be 12% lower following NZBC intake in both young trained and recreationally active football players ($p = 0.06$, 12 out of 24 participants showed a lower fatigue index). It has been reported that fatigue index during the running-based anaerobic sprint test (6 × 35-m sprints with 10 s passive recovery) is correlated with activity level of the *m.rectus femoris* and *m.biceps femoris* muscles, but not with *m.vastus lateralis* in professional football players (Brocherie et al., 2015). Thus, it is possible that the intake of NZBC in the Godwin et al. (2017) study influenced some football players to better maintain activity level in some lower limb muscles during repeated sprint running. Moreover, the authors found that there was an increase in ratings of perceived exertion (RPE) over time with both NZBC and placebo intake (Godwin et al., 2017), thus, suggesting that NZBC intake did not influence RPE during exercise.

To conclude, intake of NZBC for 7 days has been shown to influence running performance by increasing the number of sprints and improving time to exhaustion, whereas the acute intake has reported faster completion time during a 5-km running trial. It is speculated that NZBC intake benefits athletes by increasing muscle blood flow (Matsumoto et al., 2005) which can influence blunting of PCr degradation that causes fatigue, contribute to higher PCr resynthesis, improve removal of metabolites, inorganic phosphate and adenosine diphosphate (which would normally have a negative effect on force production), thereby, improving exercise performance. NZBC intake for 7 days may also have an effect on substrate metabolism when exercising in a fasted state. However, the evidence indicates it has no effect on blood lactate levels and ratings of perceived exertion during exercise and post-exercise recovery.

Table 2.2 Summary of studies investigating the effect of New Zealand blackcurrant on running performance.

Author and Year	Participants	Study Design	Study Duration	Study Groups	Blackcurrant Dose	Timing of last dose before exercise	Exercise Protocol	Key Findings (NZBC compared to placebo)
Perkins et al. (2015)	13 males 25 ± 4 years recreationally active	double-blind, randomised, crossover design	7 days	placebo and NZBC extract	300 mg/day (105 mg anthocyanins)	3 h before exercise	adapted NIE intermittent high-intensity test	↑↑ 10.6% total running distance (p = 0.023) ↑↑ 10.8% sprint distance (p = 0.020) ↑* 15% lactate at exhaustion (p = 0.07) † HR, oxygen uptake, and RPE
Willems et al. (2016)	13 males 22 ± 1 years recreationally active	double-blind, randomised, crossover design	7 days	placebo and NZBC extract	300 mg/day (105 mg anthocyanins)	3 h before exercise	Loughborough Intermittent Shuttle Test (Part A - 4 blocks of 15-min each) (Part B - till exhaustion)	↓↓ slowing of fastest sprint time in block 5 of LIST in the NZBC group (p = 0.05) † blood lactate values in blocks 4 and 5 compared to block 1 8 participants (out of 13) improved the time to exhaustion with NZBC extract by 15% (2 min and 20 s longer)
Godwin et al. (2017)	24 males 15 adults 20 ± 1 years recreationally active 9 youth 17 ± 0 years professionally trained football players	double-blind, randomised, crossover design	7 days	placebo and NZBC extract	600 mg/day (210 mg anthocyanins)	2 h before exercise	running based anaerobic sprint test	† maximum sprint time, minimum sprint time and mean sprint time ↓* fatigue Index in NZBC group (p = 0.06)

Hiles et al. (2020)	12 males and 6 females 27 ± 6 years recreationally active	double-blind, randomised, crossover design	7 days	placebo and NZBC extract	600 mg/day (210 mg anthocyanins)	Before running*	60-min of fasted running at 65% $\dot{V}O_{2max}$ at 34°C	† HR, rectal temperature, mean skin temperature, mean body temperature, and physiological strain
								† $\dot{V}O_2$ and † $\dot{V}CO_2$
								↓↓ RER during 50 min of exercise (p = 0.04)
								↓↓ carbohydrate oxidation (p = 0.014)
								↑↑ fat oxidation between 10–50 min (p = 0.008)
Pastellidou et al. (2021)	15 males 24.4 ± 3.6 years recreationally active	double-blind, randomised, crossover design	2 days	placebo and NZBC extract	300 mg/day (105 mg anthocyanins)	2 h before exercise	stepwise ramped incremental exercise test with speed increasing 0.5 km/step until volitional exhaustion	† critical speed and running economy
								† blood lactate
								† fat oxidation
								† $\dot{V}O_{2max}$ and HR max
Burnett and Willems (2022)	13 males 21 ± 2 years trained rugby players	double-blind, randomised, crossover design	7 days	placebo and NZBC extract	600 mg/day (210 mg anthocyanins)	2 h before exercise	running based anaerobic sprint test	† fastest 35 m sprint and sprint time
							Illinois agility test	† agility test
							seated medicine ball throw test	† upper body strength, power, and handgrip strength

Moss et al. (2023)	16 males 26 ± 5 years trained runners	double-blind, randomised, crossover design	1 day	placebo and NZBC extract	900 mg/day (315 mg anthocyanins)	2 h before exercise	10-min run at lactate threshold	† $\dot{V}O_2$, RER, $\dot{V}E$, carbohydrate oxidation, fat oxidation, heart rate, blood lactate and RPE
							5-km time-trial	faster finish time with NZBC intake (38 s, ~3%) (p = 0.001)

↑↑, significant increase; ↓↓, significant decrease; †, no significant difference; ↑*, trend to be higher; ↓*, trend to be lower; LIST, Loughborough Intermittent Shuttle Test; NZBC, New Zealand blackcurrant; RER, Respiratory Exchange Ratio; RPE, Ratings of Perceived Exertion; $\dot{V}E$, minute ventilation, $\dot{V}O_2$, volume of oxygen consumed; $\dot{V}CO_2$, volume of carbon dioxide produced; *time not defined

2.6. Mechanisms of action

Increase in skeletal muscle perfusion and fat oxidation are two possible ways by which blackcurrant anthocyanin intake can improve running and cycling performance. The following sections describe the mechanisms of action of NZBC for the increase in blood flow and changes in substrate partitioning that benefit exercise performance.

2.6.1. Blood flow

Increase in blood flow is frequently suggested as the underlying mechanism of action of NZBC intake on improving exercise performance. The *in vitro* treatment of human endothelial cells with NZBC has demonstrated an increase in the production of endothelial nitric oxide synthase (eNOS) (Edirisinghe et al., 2011). The nitric oxide (NO) released from endothelial cells via endothelial nitric oxide synthase (eNOS) can enhance peripheral blood flow to exercising muscles through relaxation of vascular smooth muscle cells and vasodilation of blood vessels (Garcia & Sessa, 2019). Blackcurrant anthocyanins have also been shown to increase eNOS mRNA expression and subsequent NO production in human endothelial cells in culture (Speciale et al., 2014) and in small animal studies (Horie et al., 2019). Furthermore, there is evidence that blackcurrant extract can activate eNOS via the Akt/PI3 kinase pathway in human umbilical vein endothelial cells (Edirisinghe et al., 2011). Another study on isolated rat aortic rings demonstrated a 37% relaxation of the intact rings by anthocyanins, possibly by the involvement of the purinergic pathway to produce NO (Mendes et al., 2003).

New Zealand blackcurrant intake also has been shown to increase delivery of blood to muscles via vasodilation (Matsumoto et al., 2005). The increase in blood flow therefore could influence oxygen delivery, fuel utilisation, phosphocreatine degradation, and lactate clearance. Matsumoto et al. (2005) observed a 22% increase in the peripheral blood flow at rest after the consumption of a single dose of 17 mg/kg BM of blackcurrant concentrate containing 10.83% of anthocyanins in healthy male participants. The change in forearm blood flow was assessed using near infrared spectroscopy (NIRS) which is a recognised, accurate and sensitive assessment of blood flow as it measures haemoglobin, deoxyhaemoglobin and total haemoglobin every 0.5 s (Matsumoto et al., 2005). The study also evaluated the effect of 7.7 mg/kg BM of blackcurrant concentrate intake for 14 days on typing work and observed the following: (i) the oxygenated haemoglobin measured by NIRS was significantly higher after intake of blackcurrant concentrate than with placebo until the fourth set of typing work and (ii) the viscoelasticity of the trapezius muscle increased significantly after the typing workload in the placebo group only, however it was not significantly different between the blackcurrant and placebo group. Thus, suggesting that intake of blackcurrant could have improved shoulder stiffness caused by typing work by increasing peripheral blood flow and reducing muscle fatigue.

More recently, studies have demonstrated an effect of NZBC intake on blood flow during isometric contractions. Fryer et al. (2020) reported that the intake of NZBC for 7 days resulted in faster time to half-recovery of tissue saturation index in rock climbers following an intermittent isometric contraction protocol. However, there was no difference in brachial artery flow, artery diameter or artery velocity in the three minutes of recovery.

Cook, Myers, Gault and Willems (2017) reported an increase in femoral artery diameter during the sustained submaximal isometric contraction with the intake of 600 mg/day of NZBC for 7 days. The increase in femoral artery diameter was accompanied by a decrease in blood pressure, mean arterial blood pressure and total peripheral resistance with an associated increase in cardiac output and stroke volume (Cook, Myers, Gault, & Willems, 2017). A similar study by Cook et al. (2023) evaluated the intake duration effects of NZBC following 1, 4 and 7 days' NZBC intake of 600 mg/day on the isometric contraction-induced cardiovascular responses in healthy male participants. The study reported that the femoral artery diameter was higher following 4 and 7 days of NZBC intake compared to baseline and higher on day 7 compared to day 1 of intake (Cook et al., 2023). Moreover, mean arterial pressure and total peripheral resistance decreased and stroke volume and cardiac output increased significantly at different times during the isometric contraction following 7 days' intake in comparison to 1-day intake of NZBC. Thus, it seems that consumption of NZBC for several days is essential to alter the isometric-contraction induced cardiovascular responses (Cook et al., 2023).

Research evaluating the effect of NZBC intake on high-intensity intermittent exercise performance have speculated that the improvement in performance could be because of the influence of NZBC on blood flow. Studies have shown an increase in distance covered during a high-intensity intermittent running protocol (Perkins et al., 2015), reduced slowing of the fastest time of sprint during repeated-sprint performance (Godwin et al., 2017), and higher cycling intensity at the onset of blood lactate accumulation (Willems et al., 2015). It is possible that the increase in blood flow could in turn (i) decrease fatigue during high-intensity intermittent exercise by blunting phosphocreatine degradation (Perkins et al., 2015), (ii) contribute to higher PCr resynthesis, and improved removal of metabolites, inorganic phosphate and adenosine diphosphate which would normally have a negative effect on force production (Godwin et al., 2017), and (iii) alter the balance of lactate appearance and removal mechanisms of blood lactate accumulation (Willems et al., 2015), thereby, improving exercise performance.

Thus, to conclude, though the influence of NZBC on blood flow is evident when relaxed and during isometric contractions, its blood flow effects during high-intensity intermittent exercise performance are currently speculative and warrant further research.

2.6.2. Fat oxidation

Animal studies have reported an increase in fat oxidation with blackcurrant supplementation (Benn et al., 2014). For example, chronic blackcurrant extract intake (0.1% of blackcurrant wt/wt for 12 weeks) in mice has been shown to elevate mRNA of genes involved with energy expenditure including peroxisome proliferator-activated receptor alpha (Benn et al., 2014). Similarly, Tsuda et al. (2005) observed up-regulation of genes associated with lipid metabolism such as hormone sensitive lipase as well as increased lipolysis activity with the treatment of adipocytes with the cyanidin 3-glucoside or cyanidin.

Four studies have reported an increase in fat oxidation in trained and recreationally active individuals following the consumption of 300, 600, and 900 mg/day NZBC for 7 days (Cook et al., 2015; Cook, Myers, Gault, Edwards, et al., 2017; Hiles et al., 2020; Strauss et al., 2018) in both fed and fasted states. The participants engaged in either 30-min incremental cycling exercise (Cook et al., 2015), 60-min running at 65% $\dot{V}O_{2max}$ (Hiles et al., 2020), or 120-min cycling at 65% $\dot{V}O_{2max}$ (Cook, Myers, Gault, Edwards, et al., 2017; Strauss et al., 2018). Though these studies have reported an increase in fat oxidation, the mechanism(s) underlying this outcome remain unclear.

The AMP-activated protein kinase (AMPK) pathway plays a role in enhancing fat oxidation and it is potentially modulated by anthocyanin intake. A study in mice reported that AMPK protein expression and phosphorylation was elevated in skeletal muscle following 5 weeks ingestion of 10 g/kg BW of an anthocyanin-rich bilberry extract (Takikawa et al., 2010). AMPK activation is essential for fat oxidation. It can induce translocation of the primary fatty acid transporter in skeletal muscle (FAT/CD36) to the plasma membrane and, therefore, increase fatty acid uptake (Luiken et al., 2003). AMPK can also inhibit the activity of acetyl-CoA carboxylase thereby suppressing malonyl-CoA production and increasing fatty acid entry into the mitochondria (Towler & Hardie, 2007). It is therefore possible that the increase in fat oxidation following anthocyanin intake can be rationalised through the effect of anthocyanins on several nodes of control related to protein activity and expression of AMPK in adipose tissue and skeletal muscle (Strauss et al., 2018).

Anthocyanins have also shown to increase fatty acid oxidation of human HepG2 cells following *in vitro* incubation (Guo et al., 2012). An increased fatty acid oxidation and a decrease in fatty acid synthesis is caused by the action of AMPK to inhibit the activity of acetyl-CoA carboxylase (ACC) 1 and ACC-2 (Towler & Hardie, 2007). Roepstorff et al. (2005) demonstrated that there was a decrease in muscle malonyl-CoA concentration associated with an increased activity of AMPK and inhibition of acetyl-CoA carboxylase resultant from its phosphorylation by AMPK following 60-min cycling at 65% $\dot{V}O_{2max}$ in moderately trained men. It has also been reported that AMPK activation can induce translocation of

FAT/CD36 allowing increased fatty acid uptake (Luiken et al., 2003). Therefore, increased whole-body fat oxidation may result from a combination of many pathways acting synergistically including activation of AMPK and up regulation of genes involved in fat oxidation and transport of fatty acids into mitochondria.

2.7. Regulation of cellular redox balance

Polyphenolics and anthocyanins have been considered as powerful dietary antioxidants due to their ability to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS). Although there has been strong evidence for anthocyanins to be antioxidants *in vitro*, their effectiveness of acting in this way in the body once consumed has been brought into question mainly because of their poor absorption and bioavailability and a lack of knowledge on the redox (simultaneous oxidation and reduction) properties of their metabolites (Stevenson et al., 2009). More recently, it has been suggested that some polyphenolic compounds including anthocyanins can act as "pro-oxidant" polyphenols due to their electrophilic properties (Andrés et al., 2023; Dangles & Fenger, 2018). The pro-oxidant effects of polyphenols encompass various processes which include the transient reduction of Cu^{2+} to Cu^{+}, the formation of ROS, and the potential disruption of cellular antioxidant defence mechanisms, such as those involving glutathione and glutathione-S-transferase (Sahu & Gray, 1996). The underlying mechanisms responsible for these effects involve the creation of a labile flavonoid-redox complex (Cadenas, 1997), which in turn can generate hydrogen peroxide (H_2O_2). Subsequently, this H_2O_2 production may lead to the oxidation of haemoglobin (Jia & Alayash, 2008) and the liberation of Fe^{+2} ions that can undergo auto-oxidation or partake in a redox process, thereby functioning as pro-oxidants (Bhattacharyya et al., 2014).

Two pilot studies by Hurst et al., (2019 and 2020) evaluated the effect of the pro-oxidant properties of NZBC anthocyanins on (a) plasma oxidative capacity and (b) plasma antioxidant capacity before exercise and during post-exercise recovery. The first study evaluated the effect of 0.8, 1.6, or 3.2 mg/kg NZBC intake and found a significant increase ($p < 0.05$) in plasma oxidative capacity 1 h following consumption of 1.6 and 3.2 mg/kg NZBC. This increase in plasma oxidative capability in the 1.6 and 3.2 mg/kg NZBC groups was still higher immediately post-exercise and then decreased significantly at 2 h and 6 h post-exercise recovery. Moreover, there was no change in the plasma antioxidant capacity between groups before and after the exercise trial (Hurst et al., 2019).

The second study evaluated the effect of 3.2 mg/kg NZBC (240 mg total anthocyanins) on plasma oxidative and antioxidant activity before exercise and during 2 h of post-exercise recovery after 5 weeks of NZBC intake. A trend for an increase in plasma oxidative capacity was observed before exercise in the NZBC group. After exercise, plasma oxidative capacity increased significantly in both

placebo and NZBC groups, and it was not different from each other. However, during exercise recovery, the group with NZBC intake showed a greater decrease in plasma oxidative capacity along with no changes in overall plasma antioxidant capacity compared to the placebo (Hurst et al., 2020).

The increase in plasma oxidative capacity observed in these studies combined with no change in antioxidant capacity (FRAP assay) indicated that the facilitated decrease in exercise-induced oxidative stress was unlikely to be due to the inherent antioxidant properties of the ingested blackcurrant anthocyanins. Hurst et al., (2019) suggested that the pre-exercise increase in plasma oxidative capacity observed may have been elicited by the electrophilic nature of anthocyanins which could have triggered the activation of adaptive cellular redox-sensitive processes such as nuclear redox factor2 (Nrf2) transcription. Nrf2 is considered as a 'master regulator' of antioxidant defensive responses and becomes activated under oxidative stress conditions. Upon activation, Nrf2 translocates into the nucleus and binds to the antioxidant response element (ARE) of antioxidant enzyme genes, initiating their transcription and providing an adaptive response to manage oxidative stress (Kropat et al., 2013; Yan et al., 2017). The proposed activation of Nrf2 could therefore have mediated the greater decrease in the oxidative capacity during exercise recovery reported in both studies compared to the placebo group (Hurst et al., 2019; Hurst et al., 2020). Moreover, although the data was not shown, additional experiments conducted by Hurst et al., 2020 found that NZBC anthocyanins could enhance 2-tert-butyl-1,4-hydroquinone (tBHQ)- and redox (copper II)-activation of Nrf2/ARE transcription and also upregulate other antioxidant enzymes such as heme oxygenase 1 and thioredoxin reductase (Hurst et al., 2019), demonstrating an activation of Nrf2 by NZBC and a subsequent upregulation of adaptive antioxidant defence systems.

It is therefore likely that the pro-oxidant activity of bioavailable blackcurrant-derived anthocyanins, either alone or in association with exercise-induced ROS, lead to the activation of cellular stress sensitive signalling and defence mechanisms, such as Nrf2/ARE, which enables the up-regulation of cellular antioxidant capacity and assists in the improvement of recovery from exercise-induced oxidative stress (Hurst et al., 2019). It is feasible that through this mechanism of action, rather than any other mechanism discussed here, that the consumption of NZBC would offer enhanced recovery benefits for athletes after maximal exercise, that is likely to translate into an improvement in training adaptation and performance itself.

2.8. Limitations of the research to date and future directions

Of the papers identified in this review, most studies evaluating the effects of NZBC on cycling performance assessed performance using an incremental cycling test and time-trials, and reported effects on substrate metabolism. On the other hand, the studies identified in this review on running

performance focused on sprint times and distance covered, but not sprint speed. More studies are therefore needed on the effects of NZBC that focus on other aspects of running performance such as speed, acceleration, and deceleration to evaluate the effect of NZBC on running performance. The most common intervention strategy identified in this review was the intake of NZBC in the range of 300-900 mg/day for 7 days. It is clear that the acute (single dose) consumption of NZBC can manage the oxidative stress mediated by moderate exercise (Hurst et al., 2019) but the effects of acute consumption on exercise performance warrants further investigation. Furthermore, while Hurst et al. (2019) is the only study to evaluate the effects of a range of doses of NZBC in a single study on parameters of bioavailability, antioxidant, pro-oxidant and oxidative stress to define the exact dose required, further studies are needed in this aspect and again in the context of performance mediated benefits. Additionally, given the different intensities and durations of cardiovascular cycling and running exercise regimes and the fitness levels of individuals and the stresses encountered as a result, more studies are required to reveal the breadth of benefits of NZBC in the context of managing oxidative stress, inflammation and/or exercise performance. These studies should also include with their physiological assessment of exercise performance blood markers for acute and chronic inflammation, oxidative stress, antioxidant activity, and biomarkers of adaptive defence pathways (e.g. Nrf2).

2.9. Conclusions

NZBC bioavailability studies have shown an increase in plasma anthocyanins levels as early as 30 min and peak at 1 to 2 h post consumption. Most studies on cycling and running performance explored the effect of 7-day intake of 300, 600, and 900 mg of NZBC extract containing 105, 210, and 315 mg of anthocyanins, respectively, with the last dose consumed 1 to 3 h before exercise. The studies on cycling reported an increase in fat oxidation, whereas the studies on running reported an increase in sprint distance covered and less slowing of sprint during repeated-sprint performance, and faster finish time during a 5-km running trial. The mechanism of action for increase in fat oxidation is presently rationalised from *in vitro* and animal studies, thus warrant caution when generalising to exercise-based studies. Additionally, there is evidence of NZBC intake increasing blood flow at rest and during sustained submaximal isometric contractions, however, there is no evidence to prove that NZBC influences the activity of nitric oxide synthase and increases vasodilation and blood flow during moderate- to high-intensity exercise. Furthermore, there is evidence to suggest that NZBC may trigger the Nrf2 adaptive defence pathway through a pro-oxidant activity which may influence recovery from exercise.

Chapter 3
The Effect of a Single-Dose of New Zealand Blackcurrant Powder on Substrate Oxidation during Cycling: A Study in Recreationally Active Males

Abstract

Background: Intake of New Zealand blackcurrants in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC) for 7 days has been shown to increase fat oxidation during mixed-intensity cycling exercise in recreationally active and trained athletes. Studies have also reported higher plasma glycerol and free fatty acid (FFA) levels before exercise which indicated an increase in lipolysis with NZBC intake. However, in these studies the last dose was provided 2 h prior to exercise, thus making it unclear if the increase in fat metabolism was due to the final dose or the result of the accumulated repeated dosing.

Aim: To evaluate the effect of a single dose of NZBC powder (12 g) on fat oxidation and FFA levels during a 90-min mixed-intensity cycling protocol in recreationally active males. We hypothesised that a single dose of NZBC would increase fat oxidation and serum FFA concentration during exercise.

Methods: Fourteen recreationally active males participated in a double blind, randomised controlled crossover study with each trial separated by 7 days. Although only two conditions were being tested: (i) placebo (PLA) and (ii) NZBC (12 g, 120 mg of anthocyanins), the trial was repeated four times with two trials each for PLA and NZBC (to reduce intra-individual variation). Participants reported to the laboratory in the afternoon after observing at least a 3-h fast and consumed one of the study drinks 1-h before the cycling. Blood samples were taken before, during, and immediately post-exercise to measure serum FFA, and substrate utilisation was assessed by indirect calorimetry during exercise.

Results: Oxygen consumption increased over time ($p < 0.001$), however it was not significantly different between PLA and NZBC treatments ($p = 0.150$). Fat oxidation and % fat oxidation increased over time and were matched with decreased carbohydrate oxidation, % carbohydrate oxidation, and respiratory exchange ratio with both PLA and NZBC treatments ($p < 0.001$), but they were not significantly different between treatments ($p > 0.05$). Furthermore, serum FFA concentration increased with time during cycling ($p < 0.001$) but was not different between treatments ($p = 0.113$).

Conclusion: Consumption of a single dose of NZBC powder containing 120 mg anthocyanins had no effect on substrate oxidation and serum FFA concentration during mixed-intensity cycling in recreationally active males.

Keywords: Polyphenols, substrate partitioning, gas analysis, lipid metabolism, sports, nutrition

3.1. Introduction

New Zealand blackcurrants (*Ribes nigrum*) are one of the richest sources of anthocyanins, and mainly contain anthocyanins delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside. New Zealand blackcurrant products used in sports performance trials vary from study to study and are usually in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC). Consumption of 300 mg/day of NZBC for 7 days has been shown to increase fat oxidation during moderate-intensity cycling exercise in recreationally active and trained athletes (Cook et al., 2015).

Fat oxidation is crucial for athletes due to its significant role in enhancing endurance performance and overall metabolic efficiency (San-Millán & Brooks, 2017). The ability to oxidise fat efficiently allows athletes to sustain prolonged periods of exercise by utilising fat as a primary energy source, thereby sparing glycogen stores for more intense efforts (Alghannam et al., 2021). This metabolic adaptation is particularly beneficial for endurance athletes, as higher rates of fat oxidation are associated with improved performance in activities such as triathlons, cross-country skiing, and ultramarathons (San-Millán & Brooks, 2017). Similarly, increased rates of fat oxidation are also beneficial during high-intensity intermittent sports such as football and field hockey, as increased fat oxidation during the low-intensity periods (such as active and passive recovery) can help faster resynthesis of phosphocreatine (PCr) - critical for short, repeated sprints (Ali et al., 2007).

Consumption of NZBC for 7 days with the final dose consumed 2 h before exercise has been shown to increase fat oxidation by 13-27%, 21-24% and 27% during moderate-intensity cycling (30 min at 45-65% $\dot{V}O_{2max}$ and 120 min cycling at 65% $\dot{V}O_{2max}$) (Cook et al., 2015; Cook, Myers, Gault, Edwards, et al., 2017a; Strauss et al., 2018). However, this intervention protocol of a 7-day intake period with the final dose 2 h prior to exercise raises the question if the increase in fat oxidation was due to the final dose or the result of the accumulated repeated dosing.

At present there is limited evidence on the effect of NZBC intake when consumed acutely. A single dose of 300 and 600 mg/day NZBC intake when consumed 2 h prior to exercise failed to show an effect on fat oxidation during 10 min of cycling at 65% of $\dot{V}O_{2max}$ in endurance trained cyclists (Montanari et al., 2021). Since the reliance on fat oxidation is higher during long duration moderate-intensity exercise, it is possible that the short 10-min cycling protocol was inadequate to evaluate acute effect on NZBC intake on fat oxidation, and hence further research is warranted.

Strauss et al. (2018) also observed that pre-exercise plasma concentrations of free fatty acids and glycerol were significantly higher following NZBC supplementation, indicating a potential influence of NZBC on fat metabolism. Therefore, the aim of this study was to evaluate the effect of a single dose

of NZBC drink containing 120 mg of anthocyanin on substrate oxidation and free-fatty acid levels during 90 min of mixed-intensity cycling in recreationally active males.

This study was part one of a two-part trial evaluating the individual and additive effect of NZBC and caffeine intake on high-intensity intermittent running performance in recreationally active males in a fatigued-state. The cycling protocol implemented in this study is the muscle glycogen depletion protocol, used to cause fatigue in individuals taking part in the running protocol the next day (part two).

3.2. Materials and methods

3.2.1. Participants

Fourteen healthy male participants were recruited from football clubs around North Shore, Auckland, New Zealand. The characteristics of the participants are described in Table 3.1. Participants were recreationally active with experience in team sports with high-intensity intermittent running but were not familiar with cycling. Participants were asked to refrain from taking anthocyanin-rich foods 2 days before the study and any additional supplements (e.g. vit C) during the study. This study was approved by the Massey University Human Ethics Southern A Committee (Ohu Matatika 1; approval number 21/09) and registered with the Australia New Zealand Clinical Trials Registry (ACTRN12621001394831). The participants provided written informed consent before their familiarisation visit and completed a medical history questionnaire.

Table 3.1 Participant characteristics

Age (years)	29.5 ± 9.3
Height (cm)	173.0 ± 23.2
Weight (kg)	76.6 ± 8.0
Maximum power (Watts)	283.4 ± 31.2
60% of maximum power (Watts)	170.0 ± 18.7
Sprint power (Watts)	336.7 ± 35.9

3.2.2. Study summary

Participants attended the initial familiarisation visit for the assessment of maximum power output, and then reported to the lab four times to complete one of the four trials with each visit. The study was a double blind randomised controlled crossover study with each trial separated by 7 days. Although only two treatments were being tested: (i) placebo (PLA) and (ii) NZBC, the trial was repeated

four times with two trials each for PLA and NZBC (to reduce intra-individual variation). The average of the variables assessed during the two trials was used for statistical analysis.

3.2.3. Familiarisation visit

Baseline assessments of body mass and height were performed after obtaining written consent from the participants. Participants enagaged in an incremental exercise test (ramp test) on a stationary cycle ergometer (Velotron Pro cycle ergometer, Racermate Inc., USA) to assess maximum power output (W_{max}) (Kuipers et al., 1985). Participants wore a chest strap heart rate monitor (T31 coded™ transmitter, Heart Rate Sensor, Polar, Finland) and began cycling at 100 W for a 5-min warm-up. Post warm-up, the workload increased by 50 W every 2.5 min until the heart rate of 160 beats per minute (bpm) was reached (Vøllestad et al., 1992). Thereafter, workload was increased by 25 W every 2.5 min until participants reached volitional exhaustion. The cycling power at which the particpants reached volitional exhaustion was considered as maximum power, and this resistive load was doubled to obtain the maximum sprint power for the muscle glycogen depletion protocol (Bowtell et al., 1999; Vøllestad et al., 1992). After a short resting period, participants were introduced to gas analysis using Quark RMR Metabolic Cart (COSMED Srl, Rome, Italy). Once they got familiarised with breathing through the mouthpiece, the session was concluded. Participants were given instructions for dietary standardisation and maintaining their physical activity between visits before leaving the laboratory.

3.2.4. Physical activity and dietary standardisation

Participants reported to the laboratory in the afternoon after fasting for at least 3 h before each trial. Participants were asked to restrict alcohol and were given a list foods containing caffeine, supplements, and high amounts of nutritional polyphenolic compounds and antioxidants to avoid 48 h before the trial. They were also asked to record dietary intake over the 24-h period prior to the first main trial and to replicate their intake prior to the other three trials. Food diaries were analysed using Foodworks (Xyris, Brisbane, Austrialia) for carbohydrate, fat, protein and total energy intake (average intake for the two trials for placebo and NZBC). There were no differences in carbohydrate, fat, protein and total energy intake (paired t-test, $p > 0.05$) between the experimental visits (Table 3.2). Participants were also instructed to keep their weekly exercise schedule as consistent as possible.

3.2.5. Experimental procedures

For the main visits, participants reported to the lab in the afternoon after observing at least a 3-h fast and baseline tests were performed. Participants were asked to provide a mid-stream urine sample to determine hydration status by measuring urine specific gravity (USG) using a clinical refractometer (Atago 2773 MASTER-SUR/NM Clinical Refractometer, Atago Co., LTD., Japan). If found dehydrated

(i.e. USG > 1.020) participants consumed 200 ml of water at every trial to maintain consistency in testing procedure.

Table 3.2 Dietary intake 24 h before experimental visits

	Placebo	NZBC
Carbohydrate (g/day)	284.5 ± 38.5	276.9 ± 52.2
Protein (g/day)	138.0 ± 32.5	145.4 ± 31.7
Fat (g/day)	107.0 ± 29.8	117.5 ± 34.1
Total energy intake (kcal/day)	2757.0 ± 398.9	2845.0 ± 464.4
Total energy intake (kJ/day)	11535.3 ± 95.3	11903.5 ± 111.0

Data reported as mean ± SD from 10 participants (incomplete data from 4 participants)

3.2.6. Cardiovascular measures

Participants were asked to remain in a supine position for assessment of blood pressure and heart rate (Omron, Healthcare CO. Ltd.; Kyoto, Japan) and stroke volume and systemic vascular resistance (Uscom 1A, Uscom Ltd, Sydney, Australia). The assessment was done at baseline and at 1-h after study treatments or samples were consumed.

3.2.7. Intervention

After completing baseline cardiovascular measures, participants consumed either 12 g of NZBC powder or placebo mixed with 100 ml of water. The placebo (PLA) contained the same quantity of maltodextrin as NZBC powder with added fructose, glucose, and colour to match the nutritional value and apperance of NZBC powder (Table 3.3). Both placebo and the study NZBC powder were supplied by the study sponsor. Appendix 2.11 describes the randomisation for the study.

Table 3.3 Nutritional value of the NZBC powder and placebo (12 g)

	NZBC	Placebo
Energy (kJ)	188	188
Energy (kcal)	45	45
Total carbohydrates (g)	10.3	10.3
Total added sugar (g)	4.5	10.3
Vitamin C (mg)	70	0
Anthocyanins (mg)	120	0

* % Daily Values are based on a 2000 calorie diet; n/a Daily Value not established
Other ingreidents: Maltodextin, monk fruit extract, natural flavours (No added colors or preservatives)

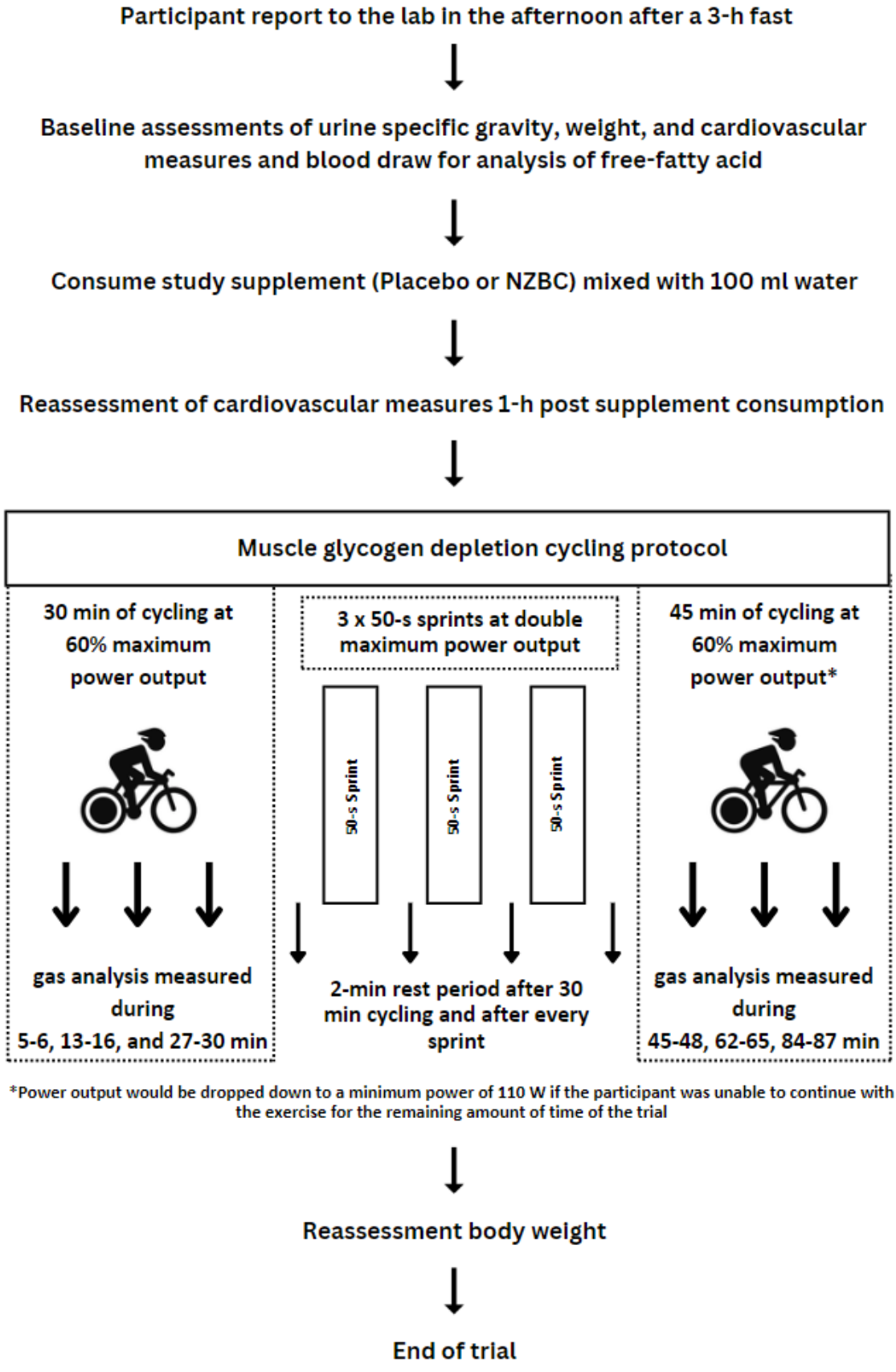

Figure 3.1 Study design to evaluate the effect of NZBC (containing 120 mg of anthocyanins) on substrate oxidation during moderate-intensity cycling trial.

3.2.8. Blood dispensing and analysis

An 18-gauge, 1.3-mm intravenous cannula (Insyte, Becton Dickson, NJ, USA) was inserted into a medial antecubital vein of the non-dominant hand of the participant. Blood samples were taken at baseline, 1-h post supplement, before starting sprint phase of cycling, and then immediately after completing the 90-min cycling protocol. Regular flushing with saline (0.9% sodium chloride, Fresenius Kabi, Auckland, New Zealand) was used to keep the cannula free from blood clots. Two millilitres of blood sample was collected in the serum tube. The sample was allowed to clot for 20 min before being centrifuged for 10 min at 1250 × g (MF 50, Hanil Science Industrial Co. Ltd, Korea). The serum sample was later stored at −80°C for analysis of free fatty acids (FFA).

Serum FFA were analysed using the Randox non esterified fatty acids kit (FA 115) (Randox Laboratories Limited, Japan) according to the manufacturer's instructions in a clinical chemistry analyser (RX Daytona plus, Randox Laboratories Limited, Japan) at the Nutrition Laboratory, School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand.

3.2.9. Cycling exercise protocol

The exercise protocol was designed to reduce the glycogen content in both type I and type II muscle fibres and was based on a model suggested by Vøllestad et al. (1992) (Figure 3.1). Subjects initially performed 30 min of exercise at an intensity close to 60% of their maximum power output. Following a 2-min rest, subjects then performed three 50-s 'sprints' at double the resistive load, with 2 min of rest between each sprint. After another 2 min of rest, they were required to cycle for a further 45 min at 60% maximum power output to further reduce glycogen. They were allowed to drop the intensity to a maximum of 110 W over the 45-min period if they were unable to maintain their prescribed intensity of 60% of maximum power output (Bowtell et al., 1999). The participants were provided with 2 ml/kg BM of water before and after every 15 min of exercise to offset severe dehydration (Figure 3.1).

3.2.10. Gas analysis

Expired gas was collected three times each before and after the 3 x 50-s sprints using online gas analysis. The first sample was collected for 2 min, and the five subsequent times were collected for 6 min each. The gas samples were analysed for minute ventilation, oxygen consumption, and carbon dioxide production. Values of energy expenditure were calculated based on the following equation by Elia and Livesey (1992) and were normalised by body weight and minute:

$$\text{Energy Expenditure (kcal)} = 3.815 + 1.232 \times \text{Respiratory Exchange Ratio (RER)}$$

The partitioning between carbohydrate and fat oxidation was based on the RER table from (Lusk, 1924).

3.2.11. Perceptual measures

Perceptual measures were assessed using the feeling scale (FS), felt arousal scale (FAS), and ratings of perceived exertion (RPE) scale throughout the 90-min cycling trial. The FS that assess responses towards a particular feeling, is a 11-point scale ranging from very bad (− 5), bad (-3), fairly bad (-1), neutral (0) to fairly good (+ 1), and good (+ 3), and very good (+ 5) (Hardy & Rejeski, 1989). The FAS measures perceived activation along a 6-point scale, ranging from low arousal (1 point) indicating boredom or calmness to high arousal (6 points) suggested by anger or anxiety (Svebak & Murgatroyd, 1985). The RPE is a 15-point scale to assess subject level of exertion intensity. The score ranges from 6 to 20, with 6 being no exertion at all to 20 being maximum exertion (Borg, 1982).

3.2.12. Statistical analysis

Due to equipment failure, data from 11 out of 14 participants was collected and analysed. The data were compared using a two-way ANOVA with repeated measures (IBM SPSS Statistic (v. 28.0.1, IBM, USA) to examine main effects of i) treatment and ii) time and iii) interaction of treatment*time. Bonferroni's pairwise comparisons with 95% confidence were conducted to identify specific differences between groups and timepoints. Differences were considered significant at an alpha level of $p < 0.05$. To determine the effect size of responses, partial eta^2 (η^2) was also calculated using SPSS. The threshold values for effect size were interpreted as small (0.01), medium (0.06), and large effects (0.14). Data is presented as mean ± SD.

3.3. Results

3.3.1. Substrate oxidation during cycling

Oxygen consumption increased over time with both PLA and NZBC intake (p = 0.013) but was not different between treatments (p = 0.469). There was a gradual increase in energy expenditure followed by a decrease at the 60-min time-point in both PLA and NZBC trials (p = 0.014). However, there was no effect of treatment and interaction effect for treatment*time for oxygen consumption and energy expenditure (Table 3.4, Figure 3.2).

Fat oxidation and % fat oxidation increased over time with a matched decrease in carbohydrate oxidation, % carbohydrate oxidation, and respiratory exchange ratio (RER) with both PLA and NZBC intake (p < 0.001), however, they were not different between treatments (p = 0.345, p = 0.344, p = 0.288, p = 0.344, and p = 0.477). There was no interaction effect for treatment*time for fat oxidation, carbohydrate oxidation, and respiratory exchange ratio (Table 3.4, Figure 3.3).

Table 3.4 Oxygen consumption, substrate oxidation, & energy expenditure during 90-min cycling protocol after a single dose of placebo and NZBC powder.

		Before 3 x 50-s sprints			After 3 x 50-s sprints			Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *Time (*p* value and effect size)
		5-6 min	13-16 min	27-30 min	45-48 min	62-65 min	84-87 min			
Oxygen consumption ($ml \cdot min^{-1} \cdot kg^{-1}$)	PLA	29.3 ± 2.5	30.2 ± 2.4	31.0 ± 2.5	32.7 ± 2.18	29.6 ± 2.41	27.9 ± 3.6	$p = 0.469$ $\eta^2 = 0.060$	**$p = 0.013$** $\eta^2 = 0.427$	$p = 0.625$ $\eta^2 = 0.050$
	NZBC	28.7 ± 2.0	29.6 ± 1.9	30.0 ± 1.9	31.8 ± 2.06	29.0 ± 1.98	27.6 ± 2.7			
Respiratory exchange ratio	PLA	1.14 ± 0.13	1.09 ± 0.12	1.07 ± 0.12	0.99 ± 0.09	0.99 ± 0.11	0.98 ± 0.11	$p = 0.477$ $\eta^2 = 0.058$	**$p < 0.001$** $\eta^2 = 0.904$	$p = 0.445$ $\eta^2 = 0.047$
	NZBC	1.12 ± 0.08	1.08 ± 0.08	1.05 ± 0.08	0.97 ± 0.06	0.98 ± 0.08	0.97 ± 0.09			
Energy expenditure ($kcal \cdot kg^{-1} \cdot min^{-1}$)	PLA	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.02	$p = 0.317$ $\eta^2 = 0.111$	**$p = 0.014$** $\eta^2 = 0.421$	$p = 0.628$ $\eta^2 = 0.047$
	NZBC	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.14 ± 0.01			
Fat oxidation (kcal/min)	PLA	0.06 ± 0.12	0.32 ± 0.46	0.69 ± 0.89	2.10 ± 1.73	2.11 ± 1.83	2.27 ± 1.91	$p = 0.345$ $\eta^2 = 0.099$	**$p < 0.001$** $\eta^2 = 0.698$	$p = 0.500$ $\eta^2 = 0.069$
	NZBC	0.13 ± 0.21	0.39 ± 0.58	0.85 ± 1.11	2.44 ± 1.27	2.25 ± 1.99	2.62 ± 1.98			
Carbohydrate oxidation (kcal/min)	PLA	11.54 ± 1.06	11.48 ± 1.03	11.36 ± 1.29	10.40 ± 2.21	9.30 ± 2.68	8.46 ± 2.69	$p = 0.288$ $\eta^2 = 0.124$	**$p < 0.001$** $\eta^2 = 0.672$	$p = 0.524$ $\eta^2 = 0.064$
	NZBC	11.19 ± 0.77	11.20 ± 0.85	10.84 ± 1.36	9.74 ± 1.42	8.94 ± 2.43	7.98 ± 1.77			
Fat oxidation (%)	PLA	0.5 ± 1.1	2.7 ± 3.8	5.6 ± 7.3	16.9 ± 14.26	19.1 ± 17.9	21.56 ± 19.2	$p = 0.344$ $\eta^2 = 0.100$	**$p < 0.001$** $\eta^2 = 0.649$	$p = 0.511$ $\eta^2 = 0.067$
	NZBC	1.2 ± 1.9	3.3 ± 4.8	7.3 ± 9.7	20.0 ± 10.51	20.6 ± 19.0	23.94 ± 16.7			
Carbohydrate oxidation (%)	PLA	99.5 ± 1.1	97.3 ± 3.8	94.4 ± 7.3	83.1 ± 14.26	80.9 ± 17.9	78.44 ± 19.2	$p = 0.344$ $\eta^2 = 0.100$	**$p < 0.001$** $\eta^2 = 0.649$	$p = 0.511$ $\eta^2 = 0.067$
	NZBC	98.9 ± 1.9	96.7 ± 4.8	92.7 ± 9.7	80.0 ± 10.51	79.4 ± 19.0	76.06 ± 16.7			

PLA, Placebo; NZBC, New Zealand blackcurrant. Values are mean ± SD. *n* = 11 (due to equipment failure)

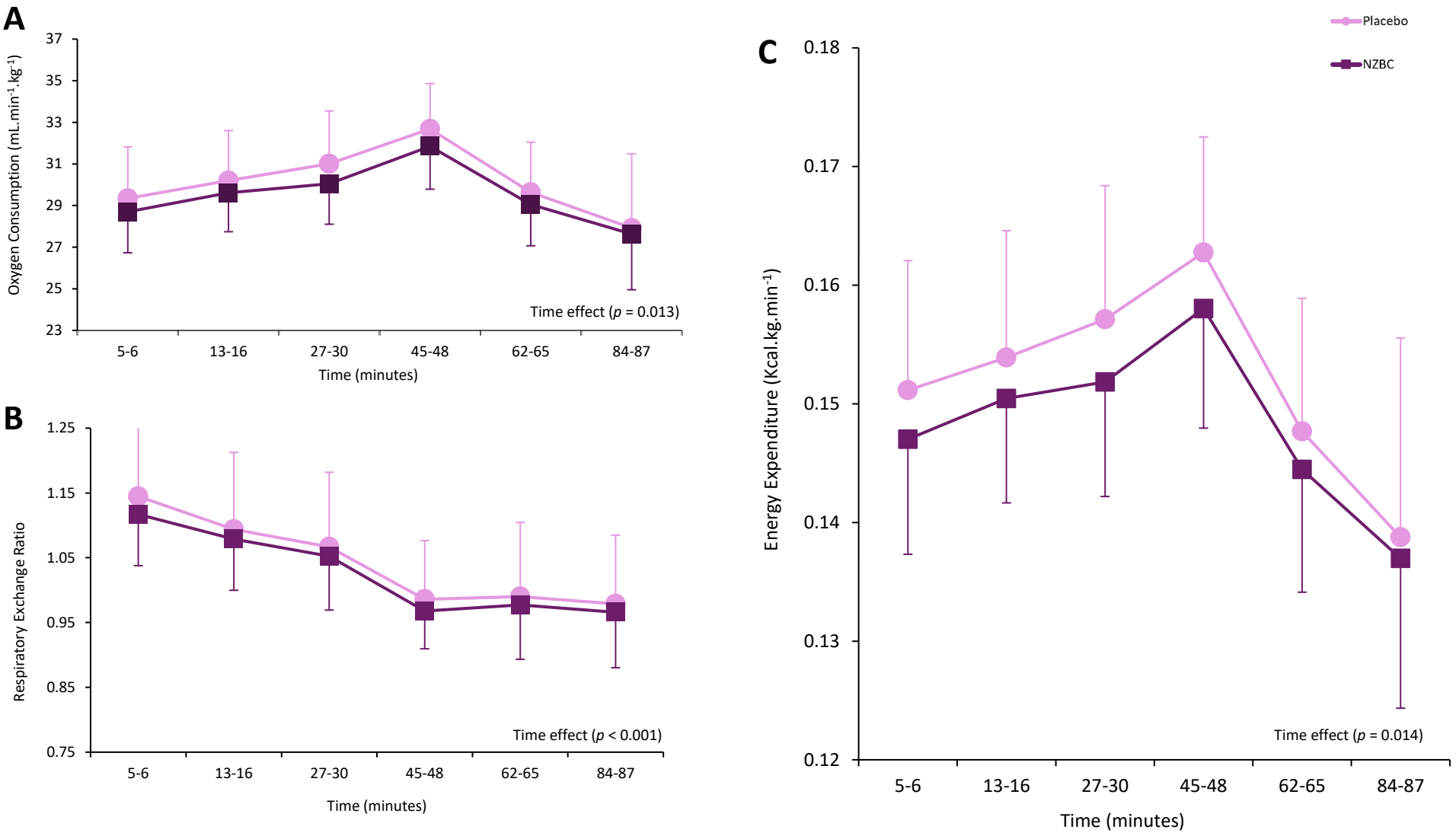

Figure 3.2 (A) Oxygen consumption, (B) respiratory exchange ratio, and (C) energy expenditure during 90 min of cycling following a single dose of placebo and NZBC powder. Values are presented as mean ± SD. *n* = 11

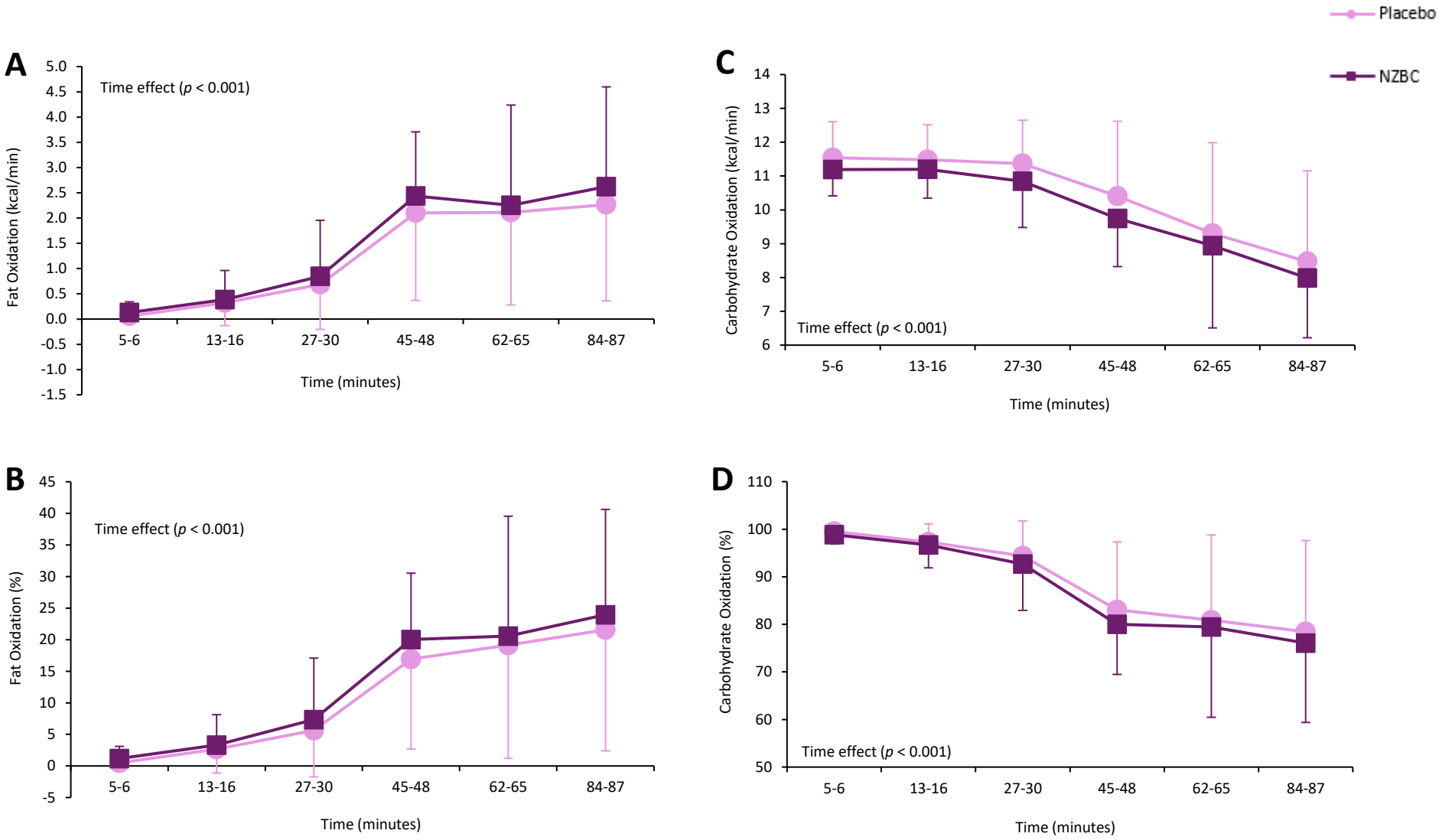

Figure 3.3 (A) Fat oxidation (kcals/min), (B) Fat oxidation (%), (C) carbohydrate oxidation (kcals/min), and (D) carbohydrate oxidation (%) during 90 min of cycling following a single dose of placebo and NZBC powder. Values are presented as mean ± SD. *n* = 11

3.3.2. Free fatty acid concentration

Serum FFA concentration increased with time during cycling ($p < 0.001$) and was not different between treatments ($p = 0.113$). No interaction effect for treatment*time was observed (Table 3.5, Figure 3.4).

Table 3.5 Serum free-fatty acid concentration (mmol/l) at before, during, and immediately post cycling after a single dose of placebo and NZBC powder.

		Baseline	1-h post intake	Before sprints	Post cycling	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *Time (*p* value and effect size)
Serum free-fatty acid (mmol/l)	PLA	0.29 ± 0.17	0.21 ± 0.10	0.35 ± 0.26	0.90 ± 0.46	$p = 0.113$	**$p < 0.001$**	$p = 0.242$
	NZBC	0.31 ± 0.25	0.23 ± 0.13	0.46 ± 0.28	1.13 ± 0.61	$\eta^2 = 0.182$	$\eta^2 = 0.828$	$\eta^2 = 0.104$

PLA, Placebo; NZBC, New Zealand blackcurrant. Values are mean ± SD.

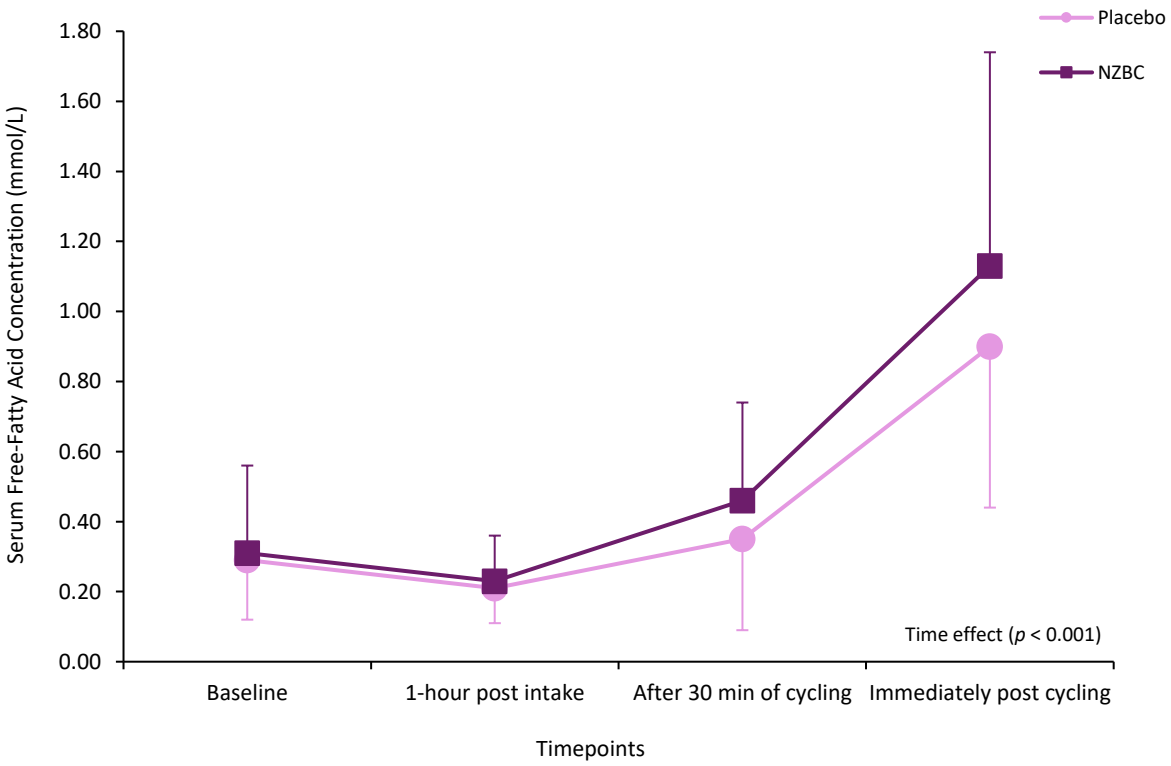

Figure 3.4 Serum free-fatty acid concentration (mmol/l) at baseline, 1-h post intake, before starting 3 x 50 sec sprints and immediately post cycling after a single dose of placebo (PLA) or NZBC powder. Values are presented as mean ± SD. *significantly higher in NZBC group compared to PLA, effect of treatment ($p = 0.035$).

3.3.3. Heart rate

Heart rate increased over time ($p < 0.001$) during cycling, however it was not different with the intake of NZBC and PLA. Additionally, no interaction effect for treatment*time was observed for both treatments (Table 3.6).

Table 3.6 Heart rate before and during 90-min cycling protocol after a single dose of placebo and NZBC powder.

	Treatment	Baseline	1-h post intake	During warm up	During 30 min of cycling	During sprints	During 45 min of cycling post sprints	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *Time (*p* value and effect size)
Heart rate (bpm)	PLA	61.0 ± 5.3	56.5 ± 4.9	78.6 ± 7.3	140.6 ± 12.3	137.7 ± 12.9	148.5 ± 9.3	$p = 0.388$	$\mathbf{p < 0.001}$	$p = 0.497$
	NZBC	60.7 ± 7.1	55.5 ± 6.1	77.5 ± 6.3	138.8 ± 11.7	140.1 ± 10.2	145.4 ± 13.7	$\eta^2 = 0.108$	$\eta^2 = 0.976$	$\eta^2 = 0.104$

PLA, Placebo; NZBC, New Zealand blackcurrant. Values are mean ± SD.

3.3.4. Perceptual measures

3.3.4.1. Feeling scale (FS) and felt arousal scale (FAS)

Both FS and FAS changed with time ($p < 0.001$) with no significant differences with the intake of NZBC and PLA. However, there was a trend for FS to be lower with PLA intake ($p = 0.068$, $\eta^2 = 0.234$). There was no interaction effect of treatment*time observed for both FS and FAS with the intake of NZBC and PLA (Table 3.7).

The FS and FAS values were also plotted in a circumplex model providing a visual description of changes in affect/pleasure and arousal throughout the trial (Figure 3.5). In both PLA and NZBC trial, participants' data indicated they were in the "low arousal and pleasure" quadrant before cycling and moved to "high arousal and pleasure" quadrant 30 min into cycling. However, by the end of the cycling protocol, participants were in the state of "high arousal and displeasure" for both groups (Figure 3.7).

Table 3.7 Feeling scale (affect or pleasure) and felt arousal scale ratings (arousal or activation) before and during 90-min mixed-intensity cycling in placebo and NZBC trials.

		1-h post Intake	30-min after cycling	Post sprints	45-min after sprints	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *Time (*p* value and effect size)
Feeling scale (+5 to -5)	PLA	2.3 ± 1.0	0.9 ± 1.6	-1.1 ± 2.1	-0.4 ± 1.8	$p = 0.068$	**$p < 0.001$**	$p = 0.292$
	NZBC	2.5 ± 1.2	0.6 ± 1.6	-1.4 ± 1.8	-1.3 ± 1.8	$\eta^2 = 0.234$	$\eta^2 = 0.631$	$\eta^2 = 0.090$
Felt arousal scale (1 to 6)	PLA	2.7 ± 0.8	3.9 ± 0.9	4.6 ± 1.4	3.9 ± 1.1	$p = 0.940$	**$p < 0.001$**	$p = 0.318$
	NZBC	2.5 ± 0.8	3.7 ± 1.0	4.7 ± 1.5	4.2 ± 1.0	$\eta^2 = 0.000$	$\eta^2 = 0.586$	$\eta^2 = 0.085$

PLA, Placebo; NZBC, New Zealand Blackcurrant. Values are means ± SD.

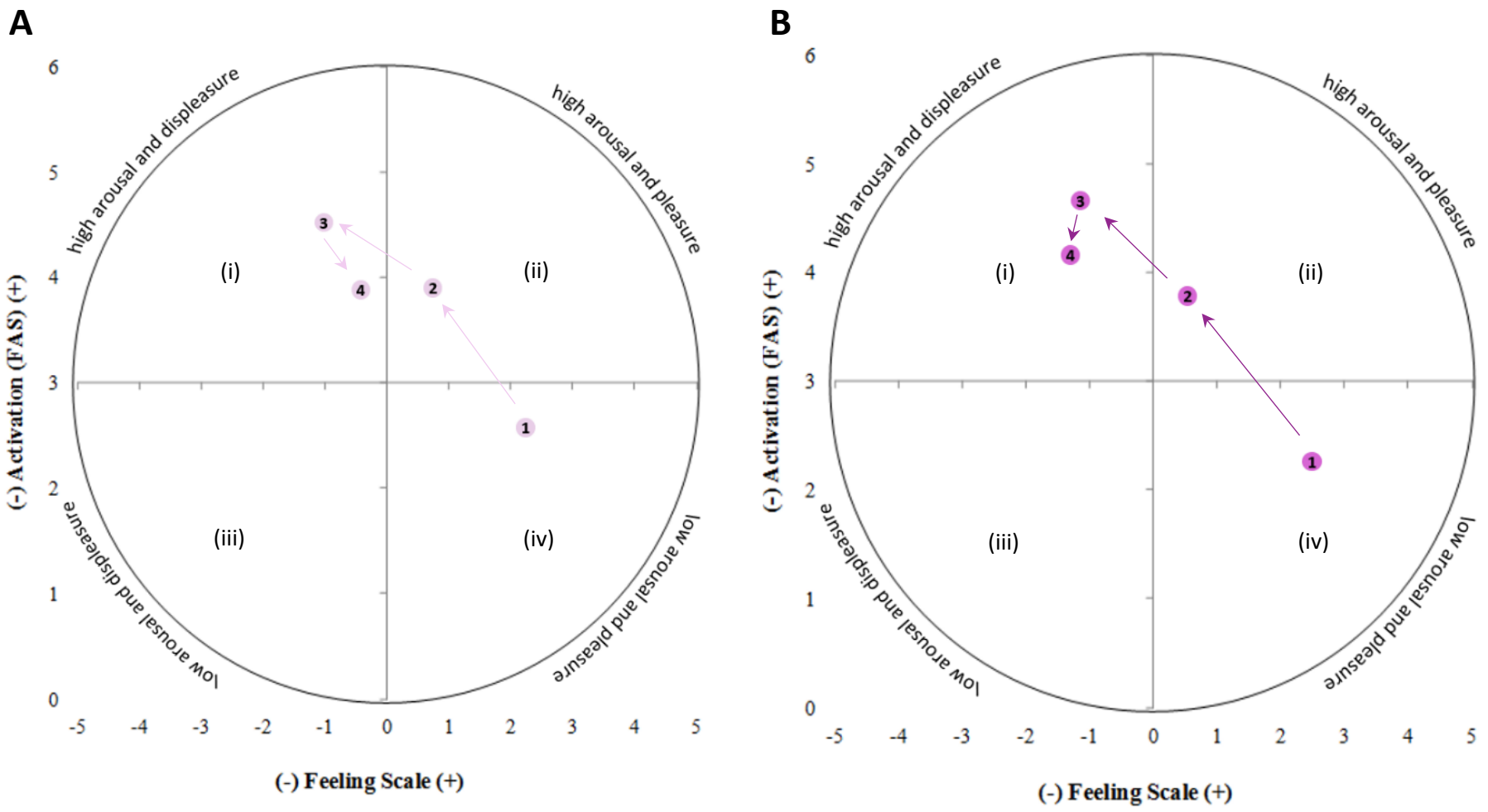

Figure 3.5 Circumplex model of affect in (A) Placebo and (B) NZBC trials based on feeling scale and felt arousal scale ratings (1) 1-hour post supplement, (2) 30 min after cycling, (3) post 3 x 50-s sprints, and (4) 45 min after sprints. Roman numerals indicate various activation and pleasure states: (i) high arousal and displeasure (e.g. anger); (ii) high arousal and pleasure (e.g. vigour); (iii) low arousal and displeasure (e.g. boredom or fatigue); (iv) low arousal and pleasure (e.g. calm).

3.3.4.2. Ratings of perceived exertion (RPE)

Ratings of perceived exertion were the lowest post-warm up and increased over time during cycling (p < 0.001); however there was no difference with the intake of PLA and NZBC (p = 0.258). No interaction effect of treatment*time was observed (Table 3.8, Figure 3.6).

Table 3.8 Ratings of perceived exertion before, during and after the 90-min cycling protocol after a single dose of placebo and NZBC powder.

		Post warm-up	30 min after cycling	Post sprints	45 min after sprint	Effect of treatment (p value and effect size)	Effect of time (p value and effect size)	Effect of treatment *Time (p value and effect size)
Ratings of perceived exertion (1 to 20)	PLA	7.8 ± 1.4	13.1 ± 1.3	17.8 ± 1.1	14.9 ± 1.6	p = 0.258	**p < 0.001**	p = 0.448
	NZBC	8.0 ± 1.4	13.7 ± 1.1	17.5 ± 1.2	15.3 ± 1.7	η^2 = 0.097	η^2 = 0.955	η^2 = 0.060

PLA, Placebo; NZBC, New Zealand blackcurrant. Values are mean ± SD.

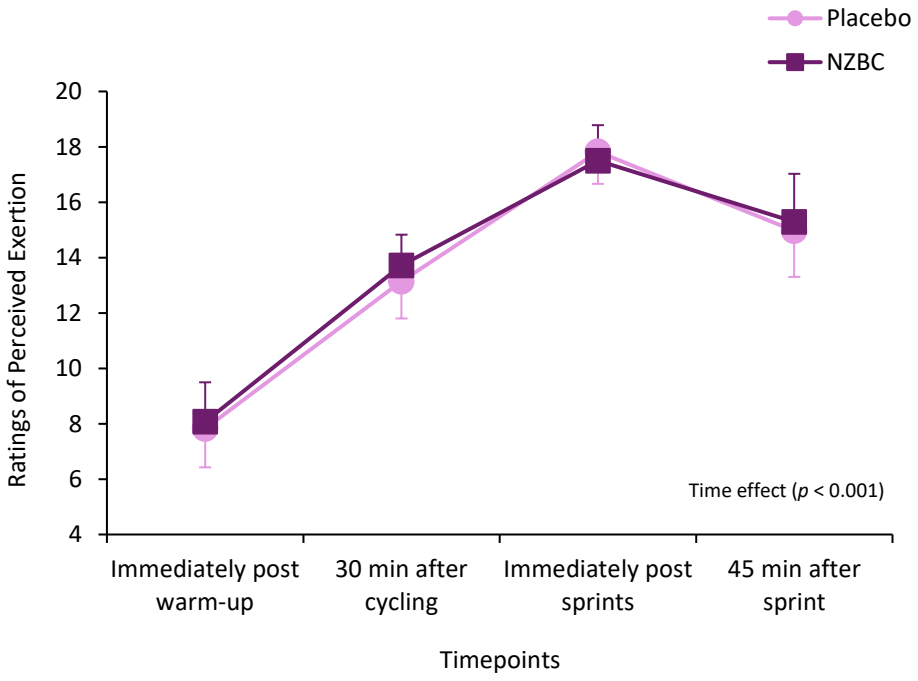

Figure 3.6 Ratings of perceived exertion before and during 90 min cycling after a single dose of placebo or NZBC powder. Values are presented as mean ± SD.

4.3.5. Cardiovascular measures at rest

Systolic blood pressure, diastolic blood pressure, and stroke volume did not change with NZBC and PLA intake (p = 0.625, p = 0.625, and p = 0.984). Systemic vascular resistance increased from baseline to 1-h post beverage intake (p = 0.009), however was not different between with the intake of NZBC and PLA (p = 0.939; Table 3.9).

Table 3.9 Cardiovascular measures at baseline and 1-h post intake of a single dose of placebo or NZBC.

		Baseline	1-h post intake	Effect of treatment (p value and effect size)	Effect of time (p value and effect size)	Effect of treatment *Time (p value and effect size)
Systolic blood pressure (mmHg)	PLA	123.0 ± 8.1	121.36 ± 7.64	p = 0.625	p = 0.621	p = 0.563
	NZBC	121.8 ± 6.9	121.54 ± 8.19	η^2 = 0.019	η^2 = 0.019	η^2 = 0.026
Diastolic blood pressure (mmHg)	PLA	74.9 ± 6.6	75.43 ± 6.74	p = 0.625	p = 0.621	p = 0.563
	NZBC	74.2 ± 5.7	74.21 ± 6.02	η^2 = 0.019	η^2 = 0.019	η^2 = 0.026
Stroke volume (ml)	PLA	75.4 ± 12.3	72.4 ± 15.3	p = 0.984	p = 0.305	p = 0.612
	NZBC	74.6 ± 9.9	73.2 ± 13.2	η^2 = 0.000	η^2 = 0.081	η^2 = 0.020
Systemic vascular resistance (dynes·sec·cm^{-5})	PLA	1814.4 ± 405.0	2111.9 ± 550.8	p = 0.939	**p = 0.009**	p = 0.489
	NZBC	1861.4 ± 343.5	2077.1 ± 489.9	η^2 = 0.000	η^2 = 0.418	η^2 = 0.038

PLA, Placebo; NZBC, New Zealand blackcurrant. Values are mean ± SD.

3.4. Discussion

This was the first study to evaluate the effects of an acute intake of NZBC containing 120 mg anthocyanins on substrate metabolism and FFA concentration during a 90-min mixed-intensity cycling protocol. The study found that the intake of a single dose of NZBC did not influence substrate oxidation and serum FFA concentration during mixed -intensity cycling in recreationally active males.

The study observed no differences in fat oxidation, carbohydrate oxidation, and respiratory exchange ratio (RER) during exercise, with the intake of NZBC. The RER was high at the beginning of the trial and stayed consistently high throughout the trial with both NZBC and PLA intake. It is possible that the CHO content of the meal consumed 3 h prior to reporting to the laboratory and of the PLA and NZBC powder (10 g) ingested 1 h before exercising, affected the RER values throughout the trial. The intake of CHO 3-4 h prior to exercise can blunt fatty acid oxidation during the exercise in detriment of CHO, and increase the RER values (Ormsbee et al., 2014; Rothschild et al., 2022). This could be due to the inhibitory effects of insulin on lipolysis, and stimulation of glucose uptake and oxidation (Ormsbee et al., 2014; Rothschild et al., 2022), which persist even after the insulin levels return to basal values before exercise (Coyle et al., 1985). Thus, masking the effect of NZBC intake on fat oxidation and leading to higher RER throughout the trial. This may have also interfered with the increase in reliance of fat oxidation as the trial progressed. Furthermore, the muscle glycogen depletion protocol used in our study could have been too anaerobically intense for our recreationally active participants as ~80% of them were football players, unaccustomed to mixed-intensity intermittent cycling. Intense bouts of activity have been shown to increase the reliance on anaerobic metabolism and lead to acidosis, promoting the formation of non-oxidative CO_2 when H^+ is buffered by HCO_3^-. This could have also increased the RER values (Jeukendrup & Wallis, 2005).

In contrast, four studies reported a significant increase in fat oxidation during moderate-intensity exercise in trained and recreationally active individuals following the consumption of 300, 600, and 900 mg/day NZBC (containing 105, 210, and 315 mg of anthocyanins) for 7 days in both fed (breakfast 2-h prior to exercise) and fasted states (Cook et al., 2015; Cook, Myers, Gault, Edwards, et al., 2017; Hiles et al., 2020; Strauss et al., 2018). Therefore, it is possible that a 7-day intake of NZBC is necessary to increase fat oxidation during moderate and mixed-intensity exercise.

Furthermore, our results did not report an increase in serum FFA concentration with 12 g of NZBC intake (containing 120 mg anthocyanins) during exercise. This result is inconsistent in comparison to the increase in the pre-exercise serum FFA concentration reported by Strauss et al. (2018) following the intake of 600 mg/day of NZBC for 7 days (210 mg anthocyanins/day). However, the comparison of our study to that of Strauss et al. (2018) is limited as Strauss et al. (2018) only evaluated serum FFA

concentration before exercise. Strauss et al. (2018) also observed a 27% increase in fat oxidation rates and reported that serum FFA concentrations were moderately associated with mean average rates of fat oxidation during exercise. The authors speculated that the intake of NZBC for 7 days may have enhanced fat oxidation by influencing the genes involved in lipid metabolism (Tsuda et al., 2005). Several animal studies have shown an increase in fat metabolism following blackcurrant supplementation (Benn et al., 2014; Tsuda et al., 2005). For example, chronic blackcurrant extract intake in mice (0.1% wt/wt for 12 weeks) has been shown to elevate mRNA of genes involved with energy expenditure including peroxisome proliferator-activated receptor alpha, resulting in increased fat metabolism (Benn et al., 2014). Similarly, Tsuda et al. (2005) observed an up-regulation of genes associated with lipid metabolism such as hormone-sensitive lipase and increased lipolysis activity by the treatment of adipocytes with the cyanidin 3-glucoside or cyanidin. Further assessment of plasma glycerol, β-hydroxybutyrate, and catecholamines may provide insights into the effect of NZBC extract consumption on fatty acid metabolism during cycling (following several days of supplementation).

Our study also analysed the effect of NZBC intake on cardiovascular measures at rest as 300 mg/day of NZBC intake (containing 105 anthocyanins) for 7 days has previously shown to increase stroke volume and cardiac output by 25% and 26%, respectively, and decrease the total peripheral resistance at rest by 16% (Cook et al., 2015; Cook, Myers, Gault, Edwards, et al., 2017; Strauss et al., 2018). Acute intake of NZBC has also shown to increase peripheral blood flow by 22% at rest (Matsumoto et al., 2005), thus allowing for improved nutrient delivery and removal of by-products of metabolism. However, in the present study, systolic and diastolic blood pressure, stroke volume, and systemic vascular resistance did not change following acute NZBC intake. It is possible that a 7-day loading period is needed to elicit the vasodilatory effects of NZBC anthocyanins via an increase in nitric oxide production in addition to a decrease in total peripheral resistance, as observed by Willems et al. (2015).

This was also the first study to evaluate the effect of NZBC intake on perceptual measures during cycling. Perceived exertion plays a significant role in the ability of an individual to work at greater and/or prolonged intensity, and thus influences subsequent performance (Davis & Green, 2009; Lieberman, 2001). We observed that RPE was lowest post-warm up and increased significantly during cycling, however, was not different between the PLA and NZBC groups. As RPE does not give any indication of the participant's other emotions such as "feeling good" or "feeling bad" during exercise and it does not estimate the participant's perceived activation (arousal), we also assessed pleasure or displeasure felt during exercise, and changes in perceived arousal throughout exercise. However, there was no difference in affect or arousal with NZBC and PLA intake. It is possible that because NZBC

is not a central nervous stimulant, it did not influence RPE and affect and arousal status during exercise.

3.5. Limitations and future directions

The CHO content of the meal consumed by the participants' 3 h prior to reporting to the laboratory may have influenced fat oxidation and serum FFA concentration during exercise. Furthermore, we cannot rule out the interference by the low CHO content of the study drinks (both NZBC and PLA) on substrate oxidation during the trial. This research study assessed only serum FFA concentration as an indicator of fat metabolism, when additional assessments of plasma glycerol and β-hydroxybutyrate could have provided a more comprehensive understanding of the effects of acute NZBC consumption on lipolysis and fatty acid oxidation.

3.6. Conclusion

Ingestion of a single drink of reconstituted NZBC powder containing 120 mg anthocyanins had no effect on substrate oxidation and serum FFA concentration during mixed-intensity cycling in recreationally active males. More research is needed to (i) compare the effect of acute and chronic intake of NZBC, (ii) compare different types of NZBC supplements (extracts and powder form) with and without carbohydrates and lastly, (iii) understand the underlying mechanism of action of NZBC intake on substrate and fat metabolism during mixed-intensity exercise.

Chapter 4
Individual and Additive Effects of New Zealand Blackcurrant Powder and Caffeine Intake on High-Intensity Intermittent Running Performance

Abstract

Background: Consumption of New Zealand blackcurrant in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC) for 7 days has shown to reduce slowing of sprint speed in recreationally active males. Caffeine, on the other hand when consumed acutely, has shown to improve repeated-sprint performance in a fatigued state. Thus, combining NZBC and caffeine intake could potentially provide an additive or a synergistic effect to improve repeated-sprint performance, especially when fatigued.

Aim: To compare the individual and additive effects of a single dose of NZBC powder (12 g, 240 mg of anthocyanins) and caffeine (240 mg) intake on sprint performance using the modified Loughborough Intermittent Shuttle Test (m-LIST) in a fatigued state. We hypothesised that a single dose of NZBC with caffeine could lead to reduced slowing of sprint speed during blocks 5 and 6 of the m-LIST protocol.

Methods: Fourteen recreationally active males participated in a double blind, randomised controlled crossover trial consisting of four experimental arms: placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF) in a fatigued state. Participants reported to the laboratory fasted and consumed a standardised breakfast with the study drink 1-h before the exercise trial. Speed and distance covered for walking, sprinting, running, and jogging were assessed during m-LIST. Blood lactate and free fatty acids (FFA) levels, and perceptual measures (feeling scale, FS; felt arousal scale, FAS; and ratings of perceived exertion, RPE) were also assessed.

Results: There was an effect on treatment on average sprint speed (p = 0.049), with the intake of NZBC-CAFF sustaining a higher average sprint speed compared to NZBC during the m-LIST protocol (p = 0.025). Furthermore, there was an increase in serum FFA concentration across all treatments (p < 0.001) during the m-LIST protocol, however, NZBC-CAFF was the only treatment that showed a sustained increase in the serum FFA levels 1-h post m-LIST protocol (p = 0.042 and η^2= 0.214). There was a trend with a large effect size for an effect of treatment on RPE (p = 0.053 and η^2 = 0.206) and the values of RPE for CAFF were lower than PLA (p = 0.045).

Conclusion: The findings revealed no significant ergogenic benefits from either NZBC or caffeine, although results suggested that caffeine may help sustain higher sprint speeds. The lack of positive results aligns with previous research, indicating that longer-term NZBC intake (e.g. 7 days supplementation) may be necessary for enhancement of performance. Further research should investigate the effects of NZBC-CAFF on lipolysis and fat oxidation.

Keywords: Polyphenols, repeated sprints, sports, nutrition

4.1. Introduction

High-intensity intermittent running is essential for success in field-based sports such as European football, field hockey, and rugby. This type of exercise consists of alternating periods of short-duration sprints (≤10 s, high-intensity) and brief active recovery periods (≤ 60 s, moderate-to-low-intensity) (Glaister, 2005). Sprinting (>25.1 km/h) and high-speed running (19.8 – 25.1 km/h) in European football has significantly increased over the last 15 years and it accounts for approximately 1-3% and 7-11% of the total distance covered during a match (Chmura et al., 2018). Moreover, sprints are most frequent in the first 30 min and the last 15 min of the game (Oliva-Lozano et al., 2023) with a disproportionate number of goals scored in the last 15 min of match play (Reilly & Williams, 2003). Straight sprinting performed either by the scorer or the assisting player has been identified as the most frequent action just before scoring a goal (Faude et al., 2012; Martínez-Hernández et al., 2023). Therefore, the ability to reduce fatigue to maintain the repeated-sprint performance, especially in the last 15 min of the game may substantially affect the outcome of the game.

Fatigue during repeated-sprint exercise typically develops rapidly after the first sprint (Mendez-Villanueva et al., 2008). This is caused by various factors such as the generation of an inadequate motor command in the motor cortex (i.e. neural factors) and the accumulation of metabolites within muscle fibres (i.e. muscular factors) (Girard et al., 2011). Research has shown that consumption of ergogenic aids such as caffeine (Glaister et al., 2008) and New Zealand blackcurrant in the form of juice concentrate, powdered juice concentrate, powdered whole fruit, and powdered capsulated anthocyanins extract (NZBC) (Willems et al., 2016) can improve repeated-sprint performance.

The individual effects of NZBC and caffeine intake on high-intensity running performance have previously been assessed using the Loughborough Intermittent Shuttle Test (LIST). The LIST protocol is designed to replicate the activity pattern such as distance covered and sprint speed during a football game (Nicholas et al., 2000), along with physiological and metabolic responses that closely relate to the $\dot{V}O_{2max}$ capacity of football players (Jones et al., 2013). During the LIST, fatigue may be caused by limitations in energy supply, which include energy available from phosphocreatine hydrolysis, anaerobic glycolysis and oxidative metabolism, and a decrease in muscle excitability by the accumulation of interstitial potassium ions (Girard et al., 2011).

Consuming 300 mg/day of NZBC (containing 105 mg of anthocyanins) for 7 days with the last dose 3-h before exercise has shown to reduce the slowing of the fastest sprint between block 1 and 5 during part A and a 15% increase in time to exhaustion (8 out of 13 participants) during part B of the LIST protocol (Willems et al., 2016). Similarly, caffeine intake of 3.7 mg/kg when combined with a 6%

carbohydrate-electrolyte solution has also shown to decrease the slowing of the repeated-sprints in the last three blocks of the LIST (modified protocol) (Gant et al., 2010).

Caffeine acts through both peripheral and central mechanisms (Domínguez et al., 2021) by binding to the adenosine receptors and blocking their inhibitory neurophysiological action, and thus provides a stimulatory effect on the central nervous system (Graham, 2001). However, the mechanism of action of NZBC intake on improvements in sport performance is yet to be fully elucidated. A review examining the intake of dietary anthocyanins (cherries and NZBC) and sports performance speculated that the performance improvements in athletes may be caused by the vasoactive properties of anthocyanins (Cook & Willems, 2019). Anthocyanins have the property to increase the endothelial nitric oxide activity and subsequently enhance blood flow in the muscles (Cook & Willems, 2019). Therefore, it is possible that intake of NZBC before exercise can influence blood flow during the brief recovery periods of the high-intensity intermittent running protocol. The increase in blood flow can benefit sprint performance by allowing higher phosphocreatine resynthesis and improved removal of metabolites such as hydrogen ions, inorganic phosphate and adenosine diphosphate that contribute to fatigue (Godwin et al., 2017).

Training for field sports such as rugby often takes place less than 48 h post-match and athletes are expected to train for two or more consecutive days during a week (Baker, 2001). It is likely excessive training with inadequate recovery periods can lead to accumulated fatigue, compromise neuromuscular performance (McLean et al., 2010; Pointon & Duffield, 2012; Webb et al., 2013), and cause under-performance on match-day (Johnston, Gabbett, et al., 2013; Johnston, Gibson, et al., 2013). It is possible that combining two ergogenic aids with different mechanisms of action, such as caffeine (a central nervous system stimulant) (Hulton et al., 2020) and NZBC (with vasoactive properties) (Matsumoto et al., 2005), could potentially provide an additive or a synergistic effect to improve exercise performance, especially when fatigued.

The aim of the present study was therefore, to examine the individual and additive effects of NZBC powder and caffeine intake on sprint performance using a modified LIST (m-LIST) protocol. The m-LIST protocol consists of 4 × 15-min blocks of exercise marked as “prescribed-pace” (blocks 1 to 4) and is followed 2 × 15-min “self-paced” blocks (blocks 5 and 6) (Ali et al., 2014). The prescribed-pace component (60 min) is marked as a fatiguing preload and is consistent with the distance covered between trials. However, the “self-paced” component requires participants to perform the same activity patterns but at a self-selected pace and thus, quantifies endurance performance between trials (Ali et al., 2014). The m-LIST protocol assesses speed, distance covered, sprint time, and reaction time for sprinting from blocks 1 to 6; and speed and distance covered for walking, jogging, and running during blocks 5 to 6. Therefore, represents a prescribed-pace phase with sprint performance

evaluation, followed by the self-paced phase that examines these both speed and distance covered in the last 30 min of the game when fatigue is most evident, and when match outcomes are decided (Ali et al., 2014).

We hypothesised that a single dose of NZBC with caffeine when consumed in a fatigued state would lead to reduced slowing of sprint performance during blocks 5 and 6 of the m-LIST protocol. In order to get the participants in a fatigued state, we implemented a 90-min muscle glycogen depletion protocol (cycling) on the previous day of the study.

4.2. Materials and methods

4.2.1. Sample size determination

To determine the necessary sample size, a power analysis using G-Power 3.1.9.7 was conducted, focusing on the primary outcome measure of sprint performance. Comparing caffeine and placebo trials from previous research involving well-trained male football players (Gant et al., 2010) revealed a 4.5% improvement in mean 15-m sprint performance during blocks 5 and 6 of LIST. Utilising G*Power with an alpha level of 0.05 and a power of 0.80, based on data from the study by Gant et al. (2010), our estimated sample size was 12 participants. However, to account for any dropouts we recruited 14 participants for this study.

4.2.2. Participants

Fourteen healthy male participants were recruited from local football clubs around Auckland, New Zealand. The characteristics of the participants are described in Table 4.1. Participants were recreationally active with experience in team sports with high-intensity intermittent running, but were not familiar with cycling. This study was approved by the Massey University Human Ethics Southern A Committee (Ohu Matatika 1) (approval number 21/09) and registered with the Australia New Zealand Clinical Trials Registry (ACTRN12621001394831). The participants provided written informed consent before their familiarisation visit and completed a medical history questionnaire.

Table 4.1 Participant characteristics (mean ± SD)

Age (years)	29.5 ± 9.3
Height (cm)	173.0 ± 23.2
Body mass (kg)	76.6 ± 7.9
Maximal oxygen uptake ($\dot{V}O_{2max}$; ml·kg^{-1}·min^{-1})	48.8 ± 4.2

4.2.3. Study design

Participants attended an initial two-part familiarisation visit for the assessment of $\dot{V}O_{2max}$, and then reported to the laboratory for four main trials, with each trial taking place over two days. On day 1 of the trial, participants reported to the laboratory in the afternoon/early evening to participate in the glycogen depletion fatiguing cycling protocol and then the following morning on Day 2 for the running trial (m-LIST). The study was a double blind, randomised, crossover trial (Latin square design) and was separated by a minimum of 7 days for a wash-out period. The four running trials tested four study treatments: (i) placebo (PLA), (ii) NZBC, (iii) caffeine (CAFF), and iv) NZBC and caffeine (NZBC-CAFF) (Table 4.2). The data was collected over a period of 18 months from Januray 2022 to June 2023.

Table 4.2 Latin Square design for the study

Condition	Day 1	Fatiguing exercise	Day 2	Performance metrics/exercise
NZBC	NZBC + 0 mg caffeine	90-min cycling	NZBC + 0 mg caffeine	90-min m-LIST
Placebo	Placebo + 0 mg caffeine	90-min cycling	Placebo + 0 mg caffeine	90-min m-LIST
Caffeine	Placebo + 0 mg caffeine	90-min cycling	Placebo + 240 mg caffeine	90-min m-LIST
NZBC + Caffeine	NZBC + 0 mg caffeine	90-min cycling	NZBC + 240 mg caffeine	90-min m-LIST

NZBC, New Zealand blackcurrant powder

4.2.4. Familiarisation visit

Participants were asked to report to the laboratory in comfortable athletic wear and running shoes and were later taken to the Massey University Recreation Centre, Albany Campus, Auckland for their familiarisation visit. The visit was divided into three parts: (i) maximal multistage 20-m shuttle run test ('beep' test) to estimate their $\dot{V}O_{2max}$ (Léger & Lambert, 1982), (ii) undertaking the m-LIST protocol for 30 min to allow adequate understanding of the running patterns and the experimental procedures (Ali et al., 2014), and (iii) an incremental exercise test (ramp test) on a stationary cycle ergometer (Velotron Pro cycle ergometer, Racermate Inc., USA) to estimate maximum power output for the

muscle glycogen deleption protocol for day 1 of the study (Kuipers et al., 1985). The value obtained from the $\dot{V}O_{2max}$ test was used to establish running speeds for the m-LIST protocol.

4.2.5. Fatiguing protocol

Participants engaged in ~90 min of moderate-intensity intermittent cycling exercise on a stationary cycle ergometer (Velotron Pro cycle ergometer, Racermate Inc., USA) between 3 and 7 pm the day before the running exercise to reduce their glycogen stores and to induce 'prior exercise' fatigue (Bowtell et al., 1999). The fatiguing protocol simulated the training conditions that may lead to accumulated fatigue in athletes undertaking regular training. The cycling protocol consisted of 30 min of exercise at an intensity of 60% of the participant's maximum power output. Once completed, participants rested for 2 min and then performed three 50-s 'sprints' at double the resistive load, with 2 min rest between each sprint. After another 2 min of rest, participants continued to cycle for a further 45 min at 60% maximum power output to further reduce glycogen stores. Participants were allowed to reduce the intensity to a maximum of 110 W over this period if they were unable to maintain their prescribed intensity of 60% of maximum power output (Bowtell et al., 1999). The maximum power output was established using an incremental exercise test (ramp test) on a stationary cycle ergometer (Velotron Pro cycle ergometer, Racermate Inc., USA) during the familiarisation visit (Kuipers et al., 1985). The participants were provided with 2 ml/kg BM of water before and after every 15 min of exercise to offset dehydration.

3.2.6. Dietary standardisation

Participants were asked to refrain from consuming alcohol, foods containing caffeine, and high amounts of nutritional polyphenolic compounds and antioxidants 48 h before their cycling protocol and till the completion of their running trial. They were provided with a standardised dinner the day before the trial and breakfast on the day of the trial. Dinner included beef lasagna, garlic bread, salad greens, and a banana at a serving size of 14.84 kJ/kg (for example, participant weighing 70 kg would require ~2930 kJ for dinner and this energy would come from 350 g beef lasagna, 70 g of garlic bread, 110 g banana, and 50 g of salad greens). The breakfast included 50 g of muesli, 100 g of yogurt, and 30 ml of milk containing ~1770 kJ, 30 g of carbohydrate, 15 g of protein, and 10 g of fat.

4.2.6. Experimental procedures

For the running exercise, participants reported to the lab early in the morning after completing at least an 8-h fast and were asked to provide a mid-stream urine sample to determine hydration status based on urine specific gravity (USG) (Atago 2773 MASTER-SUR/NM Clinical Refractometer, Atago Co., LTD., Japan). If found to be dehydrated (i.e. USG > 1.020) participants consumed 200 ml of water before

their cardiovascular assessement (and consumed 200 ml of water at every exercise visit to maintain consistency in testing procedure). The USG test was followed by assessment of cardiovascular measures and consumption of the study drink along with breakfast. Cardiovascular measures were reassessed one hour later and this was followed by a standardised 10-min warm-up and the m-LIST protocol. Ambient temperature (19.0 to 21.0 °C) and humidity (PLA: 65 to 75 %) were similar between experimental trials.

4.2.7. Cardiovascular measures

Participants were asked to remain in a supine position for assessment of blood pressure and heart rate (Omron, Healthcare CO. Ltd.; Kyoto, Japan) and stroke volume and systemic vascular resistance (Uscom 1A, Uscom Ltd, Sydney, Australia). The assessment was carried out at baseline, 1-h after study drinks were consumed, and 1-h after completion of exercise. Heart rate was also assessed through-out the m-LIST protocol using a heart rate monitor (Polar M200, Polar, Finland).

4.2.8. Intervention

After completing baseline cardiovascular measures, participants consumed either 24 g of NZBC powder with or without 240 mg of caffeine or placebo mixed with 200-ml of water. The placebo (PLA) contained the same amount of maltodextrin as NZBC powder with added fructose, glucose, and colour to match the nutritional value and appearance of the NZBC powder (Table 4.2). For the caffeinated versions of the same NZBC and PLA powders, 240 mg of caffeine was added (Table 4.3). All four powders were supplied by the study sponsor. Appendix 2.11 describes the randomisation for the study.

Table 4.3 Nutritional Content of NZBC, caffeinated NZBC, caffeinated placebo, and placebo powder (24g)

	NZBC Powder	**Caffeinated NZBC Powder**	**Caffeiante Placebo Powder**	**Placebo Powder**
Energy (kJ)	377	377	377	377
Energy (kcals)	90	90	90	90
Total carbohydrates (g)	20.6	20.6	20.6	20.6
Total added sugar (g)	9	9	20.6	20.6
Vitamin C (mg)	140	140	0	0
Anthocyanins (mg)	240	240	0	0
Caffeine (from green coffee beans) (mg)	0	240	240	0

* % Daily Values are based on a 2000 calorie diet; n/a Daily Value not established; Other ingredients: Maltodextin, monk fruit extract, natural flavours (No added colours or preservatives)

4.2.9. Modified Loughborough Intermittent Shuttle Test (m-LIST)

The modified LIST (m-LIST) protocol is a validated and reliable protocol that consists of 6 x 15-min blocks divided into four blocks of "prescribed-pace" activity (blocks 1 – 4; participants exercise based on audible signals) followed by two blocks of "self-paced" exercise (blocks 5 – 6; no audible signals) with a 3 min rest period between each block (Ali et al., 2014). Each block consisted of repeated sequences called cycles of 3 x 20-m walks at 5.4 km/h, 1 x 15-m sprint, 3 x 20-m run at a speed equivalent to 95% of $\dot{V}O_{2max}$ and 3 x 20-m jog at a speed equivalent to 55% $\dot{V}O_{2max}$. A proprietary mobile application (app) produced audio signals guiding participants' speeds during each block. Sprint times were measured using infrared photoelectric cells present in the sprint gates, transmitted wirelessly to the app. Participants completed ~11 cycles per block from blocks 1 to 4 as they were "prescribed pace" activity, and they received audio signals for repeated cycles. However, for "self-paced" blocks 5 and 6, participants continued the repeated sequence until they received a signal for cessation of exercise at the end of 15 min for both the blocks. Participants were instructed to replicate intensities and exercise patterns observed during the prescribed-pace blocks (for more detailed information see Ali et al. (2014)). Participants consumed water at a rate of 2 ml/kg BM after warm-up, followed by 2 ml/kg BM after every block from blocks 1 to 5.

The m-LIST protocol assessed for measures of speed, distance covered, sprint time, reaction time, and movement (sum of sprint and reaction time) for sprint performance from blocks 1 to 6, and speed and distance covered for walking, jogging, and running during blocks 5 to 6 Ali et al. (2014).

Before starting the m-LIST protocol participants underwent a standardised 10-min warm-up consisting of a total of 640-m of jogging, running, and sprinting as well as dynamic and static flexibility exercises of lower limbs and core muscles (for more details see appendix 2.10).

4.2.10. Countermovement Jump

Countermovement jump was assessed using the My Jump 2: measure your jump app (Apple Store, v. November 2022) to evaluate the changes in explosive leg power after warm-up and after every block of exercise during the m-LIST protocol.

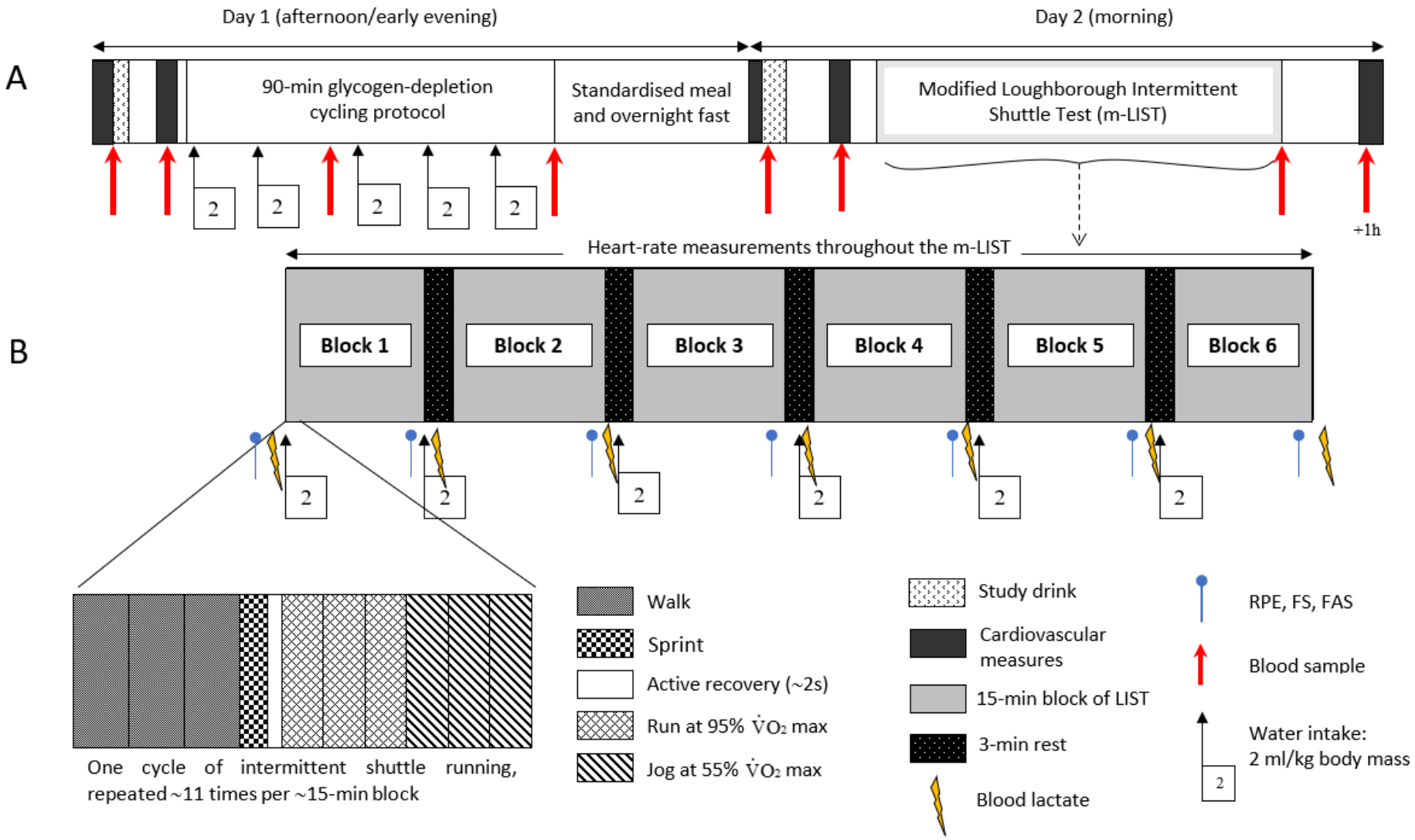

Figure 4.1 Schematic representation of A) the overall experimental protocol and B) the Loughborough Intermittent Shuttle Running Test

4.2.11. Blood sampling and lactate measurement

Blood samples were collected by venepucture from a vein within the antecubital area. Samples were taken at baseline, 1-h after consumption of the study drinks, immediately after exercise, and 1-h after exercise. Eight millilitres of blood was collected in the heparin collection tube (BD Vacutainers®, USA) and the samples were centrifuged for 10 min at 1250 × g (MF 50, Hanil Science Industrial Co. Ltd, Korea) to obtain plasma. Two millilitres of blood sample was collected in the serum collection tube (BD Vacutainers®, USA) and the samples were allowed to clot at room temperature for 20 min before being centrifuged for 10 min at 1250 × g). The samples were aliquoted and were later stored at −80°C (Thermo Scientific TSU TSU600D -80°C Upright ULT Ultra-Low Temperature Freezer) for analysis of caffeine metabolites (plasma samples) and non-esterified free-fatty acids (FFA; serum samples).

Blood lactate was assessed after completion of each block of the m-LIST protocol (Lactate Pro, Arkray, Shiga, Japan).

4.2.12. Blood analysis

Plasma caffeine and caffeine metabolites (paraxanthine and theophylline) were analysed by reversed-phase HPLC using a LC-20 liquid chromatograph equipped with an SPD-M20A photodiode array detector (Shimadzu Inc., Kyoto, Japan) (Holland et al., 1998). Frozen plasma samples were thawed and deproteinised by adding 400 µl of 0.8 M perchloric acid to 400 µl of the sample. Samples were then vortexed for 10 s and then centrifuged for 10 min at 9900 x g. The supernatant was removed and put in a glass vial and used for reversed-phase HPLC.

Serum free-fatty acids were analysed using the Randox non esterified fatty acids kit (FA 115, Randox Laboratories Limited, Japan) according to the manufacturer's instructions in a clinical chemistry analyser (RX Daytona plus, Randox Laboratories Limited, Japan) at the Nutrition Laboratory, School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand.

4.2.13. Perceptual Measures

Perceptual measures were assessed using the feeling scale (FS), felt arousal scale (FAS), and ratings for perceived exertion (RPE) before, during, and after the m-LIST protocol. The FS is a 11-point scale ranging from very bad (− 5), bad (-3), fairly bad (-1), neutral (0) to fairly good (+ 1), and good (+ 3), and very good (+ 5) (Hardy & Rejeski, 1989). The FAS measures perceived activation along a 6-point scale ranging from low arousal (1 point) indicating boredom or calmness to high arousal (6 points) suggested by anger or anxiety (Svebak & Murgatroyd, 1985). The RPE is a 15-point scale to assess subject level of exertion intensity. The score ranges from 6 to 20, with 6 being no exertion at all to 20 being maximum exertion (Borg, 1982).

4.2.14. Statistical analysis

The data were compared using a two-way ANOVA with repeated measures (IBM SPSS Statistic (v. 28.0.1, IBM, USA) to examine main effects of i) treatment and ii) time and iii) interaction of treatment*time. Bonferroni's pairwise comparisons with 95% confidence were conducted to identify specific differences between groups and timepoints. Differences were considered significant at an alpha level of $p < 0.05$. To determine the effect size of responses, partial eta^2 (η^2) was also calculated using SPSS. The threshold values for effect size were interpreted as small (0.01), medium (0.06), and large effects (0.14). Data is presented as mean ± SD.

4.3. Results

4.3.1. Sprint performance during modified Loughborough Intermittent Shuttle Test

There was an effect of treatment on average sprint speed ($p = 0.049$), with pairwise comparison demonstrating that NZBC-CAFF treatment sustained higher average sprint speed compared to NZBC during the m-LIST protocol ($p = 0.025$). Furthermore, there was a trend for CAFF ($p = 0.059$) and NZBC-CAFF ($p = 0.061$) treatments to sustain higher average sprint speeds compared to NZBC. However, the average sprint speed reduced significantly for all four treatments as the trial progressed from blocks 1 to 6 ($p = 0.032$, $\eta^2 = 0.341$). No interaction effect for treatment*time was observed for sprint speed.

Distance covered during the "prescribed-pace" blocks 1 to 4 (165 m, 11 x 15-m sprints per block) was constant for all four treatments and it decreased during the "self-pace" blocks 5 and 6 ($p = 0.001$**,** $\eta^2 = 0.662$) for all four treatments (block 5, PLA: 0.15 ± 0.01 km, NZBC: 0.15 ± 0.01 km, CAFF: 0.15 ± 0.01 km, and NZBC-CAFF: 0.15 ± 0.01 km; and block 6, PLA: 0.15 ± 0.01 km, NZBC: 0.15 ± 0.01 km, CAFF: 0.15 ± 0.01 km, and NZBC-CAFF: 0.16 ± 0.01 km; $p = 0.904$, $\eta^2 = 0.010$). No interaction effect for treatment*time was observed for distance covered.

There was an effect of treatment on sprint time ($p = 0.031$, $\eta^2 = 0.336$) with intake of CAFF and NZBC-CAFF sustaining faster sprint times compared to PLA and NZBC. There was no effect of time on sprint time for all four treatment. Reaction time and movement time increased with time ($p < 0.001$, $\eta^2 = 0.927$ and $p < 0.001$, $\eta^2 = 0.814$) but were not different between treatments. No interaction effect for treatment*time was observed for reaction, sprint, and movement time (Table 4.4).

Table 4.4 Sprint performance during blocks 1-6 of the modified Loughborough Intermittent Shuttle Test.

		Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Average Sprint speed (km/h)	PLA	18.1 ± 2.4	18.2 ± 2.5	17.7 ± 2.4	17.5 ± 2.2	17.0 ± 0.3	16.7 ± 3.3			
	NZBC	17.8 ± 2.4	17.7 ± 2.6	17.2 ± 2.5	16.9 ± 2.4	16.4 ± 3.1	16.4 ± 3.3	**$p = 0.049$**	**$p = 0.032$**	$p = 0.367$
	CAFF	19.0 ± 2.2	18.7 ± 2.1	18.2 ± 2.1	18.0 ± 2.2	17.5 ± 2.8	17.3 ± 2.8	$\eta^2 = 0.259$	$\eta^2 = 0.341$	$\eta^2 = 0.096$
	NZBC-CAFF	18.4 ± 2.2	18.1 ± 2.4	18.3 ± 2.4	18.0 ± 2.3	17.1 ± 3.1	17.4 ± 3.1			
Sprint time (s)	PLA	2.7 ± 0.8	2.5 ± 0.9	2.7 ± 1.0	2.9 ± 0.9	2.6 ± 1.1	3.0 ± 0.6			
	NZBC	2.5 ± 1.0	2.5 ± 1.0	2.7 ± 1.1	2.8 ± 0.8	3.0 ± 0.6	2.9 ± 0.7	**$p = 0.031$**	$p = 0.221$	$p = 0.315$
	CAFF	2.3 ± 1.0	2.3 ± 1.0	2.5 ± 1.0	2.4 ± 1.0	2.2 ± 1.2	2.3 ± 1.0	$\eta^2 = 0.336$	$\eta^2 = 0.178$	$\eta^2 = 0.135$
	NZBC-CAFF	2.4 ± 1.0	2.4 ± 1.0	2.5 ± 1.0	2.5 ± 1.0	2.9 ± 0.3	2.7 ± 0.5			
Reaction time (s)	PLA	1.0 ± 0.4	1.2 ± 0.6	1.1 ± 0.7	1.1 ± 0.8	2.1 ± 0.4	2.5 ± 0.7			
	NZBC	1.0 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	2.5 ± 0.8	2.5 ± 0.8	$p = 0.719$	**$p < 0.001$**	$p = 0.325$
	CAFF	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.5	2.6 ± 1.1	2.5 ± 0.7	$\eta^2 = 0.070$	$\eta^2 = 0.927$	$\eta^2 = 0.171$
	NZBC-CAFF	1.1 ± 0.3	1.2 ± 0.4	1.2 ± 0.6	1.1 ± 0.5	2.0 ± 0.7	2.2 ± 0.8			
Movement time (s)	PLA	3.6 ± 1.1	3.6 ± 1.1	3.8 ± 1.1	3.8 ± 1.2	4.3 ± 1.3	5.0 ± 0.9			
	NZBC	3.6 ± 1.0	3.6 ± 1.0	3.8 ± 1.1	3.8 ± 1.2	4.8 ± 1.0	4.8 ± 1.0	$p = 0.138$	**$p < 0.001$**	$p = 0.316$
	CAFF	3.3 ± 1.3	3.3 ± 1.4	3.4 ± 1.4	3.5 ± 1.5	4.3 ± 1.3	4.2 ± 1.3	$\eta^2 = 0.109$	$\eta^2 = 0.814$	$\eta^2 = 0.107$
	NZBC-CAFF	3.4 ± 1.4	3.5 ± 1.4	3.5 ± 1.5	3.5 ± 1.5	4.7 ± 0.7	4.7 ± 0.7			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

4.3.2. Walk, run, and jog speed and distance covered during the "self-paced" blocks 5 and 6

Average walking and jogging speed did not change over time for all four groups, however there was a decrease in walking and jogging distance covered as the trial progressed from blocks 1 to 6 ($p < 0.003$, $\eta^2 = 0.551$ and $p < 0.001$, $\eta^2 = 0.884$). There was a decrease in average running speed and running distance covered as the trial progressed from blocks 1 to 4 to blocks 5 and 6 for all treatments (Table 4.5). No interaction effect for treatment*time was observed for average walk, jog, and run speed and distance covered.

Table 4.5 Average walk, run, and jog speed and distance covered during the prescribed and self-paced blocks of the modified Loughborough Intermittent Shuttle Test.

			Block 1 - 4 (prescribed-pace)	Block 5 (self-paced)	Block 6 (self-paced)	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Walk	Distance covered (km)	PLA	0.66 ± 0	0.63 ± 0.03	0.62 ± 0.03	$p = 0.126$ $\eta^2 = 0.180$	**$p = 0.003$ $\eta^2 = 0.551$**	$p = 0.218$ $\eta^2 = 0.124$
		NZBC	0.66 ± 0	0.62 ± 0.03	0.63 ± 0.03			
		CAFF	0.66 ± 0	0.63 ± 0.03	0.63 ± 0.03			
		NZBC-CAFF	0.66 ± 0	0.62 ± 0.03	0.62 ± 0.03			
	Average speed (km/h)	PLA	5.4 ± 0	5.3 ± 0.6	5.3 ± 0.6	$p = 0.453$ $\eta^2 = 0.052$	$p = 0.520$ $\eta^2 = 0.036$	$p = 0.449$ $\eta^2 = 0.055$
		NZBC	5.4 ± 0	5.4 ± 0.2	5.3 ± 0.4			
		CAFF	5.4 ± 0	5.5 ± 0.2	5.4 ± 0.3			
		NZBC-CAFF	5.4 ± 0	5.5 ± 0.2	5.4 ± 0.3			
Run	Distance covered (km)	PLA	0.66 ± 0	0.60 ± 0.05	0.61 ± 0.03	$p = 0.367$ $\eta^2 = 0.088$	**$p < 0.001$** $\eta^2 = 0.740$	$p = 0.760$ $\eta^2 = 0.542$
		NZBC	0.66 ± 0	0.60 ± 0.03	0.59 ± 0.03			
		CAFF	0.66 ± 0	0.60 ± 0.04	0.61 ± 0.04			
		NZBC-CAFF	0.66 ± 0	0.60 ± 0.03	0.62 ± 0.03			
	Average speed (km/h)	PLA	12.6 ± 0.5	11.9 ± 1.2	11.7 ± 1.1	$p = 0.465$ $\eta^2 = 0.059$	**$p < 0.008$** $\eta^2 = 0.388$	$p = 0.700$ $\eta^2 = 0.038$
		NZBC	12.6 ± 0.5	11.9 ± 0.1	11.7 ± 1.0			
		CAFF	12.6 ± 0.5	12.1 ± 1.0	11.9 ± 1.3			
		NZBC-CAFF	12.6 ± 0.5	12.1 ± 1.0	12.0 ± 1.0			
Jog	Distance covered (km)	PLA	0.66 ± 0	0.59 ± 0.04	0.59 ± 0.04	$p = 0.394$ $\eta^2 = 0.082$	**$p < 0.001$** $\eta^2 = 0.884$	$p = 0.526$ $\eta^2 = 0.067$
		NZBC	0.66 ± 0	0.58 ± 0.03	0.59 ± 0.03			
		CAFF	0.66 ± 0	0.59 ± 0.03	0.59 ± 0.03			
		NZBC-CAFF	0.66 ± 0	0.60 ± 0.03	0.60 ± 0.03			
	Average speed (km/h)	PLA	10.0 ± 0.3	10.0 ± 0.8	9.9 ± 0.9	$p = 0.882$ $\eta^2 = 0.011$	$p = 0.394$ $\eta^2 = 0.059$	$p = 0.738$ $\eta^2 = 0.032$
		NZBC	10.0 ± 0.3	9.9 ± 0.6	9.8 ± 0.7			
		CAFF	10.0 ± 0.3	9.9 ± 0.8	10.0 ± 0.9			
		NZBC-CAFF	10.0 ± 0.3	9.9 ± 0.7	9.9 ± 0.7			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + caffeine. Values are mean ± SD.

4.3.3. Countermovement jump

Countermovement jump height did not change as the m-LIST progressed from block 1 to 6 ($p = 0.394$, $\eta^2 = 0.078$) and was not different between treatments ($p = 0.403$, $\eta^2 = 0.077$). There was no interaction effect for treatment*time (Table 4.6).

4.3.4. Blood lactate

There was an effect of treatment on blood lactate concentrations ($p = 0.041$, $\eta^2 = 0.295$) and pairwise comparison showed that the blood lactate concentration was higher with the intake of NZBC-CAFF compared to PLA ($p = 0.039$). There was no effect of time and no interaction effect for treatment*time (Table 4.7).

Table 4.6 Countermovement jump after warm-up and after every block during modified Loughborough Intermittent Shuttle Test.

		After warm-up	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Countermovement jump (cm)	PLA	38.7 ± 5.9	38.8 ± 7.3	39.0 ± 8.2	39.0 ± 7.9	38.4 ± 8.5	38.9 ± 7.7	38.9 ± 8.5			
	NZBC	39.3 ± 8.7	40.0 ± 8.9	39.0 ± 8.6	38.0 ± 9.0	38.9 ± 8.0	38.2 ± 8.8	39.6 ± 8.3	*p* = 0.403	*p* = 0.394	*p* = 0.761
	CAFF	40.2 ± 9.7	40.6 ± 9.0	40.5 ± 9.3	39.5 ± 10.6	40.1 ± 10.4	39.4 ± 9.5	40.2 ± 10.7	η^2 = 0.077	η^2 = 0.078	η^2 = 0.045
	NZBC-CAFF	39.4 ± 9.0	40.6 ± 9.4	39.6 ± 8.9	39.1 ± 8.8	39.1 ± 8.8	38.5 ± 8.1	40.2 ± 9.2			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + caffeine. Values are mean ± SD.

Table 4.7 Blood lactate after blocks 1 to 6 during the modified Loughborough Intermittent Shuttle Test.

		Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Blood Lactate (mmol/l)	PLA	4.8 ± 2.5	4.7 ± 2.7	4.1 ± 1.4	5.0 ± 3.0	3.9 ± 3.2	3.7 ± 2.0			
	NZBC	4.1 ± 1.7	4.1 ± 2.3	4.1 ± 2.2	4.2 ± 3.1	3.4 ± 1.7	3.2 ± 1.6	***p* = 0.041**	*p* = 0.633	*p* = 0.611
	CAFF	5.1 ± 1.8	6.2 ± 3.0	5.0 ± 2.0	5.1 ± 2.3	4.6 ± 2.7	5.9 ± 4.5	η^2 = 0.295	η^2 = 0.057	η^2 = 0.074
	NZBC-CAFF	5.1 ± 1.8	5.9 ± 2.9	6.7 ± 1.9	5.2 ± 2.1	5.0 ± 2.5	4.9 ± 1.8			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + caffeine. Values are mean ± SD.

4.3.5. Cardiovascular measures

Systolic blood pressure decreased 1-h after exercise ($p < 0.001$, $\eta^2 = 0.630$) in all four groups but was not different between treatments. The diastolic blood pressure decreased with time ($p = 0.012$) with an effect on treatment ($p = 0.026$, $\eta^2 = 0.237$) where CAFF and NZBC-CAFF sustained higher diastolic blood pressure compared to NZBC ($p = 0.036$ and $p = 0.045$).

Stroke volume and systemic vascular resistance (SVR) decreased over time ($p = 0.004$, $\eta^2 = 0.343$ and $p < 0.001$, $\eta^2 = 0.583$) with all four treatments, but were not different between treatments. No interaction effect for treatment*time was observed for systolic and diastolic blood pressure, stroke volume, and SVR (Table 4.8).

Heart rate increased over time ($p < 0.001$, $\eta^2 = 0.983$) during the m-LSIT protocol and was not different between the four treatments. Additionally, there was no interaction effect for treatment*time (Table 4.8).

Table 4.8 Cardiovascular measures at baseline, 1-h after consuming the study drink, and 1-h after completing exercise.

		Baseline	1-h after consuming the study drink	1-h after exercise	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Systolic Blood Pressure (mmHg)	PLA	121.4 ± 5.2	121.6 ± 9.0	112.9 ± 11.3	*p* = 0.263 η^2 = 0.096	***p* < 0.001** η^2 = 0.630	*p* = 0.102 η^2 = 0.138
	NZBC	118.4 ± 6.8	120.0 ± 8.1	111.5 ± 9.7			
	CAFF	118.9 ± 6.25	126.3 ± 8.4	113.2 ± 7.8			
	NZBC-CAFF	120.6 ± 7.8	125.6 ± 6.2	110.9 ± 8.1			
Diastolic Blood Pressure (mmHg)	PLA	73.9 ± 5.1	73.5 ± 5.6	70.6 ± 7.5	***p* = 0.026** η^2 = 0.237	***p* = 0.012** η^2 = 0.293	*p* = 0.099 η^2 = 0.141
	NZBC	75.2 ± 5.0	74.0 ± 6.5	70.1 ± 6.4			
	CAFF	75.1 ± 7.5	77.9 ± 6.5	75.6 ± 6.8			
	NZBC-CAFF	72.0 ± 6.1	76.0 ± 4.6	72.8 ± 5.8			
Stroke Volume (mL)	PLA	68.0 ± 14.3	78.1 ± 11.6	72.1 ± 13.3	*p* = 0.917 η^2 = 0.012	***p* = 0.004** η^2 = 0.343	*p* = 0.075 η^2 = 0.134
	NZBC	76.0 ± 18.3	73.1 ± 15.2	72.6 ± 16.0			
	CAFF	72.8 ± 16.3	75.6 ± 17.5	71.3 ± 15.8			
	NZBC-CAFF	73.1 ± 14.4	77.0 ± 18.6	73.5 ± 13.7			
Systemic Vascular Resistance (dynes·sec·cm^{-5})	PLA	2125.1 ± 470.4	1782.9 ± 341.4	1461.0 ± 332.1	*p* = 0.986 η^2 = 0.004	***p* < 0.001** η^2 = 0.583	*p* = 0.289 η^2 = 0.090
	NZBC	1985.5 ± 698.6	1893.5 ± 466.4	1562.2 ± 536.8			
	CAFF	1968.8 ± 445.4	1884.5 ± 499.1	1620.9 ± 484.4			
	NZBC-CAFF	1951.3 ± 461.0	1956.9 ± 696.5	1522.4 ± 439.7			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD

Table 4.9 Heart rate 1-h post study drink and during blocks 1 to 6 of the modified Loughborough Intermittent Shuttle Test.

		1-h post study drink	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	1-h post exercise	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Heart Rate (beats/min)	PLA	58.3 ± 4.2	152.0 ± 10.0	160.2 ± 7.2	161.2 ± 7.3	160.6 ± 7.0	157.4 ± 9.6	157.9 ± 7.7	77.9 ± 8.6			
	NZBC	59.1 ± 7.0	151.5 ± 10.4	156.2 ± 7.5	156.0 ± 7.9	155.5 ± 8.2	152.5 ± 10.5	152.4 ± 12.6	74.2 ± 10.5	$p = 0.407$	$\mathbf{p < 0.001}$	$p = 0.356$
	CAFF	59.2 ± 7.1	151.4 ± 14.9	157.7 ± 11.8	158.7 ± 9.4	157.1 ± 9.5	153.7 ± 9.4	154.9 ± 10.5	75.1 ± 9.0	$\eta^2 = 0.186$	$\eta^2 = 0.983$	$\eta^2 = 0.229$
	NZBC-CAFF	58.0 ± 10.9	151.7 ± 13.0	162.0 ± 5.6	161.0 ± 6.3	160.3 ± 7.5	158.4 ± 8.8	158.2 ± 12.4	75.2 ± 11.5			

PLA, Placebo; NZBC, New Zealand Blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand Blackcurrant + Caffeine. Values are means ± SD.

4.3.6. Perceptual measures

4.3.6.1. Feeling scale and felt arousal scale

There was an effect of treatment and time on feeling scale (FS) (p = 0.034, η^2= 0.216 and p < 0.001, η^2 = 0.573). Post-hoc test showed that the ratings for FS for treatments CAFF (p = 0.039) and NZBC-CAFF (p = 0.009) were significantly higher than NZBC treatment. However felt arousal scale (FAS) showed only an effect of time (p < 0.001, η^2 = 0.635) with no treatment effect as the trial progressed. The ratings for FS decreased from "feeling fairly good" at baseline to "feeling fairly bad" at the end of block 6 and returned to "feeling fairly good" 1-h after completing the m-LIST protocol. Ratings of FAS changed from "low arousal state" to "high arousal state" during the m-LIST and was "in-between low and high arousal" 1-h after completing the LIST protocol. No interaction effect of treatment*time was observed for FS and FAS (Table 4.10).

Values for FS and FAS were plotted in a circumplex model to provide a visual description of changes in pleasure and arousal throughout the trial (Figure 3.5 and 3.6). In all four treatments participants indicated that they were in the "low arousal and pleasure" state before running, "high arousal and pleasure" state during blocks 1-3, "high arousal and displeasure" state during blocks 4-6 and returned to a state of "low arousal and pleasure" at the end of exercise.

Table 4.10 Perceptual measures: feeling scale and felt arousal scale during blocks 1-6 of the modified Loughborough Intermittent Shuttle Test.

		Baseline	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	1-hour post exercise	Effect of Treatment (*p* value and effect size)	Effect of Time (*p* value and effect size)	Effect of Treatment *Time (*p* value and effect size)
Feeling Scale (-5 to +5)	PLA	1.9 ± 1.1	0.7 ± 2.2	-0.2 ± 1.7	-1.1 ± 1.8	-1.0 ± 1.8	-1.1 ± 2.2	-1.9 ± 2.3	1.6 ± 1.9			
	NZBC	1.8 ± 2.0	0.1 ± 2.1	-0.4 ± 2.6	-1.1 ± 2.4	-1.2 ± 2.4	-1.2 ± 2.2	-0.7 ± 2.8	2.0 ± 2.1	***p* = 0.034**	***p* < 0.001**	*p* = 0.095
	CAFF	1.4 ± 1.4	1.2 ± 1.3	0.3 ± 1.5	-0.3 ± 1.5	-0.5 ± 2.3	-0.8 ± 2.6	-1.1 ± 2.7	2.2 ± 1.8	η^2 = 0.216	η^2 = 0.573	η^2 = 0.127
	NZBC-CAFF	2.1 ± 1.3	1.3 ± 1.5	0.3 ± 1.3	0.1 ± 1.9	-1.0 ± 1.8	-0.9 ± 1.6	-0.9 ± 2.4	1.1 ± 1.8			
Felt Arousal Scale (1 to 6)	PLA	2.5 ± 1.0	3.9 ± 1.0	4.1 ± 1.1	4.1 ± 1.1	3.9 ± 1.2	4.0 ± 1.3	4.1 ± 1.3	2.6 ± 1.1			
	NZBC	1.9 ± 0.8	4.0 ± 1.1	4.3 ± 1.2	4.3 ± 1.2	4.3 ± 1.2	4.4 ± 1.2	4.5 ± 1.0	2.9 ± 1.2	*p* = 0.343	***p* < 0.001**	*p* = 0.413
	CAFF	2.6 ± 1.0	3.9 ± 0.8	4.0 ± 1.0	4.1 ± 1.2	4.5 ± 1.1	4.3 ± 1.2	4.4 ± 1.3	2.8 ± 1.1	η^2 = 0.087	η^2 = 0.635	η^2 = 0.078
	NZBC-CAFF	2.23 ± 1.0	3.7 ± 1.0	4.0 ± 1.1	4.3 ± 1.0	4.3 ± 0.7	4.1 ± 1.0	3.6 ± 2.2	2.8 ± 1.0			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

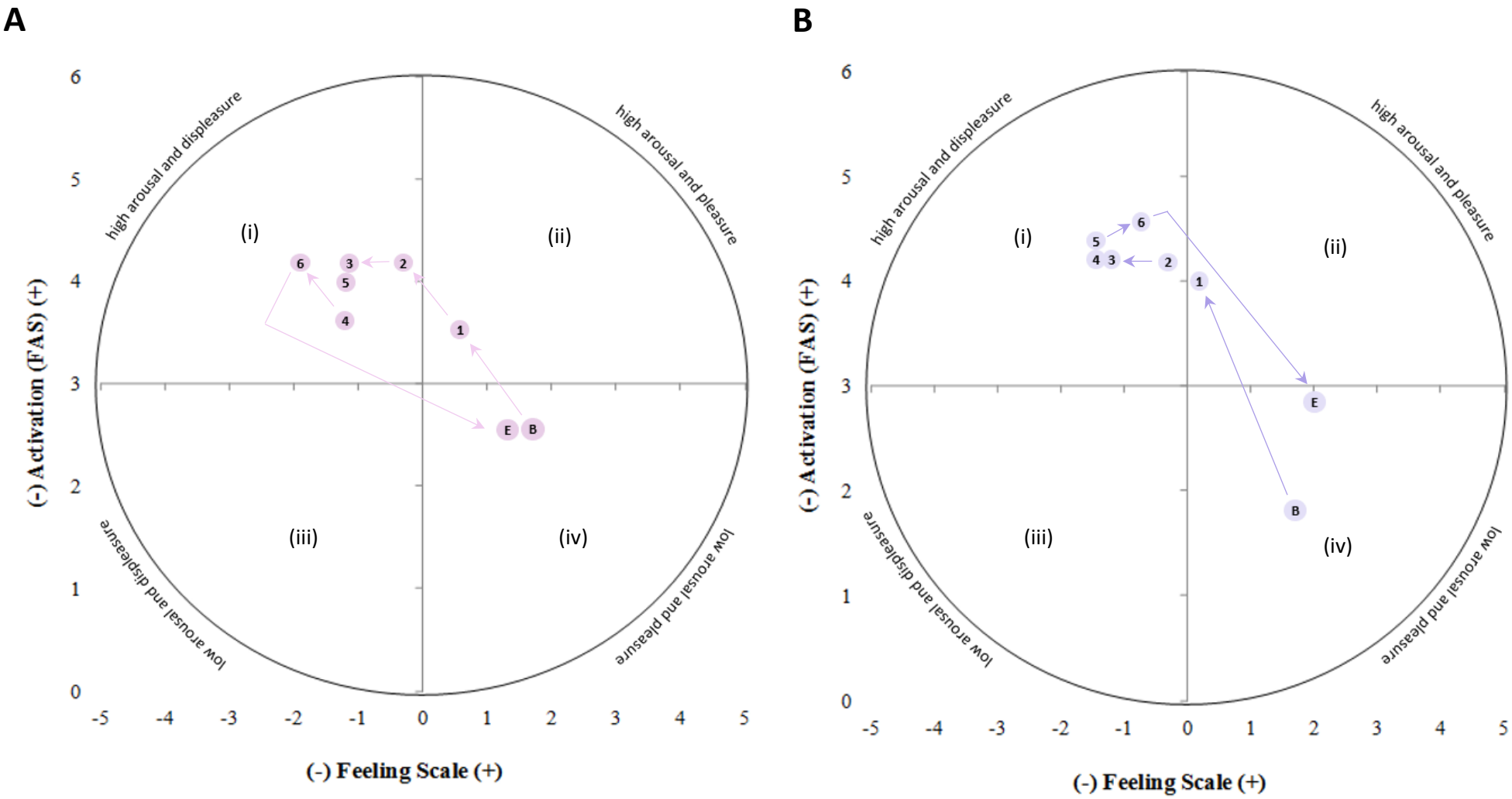

Figure 4.2 Circumplex model of affect in (A) placebo (PLA) and (B) NZBC trials based on feeling scale and felt arousal scale ratings at B - baseline, 1 - block 1, 2 - block 2, 3 - block 3, 4 - block 4, 5 - block 5, 6 - block 6, E - end of exercise. Quadrants indicate various activation and pleasure states: (i) high arousal and displeasure (e.g. anger); (ii) high arousal and pleasure (e.g. vigor); (iii) low arousal and displeasure (e.g. boredom or fatigue); (iv) low arousal and pleasure (e.g. calm).

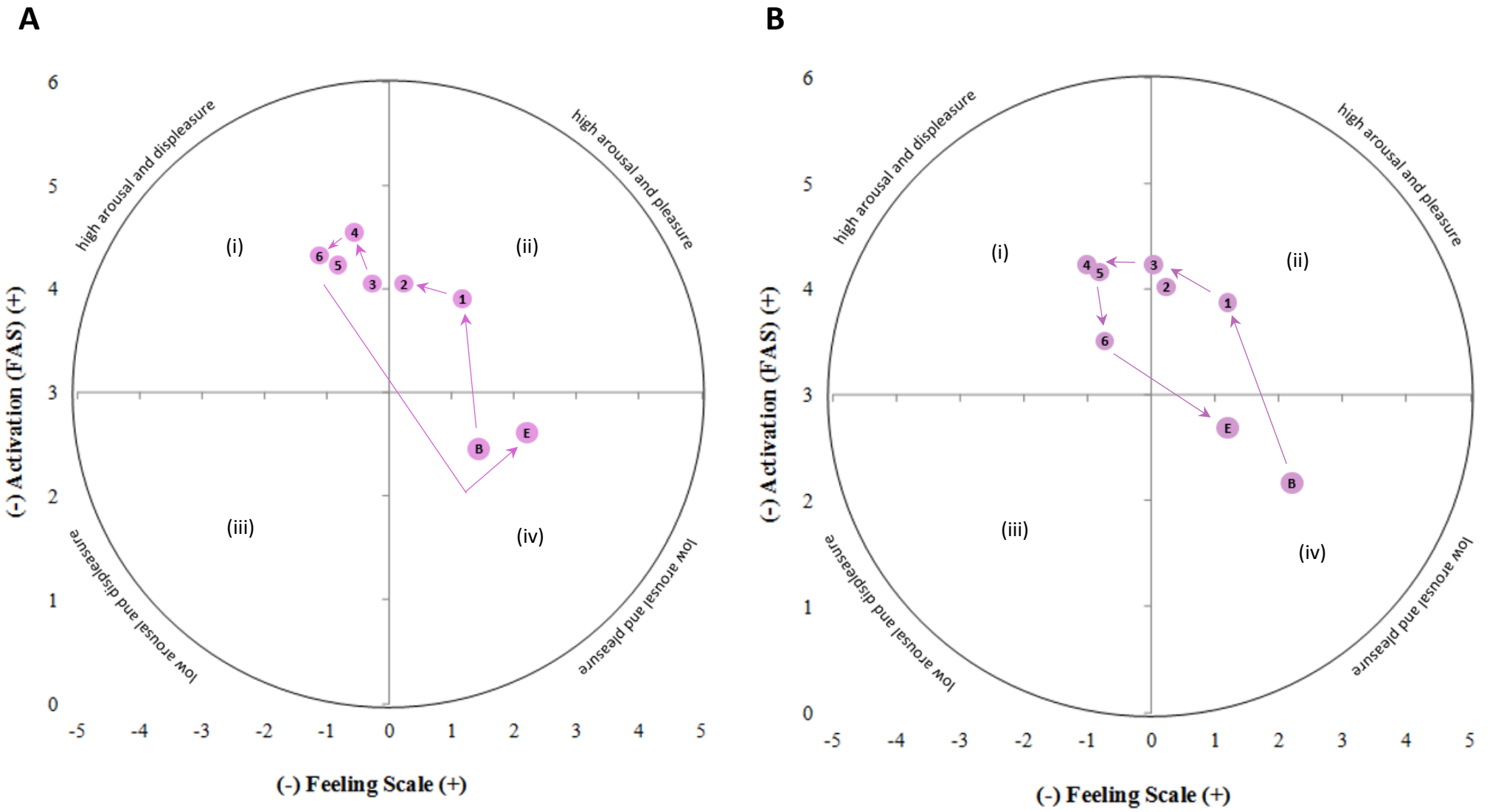

Figure 4.3 Circumplex model of affect in (A) caffeine (CAFF) and (B) NZBC + caffeine (NZBC + CAFF) trials based on feeling scale and felt arousal scale ratings at B - baseline, 1 - block 1, 2 - block 2, 3 - block 3, 4 - block 4, 5 - block 5, 6 - block 6, E - end of exercise. Quadrants indicate various activation and pleasure states: (i) high arousal and displeasure (e.g. anger); (ii) high arousal and pleasure (e.g. vigor); (iii) low arousal and displeasure (e.g. boredom or fatigue); (iv) low arousal and pleasure (e.g. calm).

4.3.6.1. Ratings of perceived exertion (RPE)

There was a trend with a large effect size for an effect of treatment on ratings of perceived exertion (p = 0.053, η^2 = 0.206). At the end of block 2, values of RPE for CAFF were lower than PLA (p = 0.045). Furthermore, there were trends for CAFF and NZBC-CAFF treatments to have lower RPE values compared to PLA (block 1: p = 0.07, block 2: p = 0.062). Overall, RPE was the lowest at the end of block 1 and continued to increase until the end of block 6, indicating an effect of time (p < 0.001). However, there was no interaction effect of treatment*time for RPE for all four groups (Table 4.11, Figure 4.4).

Table 4.11 Ratings of perceived exertion during blocks 1-6 of the modified Loughborough Intermittent Shuttle Test.

		Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**	**Effect of Treatment (p value and effect size)**	**Effect of Time (p value and effect size)**	**Effect of Treatment *Time (p value and effect size)**
RPE (1 to 20)	PLA	13.9 ± 2.1	14.9 ± 1.7	15.4 ± 1.9	15.6 ± 2.1	15.6 ± 1.9	16.7 ± 1.9	p = 0.053 η^2 = 0.206	**p < 0.001** η^2 = 0.688	p = 0.212 η^2 = 0.100
	NZBC	12.6 ± 2.5	14.2 ± 2.1	15.2 ± 1.7	15.8 ± 1.6	16.0 ± 1.8	16.5 ± 1.9			
	CAFF	12.0 ± 1.8	13.8 ± 1.6	14.6 ± 1.5	15.1 ± 2.0	15.7 ± 1.7	16.4 ± 2.1			
	NZBC-CAFF	12.1 ± 1.4	13.4 ± 1.6	13.9 ± 1.7	15.1 ± 1.8	15.4 ± 1.6	16.1 ± 2.0			

RPE, Ratings of perceived exertion; PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

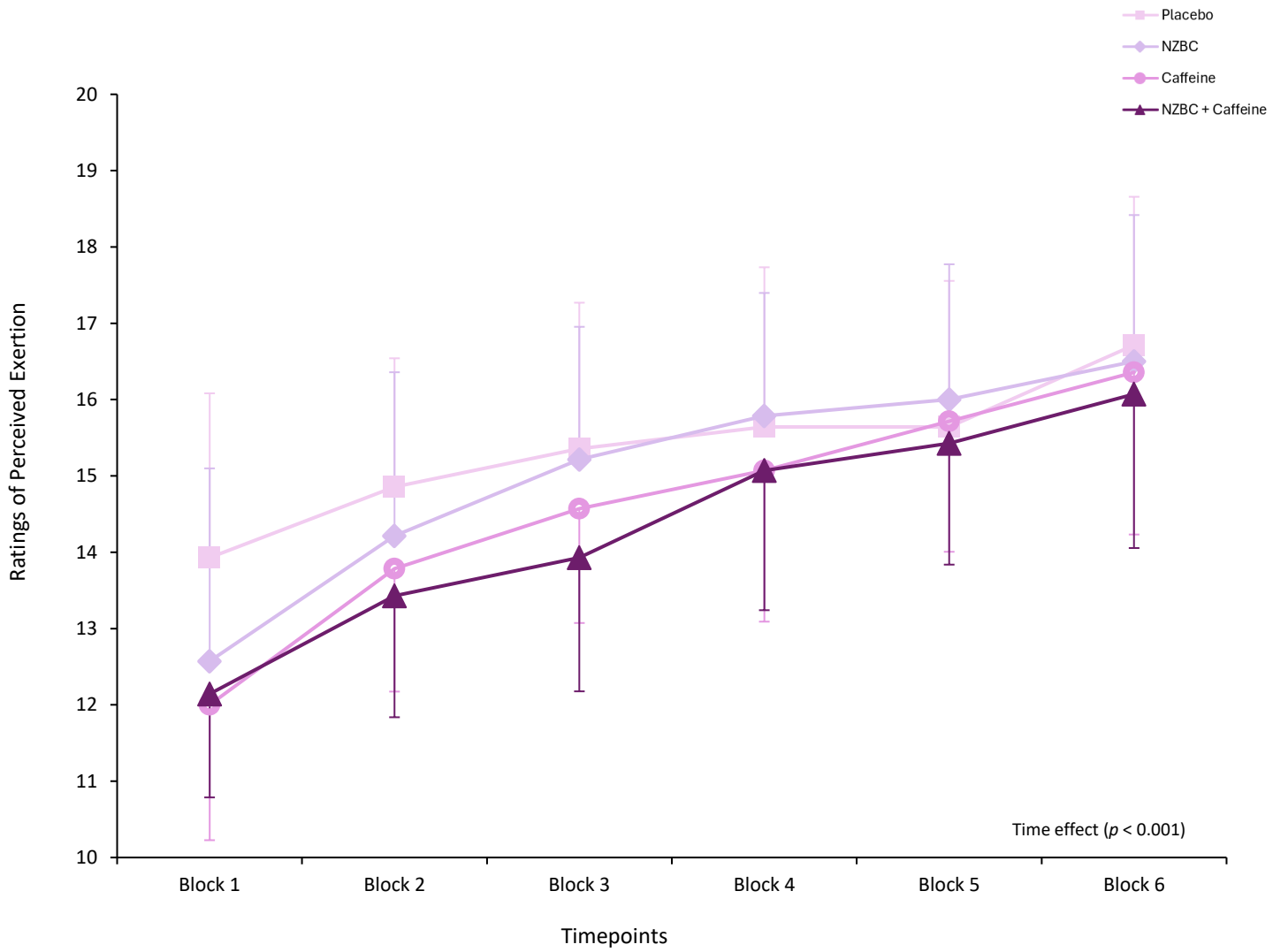

Figure 4.4 Changes in ratings of perceived exertion (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF) trials from blocks 1 to 6 during modified Loughborough intermittent Shuttle Test. *Significantly lower in NZBC-CAFF group and CAFF group compared to the PLA group.

4.3.7. Caffeine metabolites

Plasma concentrations of caffeine and its metabolites paraxanthine and theophylline increased with time in NZBC-CAFF and CAFF and were higher compared to PLA and NZBC treatments, however, they were not significantly different from each other. There was an interaction effect for treatment*time ($p < 0.001$) (Table 4.12, Figure 4.5, Figure 4.6).

Table 4.12 Caffeine metabolite concentrations at baseline (0-h), 1-h after study drink consumption (1-h), and immediately after completing the modified Loughborough Intermittent Shuttle (3-h).

		0-h	1-h	3-h	Effect of treatment (*p* value and effect size)	Effect of time (*p* value and effect size)	Effect of treatment *time (*p* value and effect size)
Paraxanthine (µg/ml)	PLA	0.31 ± 0.30	0.30 ± 0.24	0.31 ± 0.22	***p* < 0.001** η^2= 0.853	***p* < 0.001** η^2= 0.935	***p* < 0.001** η^2= 0.892
	NZBC	0.36 ± 0.32	0.37 ± 0.30	0.40 ± 0.20			
	CAFF	0.41 ± 0.30	2.39 ± 0.49	3.32 ± 0.66			
	NZBC-CAFF	0.56 ± 0.57	2.38 ± 0.84	3.19 ± 1.07			
Theophylline (µg/ml)	PLA	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	***p* < 0.001** η^2= 0.562	***p* < 0.001** η^2= 0.722	***p* < 0.001** η^2= 0.571
	NZBC	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01			
	CAFF	0.12 ± 0.01	0.15 ± 0.02	0.17 ± 0.02			
	NZBC-CAFF	0.13 ± 0.02	0.15 ± 0.03	0.17 ± 0.03			
Caffeine (µg/ml)	PLA	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	***p* < 0.001** η^2= 0.922	***p* < 0.001** η^2= 0.886	***p* < 0.001** η^2= 0.844
	NZBC	0.15 ± 0.02	0.15 ± 0.03	0.14 ± 0.03			
	CAFF	0.40 ± 0.84	3.39 ± 0.70	2.62 ± 0.56			
	NZBC-CAFF	0.51 ± 0.87	3.29 ± 0.69	2.43 ± 0.85			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

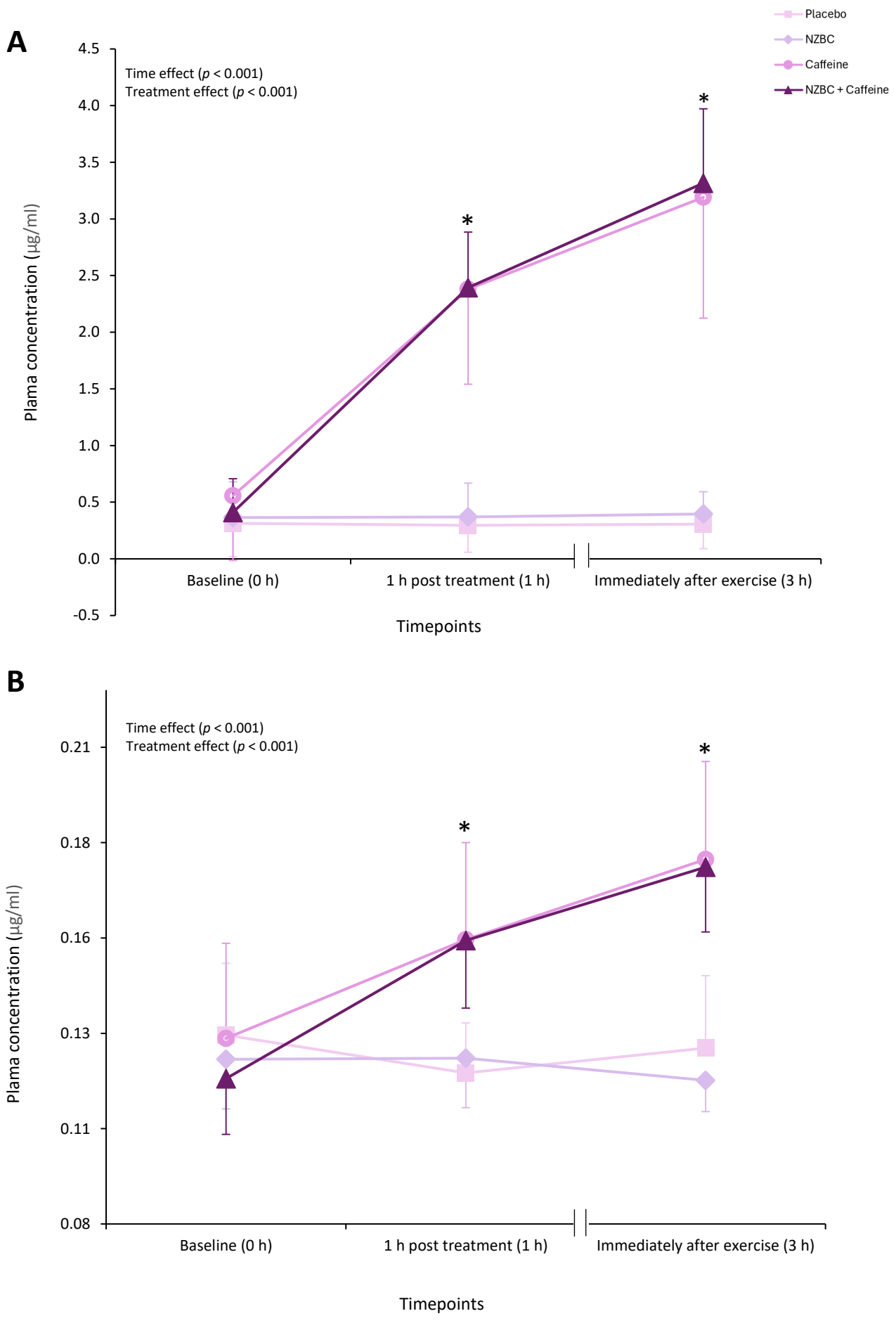

Figure 4.5 Plasma concentration of (A) paraxanthine and (B) theophylline (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC + CAFF) trials at baseline, 1-h after consuming the study drink, and immediately after exercise. *significantly higher in NZBC + CAFF group and CAFF group compared to NZBC and PLA group at that timepoint.

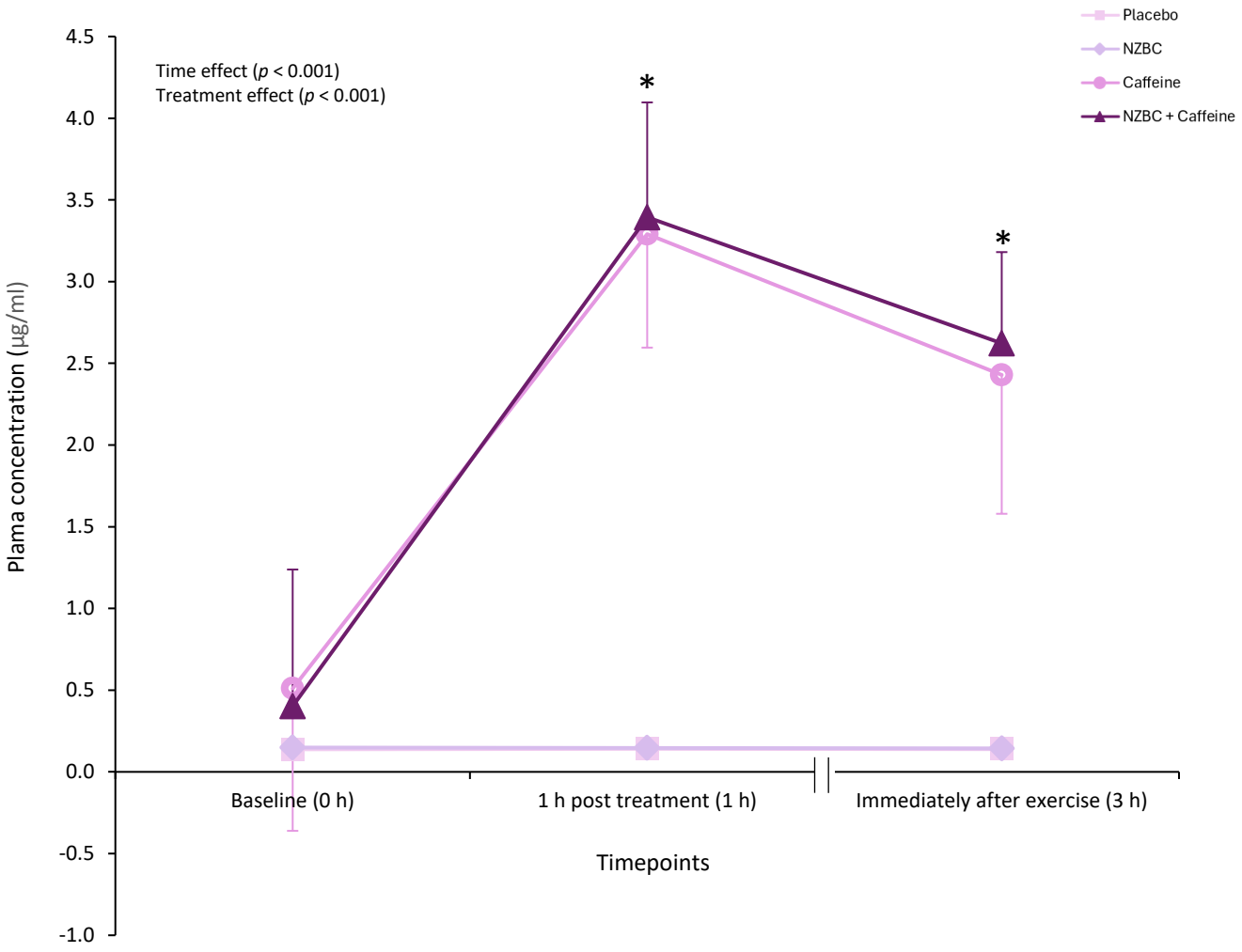

Figure 4.6 Plasma caffeine concentration (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC + CAFF) trials at baseline, 1-h after consuming the study drink, and immediately after exercise. *significantly higher in NZBC + CAFF group and CAFF group compared to NZBC and PLA group at that timepoint.

4.3.8. Free fatty acids (FFA)

Serum FFA concentrations increased with time during the m-LIST ($p < 0.001$) but were not different between treatments. However, NZBC-CAFF was the only treatment in which serum FFA concentration kept increasing 1-h post exercise ($p = 0.042$, $\eta^2 = 0.214$; Table 4.13, Figure 4.7).

Table 4.13 Free-fatty acid concentrations at baseline, 1-h after study drink consumption, and immediately after exercise, and 1-h after exercise (modified Loughborough Intermittent Shuttle Test).

		Baseline	1-h after study drink	Immediately after exercise	1-h after exercise	Effect of Treatment (*p* value and effect size)	Effect of Time (*p* value and effect size)	Effect of Treatment *Time (*p* value and effect size)
Free-fatty acids (mmol/l)	PLA	0.45 ± 0.19	0.17 ± 0.09	1.54± 0.73	1.31 ± 0.58	$p = 0.251$ $\eta^2 = 0.126$	**$p < 0.001$** $\eta^2 = 0.900$	**$p = 0.042$** $\eta^2 = 0.214$
	NZBC	0.43 ± 0.23	0.16 ± 0.05	1.32 ± 0.43	1.38 ± 0.56			
	CAFF	0.49 ± 0.31	0.21 ± 0.12	1.64 ± 0.74	1.62 ± 0.60			
	NZBC-CAFF	0.53 ± 0.23	0.22 ± 0.09	1.47 ± 0.64	1.93 ± 0.64			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

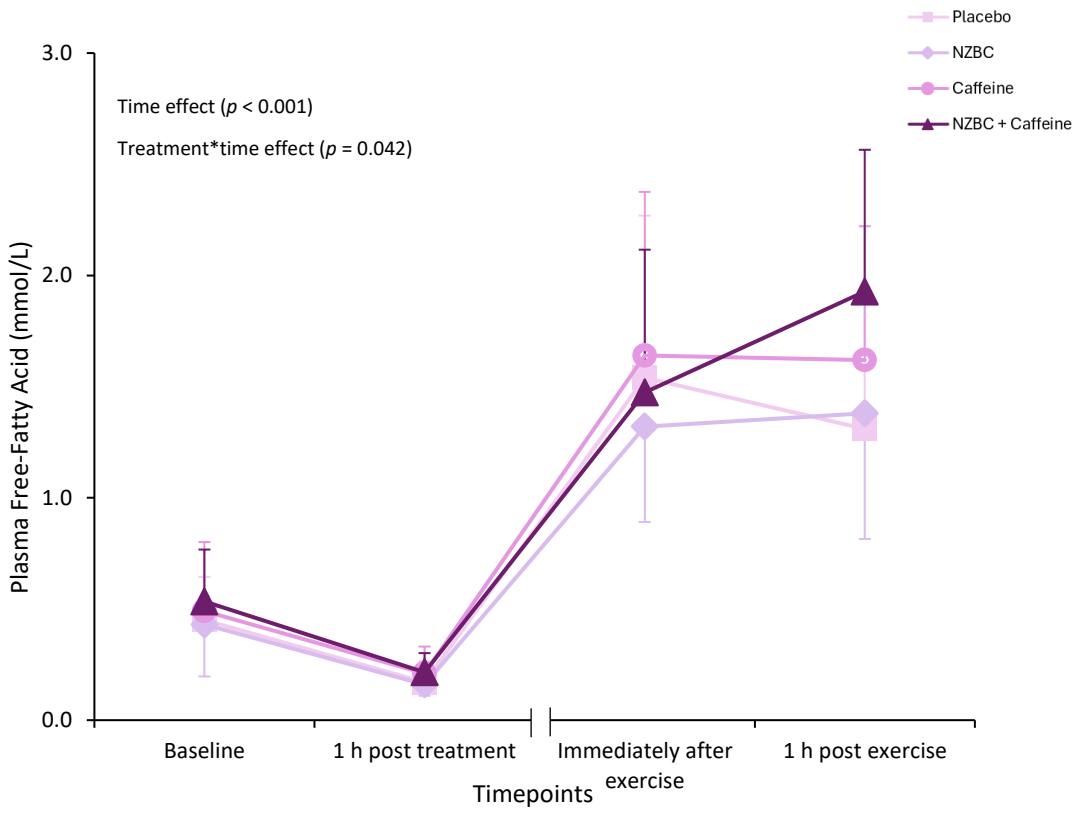

Figure 4.7 Plasma free-fatty acids concentration (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC + CAFF) trials at baseline, 1-h after consuming the study drink, immediately after exercise, and 1-h after exercise.

4.4. Discussion

This is the first study to evaluate the individual and additive effects of a single dose of New Zealand blackcurrant powder and caffeine intake on repeated-sprint performance in a fatigued state. Our study found that intake of NZBC-CAFF did not reduce slowing of sprint speed during blocks 5 and 6 of the m-LIST protocol. However, we observed an effect on treatment on average sprint speed (p = 0.049), with pairwise comparison demonstrating that NZBC-CAFF sustained higher average sprint speed compared to NZBC during the m-LIST protocol (p = 0.025). Furthermore, we observed a trend for CAFF (p = 0.059) and NZBC-CAFF (p = 0.061) treatments to sustain higher average sprint speeds compared to NZBC during the m-LIST protocol.

The average sprint speed in our study decreased as the m-LIST protocol progressed from blocks 1 to 6, indicating the occurrence of fatigue in our participants. However, it did not differ between the four treatments. Intake of NZBC-CAFF showed a higher average sprint speed compared to NZBC (p = 0.025) and there was a trend for CAFF (p = 0.059) and NZBC-CAFF (p = 0.061) treatments to sustain higher average sprint speeds compared to NZBC (but not PLA) during the m-LIST. The results for sprint performance from our study are consistent with the study by Paton et al. (2022) that found no significant additive effects of a single dose of NZBC containing 155 mg anthocyanins and 4 mg/kg body weight of caffeine intake on performance in trained male cyclists during repeated high-intensity cycling. Similarly, Paton et al. (2022) reported that acute intake of NZBC provided no benefit to exercise performance, critical speed, and time to exhaustion during high- intensity running.

At present, there is limited published information available on the acute effect of NZBC intake on running performance. Intake of a single dose of 900 mg NZBC has demonstrated a faster completion time during a 5-km time-trial in trained runners (Moss et al., 2023). In contrast, the effect of NZBC intake (300 mg/day) on exercise capacity has been more commonly evaluated using a 7-day loading period (Perkins et al., 2015; Willems et al., 2016). The study by Willems et al. (2016) observed a non-significant 15% increase in time to exhaustion in 8 out of 13 participants during Part B of the LIST (alternating run and jog until volitional fatigue) with NZBC intake. The study by Perkins et al. (2015) also reported a 10.6% increase in distance covered during an incremental high-intensity intermittent running test protocol until exhaustion with NZBC intake. Thus, it is possible that the ergogenic effects of NZBC intake on running performance could be associated with exercise capacity (i.e. time to exhaustion and time-trial) rather than a repeated-sprint performance trial that focuses on sprint speed (i.e. m-LIST). Also, a 7-day loading period for NZBC intake could be necessary to show improvements in intermittent running protocols.

The outcome of an intervention in a sports-performance trial could be influenced by (i) dose and duration of the study intervention, (ii) duration and intensity of the exercise protocol that lead to fatigue, and (iii) rested vs. fatigued-state of the participant at the start of exercise. Intake of NZBC-CAFF showed a higher average sprint speed compared to NZBC during the m-LIST protocol (p = 0.025) and there was a trend for CAFF (p = 0.059) and NZBC-CAFF (p = 0.061) treatments to sustain higher average sprint speeds compared to NZBC, which indicated that caffeine may have helped sustain higher sprint speeds. Conversely, Willems et al. (2016) and Perkins et al. (2015) observed less slowing of the fastest maximal sprint during block 5 of LIST and a trend for reduced slowing of the sprint 5 out of 6 x 35-m sprints with the consumption of 300 mg/day (105 mg anthocyanins) of NZBC for 7 days, respectively. Furthermore, intake of 3.7 mg/kg caffeine (~260 mg for a 70 kg participant) showed a decrease in slowing of the repeated-sprints in the last three blocks of the m-LIST (Gant et al., 2010). Hence, it is possible that although intake of caffeine in our study (240 mg) was similar to that by Gant et al. (2010), the single-dose of NZBC alone and with caffeine was inadequate to show an effect on repeated-sprint performance during high-intensity intermittent exercise. Thus, intake of NZBC for 7 days could be necessary to see an improvement in sprint performance.

When considering an overall change in sprint performance, intake of 600 mg/day of NZBC for 7 days had no effect on maximum, minimum, and mean sprint time in the study by Godwin et al. (2017) and intake of 300 mg/day for 7 days did not affect the average sprint times in each block in the study by Willems et al. (2016). This is consistent with the results from our study, as we reported changes in average sprint speed as overall performance per block and observed only a significant difference with NZBC-CAFF showing a higher average sprint speed compared to NZBC during the m-LIST protocol (p = 0.025). Thus, the positive outcomes mentioned earlier i.e. reduced slowing of the fastest sprint between block 1 and 5 (Willems et al., 2016), the trend for reduced slowing of the sprint 5 out of 6 x 35-m sprints (Godwin et al., 2017), and the results from our study may be considered as isolated results.

Fatigue typically develops rapidly after the first sprint and manifests as a reduction in maximal speed during repeated-sprint performance (Girard et al., 2011). Exercise parameters such as (i) longer duration of the exercise and increased number of sprints and (ii) periods of active recovery (compared to passive recovery) can increase fatigue (Girard et al., 2011). Our protocol evaluated changes in sprint speed performance during the 90-min m-LIST protocol with at least ~62-64 x 15-m sprints and recurring periods of active recovery (600-660 m of walking, running, and jogging per 15-min block) after a single-dose of NZBC-CAFF intake. The studies by Godwin et al. (2017) and Willems et al. (2016) observed changes in sprint performance in shorter duration protocols with passive/active recovery after the intake of NZBC for 7 days. Thus, it is possible that a single dose of NZBC-CAFF was also

inadequate to decrease fatigue in a 90-min high-intensity intermittent running protocol such as the m-LIST. Furthermore, we also calculated the fatigue index to evaluate the rate of decline in sprint performance using percentage decrement score (Girard et al., 2011) and found a smaller decrease in sprint speed in the NZBC-CAFF compared to CAFF through blocks 1 to 6 (one-way repeated measures ANOVA, $p = 0.048$, $\eta^2 = 0.253$; Bonferroni post-test, $p = 0.005$). However, the improvement in sprint speed fatigue index with NZBC-CAFF intake was not accompanied by changes in other sprint performance variables such as the fatigue index for reaction and movement time (see appendix 4.3). Hence, the fatigue index results should be treated with caution.

Burnett and Willems (2022); Godwin et al. (2017); Willems et al. (2016) and Strauss et al. (2018) have speculated that NZBC intake may have influenced running performance by increasing and fatty acid oxidation and peripheral blood flow (Matsumoto et al., 2005), which could subsequently contribute to higher phosphocreatine (PCr) resynthesis, reduced metabolite accumulation (Perkins et al., 2015), increased oxygen availability, and reduced the oxidative stress (Willems et al., 2016) in skeletal muscle. However, studies that evaluated muscle oxygenation during repeated cycling sprints (using near-infrared spectroscopy) have reported that despite a gradual increase in muscle deoxyhaemoglobin levels during the trial, the muscle continued to utilize a similar quantity of oxygen during each sprint (Racinais et al., 2007; Smith & Billaut, 2010). This indicates that the blood flow and subsequent oxygen delivery to the muscle may not a key determining factor of repeated-sprint performance.

Participants in other studies (Burnett & Willems, 2022; Godwin et al., 2017; Willems et al., 2016) were rested before engaging in the exercise trial compared to our participants that were in a fatigued-state at the start of the m-LIST protocol. Participants in our study engaged in a muscle glycogen-depletion cycling protocol the day before the main running trial. Saltin (1973) showed that intense training the day before a football game resulted in a pronounced decrease in muscle glycogen stores before and during the game. Low muscle glycogen stores have also shown to impair sprint performance at the end of a football game (Krustrup et al., 2006). Dietary guidelines suggest that moderately active athletes should consume 5-7 g/kg BM of CHO/day and around 1-1.2 g/kg BM of carbohydrates per hour for the first 4-6 h of post-exercise during periods of short recovery time (Burke et al., 2011; Burke et al., 2004; Thomas et al., 2016) The 24-h dietary recall of our participants indicated that that they were consuming ~3.5-4 g/kg BM of carbohydrates per day (data not shown) and the standardised meal that we provided for dinner contained 1 g/kg BM CHO (one meal). Thus, indicating that our participants were not only fatigued before the m-LIST, but also had poor glycogen stores at the start of the running trial which could have further impaired the repeated-sprint performance and affected the potential of NZBC-CAFF to reduce fatigue.

Caffeine intake before exercise has been shown to increase plasma concentrations of caffeine, paraxanthine, and theophylline and influence plasma lactate concentrations and perceptual measures during exercise. This study showed that plasma concentrations of caffeine, paraxanthine, and theophylline increased with time in the NZBC-CAFF and CAFF groups and were consistently high 1 h after consumption, during exercise, and 1 h after exercise, and these levels were significantly higher than the PLA and NZBC groups. The increase in plasma caffeine concentration was associated with lower RPE in CAFF and NZBC-CAFF treatments (relative to PLA). Perceived exertion plays a significant role in the ability of an individual to work at greater and/or prolonged intensity, and thus, influences performance. In this study, as expected, RPE increased over time for all four groups, but groups consuming caffeine showed a slower increase in RPE similar to that described by others (Ali et al., 2016). The slower increase in RPE may help explain the tendency of the NZBC-CAFF and CAFF treatments to sustain higher sprint speeds compared to NZBC and PLA treatments (Carr et al., 2008). Moreover, intake of NZBC-CAFF led to higher blood lactate concentrations compared to PLA (p = 0.039). The results from this study are consistent with studies by Anselme et al. (1992) and Schneiker et al. (2006) that showed that caffeine intake increases lactate concentration during exercise. The higher concentration of blood lactate concentrations with NZBC-CAFF treatment compared to PLA, tie in with the faster sprint speed in the caffeine trials, as shown in other studies (Foskett et al., 2009). Thus, consumption of NZBC-CAFF and CAFF could result in some potential benefits to exercising individuals by reducing perceived exertion and leading to faster sprint times.

Serum FFA concentrations increased with time during the m-LIST ($p < 0.001$) but were not different between treatments. However, NZBC-CAFF was the only treatment in which serum FFA concentration kept increasing 1-h post exercise (p = 0.042, η^2= 0.214). This indicates that the caffeine intake may affect fat metabolism during the low-intensity periods of exercise and lead to increased fat oxidation. This increase in fat oxidation can potentially enhance PCr recovery - crucial for resynthesis of ATP during short sprints. However, we did not measure substrate metabolism or PCr recovery during this study. Since this was the first study to evaluate the individual and additive effect of a single dose NZBC and caffeine intake on serum FFA during high-intensity intermittent running, more research is needed to understand these findings and their influence on exercise. Future studies should evaluate the effect of NZBC and caffeine intake on FFA and other biomarkers such as plasma glycerol and β-hydroxybutyrate to provide deeper insights on lipolysis and fat metabolism during and after exercise.

4.5. Limitations and future directions

Our study was a Latin square design with four arms which was challenging due to the heavy burden it placed on participants and also due to injuries, unavailability, and effects of the Covid-19 pandemic. This resulted in incomplete trials, and challenges to recruitment of well-trained participants (who

could not dedicate time for the trials due to their other heavy training load commitments). Second, the power analysis for sample size was based on data from well-trained male football players, which may not translate to recreationally active males in a fatigued state. We envisioned having more well-trained players for this study but, due to several reasons (including Covid-19, unavailability of lab space at appropriate times, lack of willing team coaches/managers) this was not possible. Larger participant numbers might be necessary to achieve significant results. Third, there is a possibility that consumption of a standardised breakfast may have delayed or reduced the absorption of NZBC anthocyanin in this cohort. But, without measuring plasma anthocyanin levels, the profile of plasma concentrations over time remain unknown. Lastly, we only used serum FFA concentrations as an indicator of fat metabolism. Additional assessments of plasma glycerol and β-hydroxybutyrate could have provided a more comprehensive understanding of the effects of acute NZBC consumption on lipolysis and fatty acid oxidation.

Given the apparent lack of acute effects of NZBC intake on performance, future studies should consider implementing a loading phase (e.g. up to 7 days' supplementation), followed by a final dose 1-2 h before exercise. This approach aligns with earlier studies by Willems et al. (2016), Cook et al. (2015), and Montanari et al. (2021) that demonstrated beneficial effects of NZBC intake. Future studies should also focus on assessing time to exhaustion a performance marker, since no changes in speed and distance were observed during the 90-minute m-LIST. Future studies should also evaluate plasma anthocyanin concentration before and following standardised meals as well as before, during and after exercise to evaluate the absorption of NZBC anthocyanins which may provide valuable insights to its mechanism of action.

4.6. Conclusion

This study was the first to examine the individual and combined effects of a single dose of NZBC powder and caffeine on running and sprint performance in a fatigued state. The findings revealed that intake of caffeine with and without NZBC helped sustain higher sprint speeds. It is possible that a loading period for NZBC intake (e.g. 7 days' supplementation) may be necessary for enhancement of performance. Overall, while caffeine consumption resulted in reducing perceived exertion, further research is needed to explore the effects of NZBC and caffeine consumption on fat metabolism and exercise capacity.

Chapter 5
Evaluating the Individual and Additive Effects of New Zealand Blackcurrant Powder and Caffeine Intake on Biomechanical and Kinematic Sprint Variables using Insole-Embedded Smart Inertial Measurement Units

Abstract

Background: The modified Loughborough Intermittent Shuttle Test (m-LIST) is an endurance performance test that enables the assessment of sprint and reaction time using sprint gates. However, no previous studies using the m-LIST have measured other biomechanical and kinematic sprint variables such as acceleration, deceleration, stride length, and ground contact time, that also determine performance. Adding newer and advanced technology such as the insole-embedded smart inertial measurement unit (IMU) to the m-LIST protocol can help assess biomechanical and kinematic changes that significantly influence competitive outcomes.

Aim: To evaluate the individual and additive effects of a single dose of New Zealand blackcurrant (NZBC) powder (12 g) and caffeine (240 mg) intake on sprint performance, gait, and load during the m-LIST using smart IMUs.

Methods: Fourteen recreationally active males participated in a double blind, randomised controlled crossover trial consisting of four experimental arms: placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF). Participants reported to the laboratory fasted and consumed the study drink before engaging in the m-LIST protocol. The m-LIST consisted of 6 x 15-min blocks of exercise, with each block involving repeated cycles of walk, sprint, run, and jog. The participants had 'smart IMU pods' embedded in their insoles that collected the data during the m-LIST. Upon completion of the exercise, the data was uploaded on the manufacturer's web platform for analysis.

Results: Maximum speed and average peak sprint speed decreased as the trial progressed from blocks 1 to 6 for all treatments ($p = 0.041$, $\eta^2 = 0.505$ and $p = 0.013$, $\eta^2 = 0.566$) with no effect of treatment. There was an effect of treatment on peak deceleration ($p = 0.027$, $\eta^2 = 0.448$) with no effect of time. Specifically, pairwise comparison showed that intake of CAFF had higher peak deceleration compared to NZBC intake ($p = 0.038$) during the m-LIST protocol. There was a decrease right leg stride length ($p = 0.038$, $\eta^2 = 0.542$) with a trend for an increase in average left leg ground contact time ($p = 0.070$, $\eta^2 = 0.476$) as the trial progressed form blocks 1 to 6.

Conclusion: Incorporating the smart IMU system during the m-LIST provided insights to multiple sprint variable that have not been possible previously while using sprint gates. We observed a decrease in maximum speed and average peak sprint speed as the trial progressed for all treatments along with a higher peak deceleration with CAFF treatment during the m-LIST. However, the IMUs did not accurately assess distance covered. More research is needed to test the reliability and validity of using the smart IMUs to assess running performance during the m-LIST.

Keywords: Polyphenols, performance analysis, intermittent-exercise, sports, football

5.1. Introduction

Football (soccer) consists of repeated periods of running, sprinting, and recovery, and thus, is considered a high-intensity intermittent running sport. The number of repeated sprints and periods of high-intensity running vary from game to game due to field position and competition level (Brito et al., 2024). Repeated sprints also lead to fatigue, which is defined as the failure to maintain the intensity of a given effort over time (Brito et al., 2024). Fatigue is influenced by the type of muscle contraction, duration, frequency, and intensity of the game, physiological and training status of the athlete, and environmental conditions (Brito et al., 2024). Several laboratory protocols such as the Loughborough Intermittent Shuttle-running Test (LIST; for assessment of exercise capacity) (Nicholas et al., 2000), modified-Loughborough Intermittent Shuttle-running Test (m-LIST; for assessment of endurance performance) (Ali et al., 2014), 90-min soccer-specific aerobic field test (SAFT-90) (Small et al., 2010), and ball-sport endurance and speed test (BEAST) (Williams et al., 2010) have been designed to replicate the demands of high-intensity intermittent sports, such as football. This allows for the type, intensity, and duration of the exercise to be manipulated and performed in a neutral environment, which enables a controlled assessment of the effect of fatigue on repeated-sprint performance, and provides a model to evaluate interventions designed to reduce fatigue.

Running and sprint performance during laboratory based protocols are commonly assessed using methods such as fully automatic timing systems, sprint gates, floor pods, global positioning system (GPS), and laser and radar devices, with considerable variation between methods (Haugen & Buchheit, 2016). At present, both professional sport and academic institutes use sprint gates and GPS to evaluate running and sprint performance in football players.

The LIST and the m-LIST protocols that simulate repeated-sprint and running performance for high-intensity intermittent team sports such as football, use four sprint gates and a custom-made mobile application to record the split times (Ali et al., 2014). Both the LIST and m-LIST have previously been used to assess muscle damage and fatigue during simulated football (Chou et al., 2021) as well as effectiveness of a sports supplement such as caffeine (Ali et al. (2007) and New Zealand blackcurrant products (Willems et al., 2016) on repeated-sprint performance during intervention trials. Sprint gates consist of infrared photoelectric cells that measure time and calculate speed and reaction time from split times (Ali et al., 2014), and thus cannot assess other biomechanical and kinematic sprint variables such as acceleration, deceleration, stride length, ground contact time that determine performance. Changes in these parameters are crucial, as even small performance margins can significantly influence competitive outcomes (Christensen et al., 2017). Despite the shortcomings, sprint gates are preferred for intervention studies on sports performance as they can be used in a controlled set-up such as a sports hall, an indoor training pitch, or a laboratory. On the other hand, GPS relies on using

satellite support to monitor activity and is used during training and matches that take place on-field only (Larsson, 2003). GPS can assess more variables such as distance covered, mean and maximum speed, acceleration and deceleration, impact, and body load (Cummins et al., 2013), however, the assessment of acceleration and deceleration using 1- and 5-Hz GPS units have been found to be inaccurate (Cummins et al., 2013).

Acceleration and deceleration are components of running, sprinting, and change of direction, and thus, are important factors of physical performance during a football game. For example, an ~0.8% decrease in sprint speed would increase the chances of a player to lose possession against the opponent, when both players race for the ball (Paton et al., 2001). While acceleration and deceleration influence the performance and the outcome of the play, they also expose players to high levels of mechanical stress to the musculoskeletal system and thus contribute to muscle damage and/or muscle soreness (Vanrenterghem et al., 2017). High-intensity decelerations, in particular, involve rapid decreases in momentum that generate high impact peaks and loading rates on the muscles and connective tissues (Harper & Kiely, 2018). Thus, being able to incorporate sprint variables such as maximum speed, peak sprint speed, acceleration, deceleration, and load during the performance testing would help collect a data set which evaluates several parameters of sprint performance and fatigue as well as mechanical loading that contributes to tissue damage and neuromuscular fatigue.

Use of smart insole-embedded smart inertial measurement units (IMUs) is gaining popularity in the sports performance industry. According to the manufacturers of the IMUs, their accelerometery technology has taken the best elements of GPS (speed bands and load) and kinematic analysis (step-by-step stride asymmetries and ground contact times) and provides variables similar to motion capture and dual force plate systems. Thus, it is best suitable for on-field assessments for running and jumping for team sports such as basketball and football.

In order to measure the aforementioned sprint variables that the photocells cannot assess during football simulation tests such as the m-LIST, and also, in an environment where GPS cannot be used (within a laboratory), we evaluated the feasibility of incorporating an insole-embedded smart inertial IMU system in our study. Our study used the m-LIST to explore the individual and additive effects of New Zealand blackcurrant powder (NZBC) and caffeine intake on high-intensity intermittent exercise performance. The smart IMU system consisted of IMUs embedded in insoles that measured accelerations and rotations of the feet and was used to track maximum speed, peak sprint speed, peak acceleration, peak deceleration, and total load during the individual blocks of the m-LIST protocol. We also assessed if the IMU system could detect subtle differences in sprint and running performance in recreationally trained games players with NZBC and caffeine intake.

5.2. Materials and methods

5.2.1. Sample size estimation

To determine the necessary sample size, we conducted a power analysis using online software (G-Power 3.1.9.7), focusing on the primary outcome measure of sprint performance. When comparing caffeine and placebo trials from previous research involving well-trained male football players (Gant et al., 2010) revealed a 4.5% improvement in mean 15-m sprint performance during blocks 5 and 6 of the Loughborough Intermittent Shuttle-running Test (LIST). Utilising an alpha level of 0.05 and a power of 0.80, based on data from the study by Gant et al. (2010), our estimated sample size was 12 participants.

5.2.2. Participants

Twelve healthy male participants were recruited from football clubs within Auckland (mean ± SD, age: 27.6 ± 8.0 years, weight: 76.7 ± 8.2 kg, height: 172.9 ± 25.0 cm, $\dot{V}O_{2max}$: 49.3 ± 4.4 ml·kg^{-1}·min^{-1}). Participants were recreationally active with experience in team sports with high-intensity intermittent running. This study was approved by the Massey University Human Ethics Southern A Committee (Ohu Matatika 1) (approval number 21/09) and registered with the Australia New Zealand Clinical Trials Registry (ACTRN12621001394831). Prior to testing, all participants were informed of the procedures and associated risks, after which they provided written consent and completed a medical history questionnaire.

5.2.3. Study summary

Participants attended the initial two-part familiarisation visit for the assessment of $\dot{V}O_{2max}$, and then reported to the lab four times for the four main trials: i) placebo (PLA), ii) NZBC, iii) caffeine (CAFF), and iv) NZBC and caffeine (NZBC-CAFF). The study was a double blind, randomised controlled crossover study with each trial separated by at least 7 days.

5.2.4. Familiarisation visit

Participants were asked to report to the laboratory in comfortable athletic wear and running shoes and were later taken to the Massey University Recreation Centre, Albany Campus, for their familiarisation visit. The visit was divided into two parts: i) maximal multistage 20-m shuttle run test ('beep' test) to estimate their $\dot{V}O_{2max}$ and ii) undertaking the LIST protocol for 30 min to allow adequate understanding of the running patterns and the experimental procedures in the sports hall. The value obtained from the $\dot{V}O_{2max}$ test was used to establish running speeds for the m-LIST protocol.

5.2.5. Physical activity and dietary standardisation

Participants consumed either a single dose of NZBC or placebo drink the day before the trial (depending on the study treatment the next day) and took part in a 90-min cycling protocol to standardise the amount of physical activity before the trial. Participants were asked to refrain from consuming alcohol, foods containing caffeine, and high amounts of nutritional polyphenolic compounds and antioxidants 48 h before their cycling protocol and till the completion of their running trial. Participants were provided with standardised dinner the day before the running trial and breakfast on the day of the running trial. Participants were also instructed to keep their weekly exercise schedule as consistent as possible.

5.2.6. Experimental procedures

For the main visits, participants reported to the lab in early in the morning after observing at least an 8-hour fast and baseline tests were performed. Participants were asked to provide a mid-stream urine sample to determine hydration status using urine specific gravity (USG) test (Atago 2773 MASTER-SUR/NM Clinical Refractometer, Atago Co., LTD., Japan). If found dehydrated, participants were asked to consume 200 ml of water and repeat this step at every trial to maintain consistency in testing procedure.

5.2.7. Intervention

After baseline assessment of weight and USG test, participants consumed 2 sachets (2 x 12 g) of either NZBC powder, caffinated NZBC powder, caffinated placebo, or placebo mixed with 200 ml of water. The placebo contained the same amount of maltodextrin as NZBC powder with added fructose, glucose, and colour to match the nutritional value and apperance of NZBC powder (Table 5.1). For the caffeinated versions of the same NZBC and PLA powders, 240 mg of caffeine was added to it. All four powders were supplied by the study sponsor. A standardised breakfast containing muesli, yougurt, and milk was provided after consuming the study drink.

5.2.8. Modified Loughborough Intermittent Shuttle Test (m-LIST)

Before starting the m-LIST protocol participants underwent a standardised 10-min warm-up consisting of a total 640 m of jogging, running, and sprinting as well as dynamic and static flexibility exercises of lower limbs and core muscles. The m-LIST protocol requires participants to run back and forth between two lines 20 m apart. The protocol consisted of 6 x 15-min blocks divided into four blocks of "prescribed-pace" activity (blocks 1 - 4) (participants exercise based on audible signals) followed by two blocks of "self-paced" (blocks 5, 6) running (no audible signals, but attempting to maintain the same exercise intensities as the ‘prescribed-pace’ intensity) with a 3-min rest period between each block. Each block consisted of repeated sequences of 3 x 20-m walks at 5.4 km/h, 1 x 15-m sprint, 3 x

20-m run at a speed equivalent to 95% of $\dot{V}O_{2max}$ and 3 x 20-m jog at a speed equivalent to 55% $\dot{V}O_{2max}$ max. A proprietary mobile application (app) produced audio signals guiding participants' speeds during each block. Sprint times were measured using infrared photoelectric cells present in the sprint gates, transmitted wirelessly to the app. Participants completed 11 cycles per block from blocks 1 to 4 as they were "prescribed pace" activity, and they received audio signals for repeated cycles. However, for "self-paced" blocks 5 and 6, participants continued the repeated sequence until they received a signal for cessation of exercise at the end of the 15-min mark for both the blocks. Participants were instructed to replicate intensities and exercise patterns observed during the prescribed-pace blocks (for more detailed information see Ali et al. (2014). Participants consumed water at a rate of 2 ml/kg BM after warm-up, followed 2 ml/kg BM after every block from blocks 1 to 5. Participants concluded the trial after completing the 15 min in block 6.

Table 5.1 Nutritional Content of NZBC, caffeinated NZBC, caffeinated placebo, and placebo powder (24g)

	NZBC Powder	**Caffeinated NZBC Powder**	**Caffeiante Placebo Powder**	**Placebo Powder**
Energy (kJ)	377	377	377	377
Energy (kcals)	90	90	90	90
Total carbohydrates (g)	20.6	20.3	20.6	20.6
Total added sugar (g)	9	9	20.6	20.6
Vitamin C (mg)	140	140	0	0
Anthocyanins (mg**)**	240	240	0	0
Caffeine (from green coffee beans) (mg)	0	240	240	0

Other ingredients: Maltodextin, monk fruit extract, natural flavours (No added colours or preservatives)

5.2.9. Insole-embedded smart inertial measurement unit system

In this study we used a smart insole system that uses IMUs to track and analyse biomechanics in real world settings (Plantiga Technologies Inc., Canada). The IMUs have been internally validated for gait metrics and speed (unpublished results). According to their website, their experiments showed that gait metrics were predicted with the maximum error IQR of 1.6% and 6.1% for single leg and double leg metrics respectively. Their experiments for speed validation demonstrated that speeds were predicted within -0.24% error (R2 = 1.00%, p = 0.00).

The insoles measure accelerations and rotations of the feet and can measure multiple metrics such as walk, run, consecutive countermovement jump, and more. For our study, run metrics was selected to assess sprint performance during the m-LIST protocol and it evaluated maximum speed, average peak sprint (average of top speed achieved during sprints), peak acceleration, peak deceleration, stride length, and total load among many variables during indvidual blocks.

5.2.10. Components of IMU system

The IMU system consists of four components namely, inertial measurement units (pods), insoles, docking station, and a web platform. The pods, insoles, and the docking station come in the travel safe case that allow pods to be in a sleep mode.

i. Inertial Measurement Units (pods)

The IMUs (sized 35.4mm x 28.8mm x 3.6mm) are the sensors that get embedded into the insoles and collect data samples at 416 Hz frequency using the 6-axis inertial measurement unit technology. They are time synchronised, water and impact resistant and have an 8-h recording time with local storage.

Figure 5.1 Inertial measurement units (pods)

ii. Insoles

Insoles are made using supportive ethylene-vinyl acetate copolymer foam and can be trimmed to fit custom sizing. Insoles help maintain consistent sensor placement for recording data during testing.

Figure 5.2 IMU insoles

iii. Docking station

The docking station establishes a physical connection to charge the pods and upload data after recording. It also enables both functions of charging and uploading simultaneously. The onboard LEDs indicate charge and upload status (e.g. green LEDs signify that the pods have uploaded all stored data and are fully time synched and charged).

Figure 5.3 IMU docking station

iv. Web platform

The manufacturer's web platform is where one can view, analyse, download data, and create reports. It also has features to customise data views and observe trends over time.

Figure 5.4 Web platform

5.2.10.1. Using IMUs for the m-LIST protocol

After completing the warm-up protocol, participants were asked to remove their insoles and insert the insoles embedded with fully charged IMUs into their shoes. Investigators ensured that the pods were connected to the web platform before starting the protocol. The 'running' metric option was selected through the web platform and participants began running in repeated sequence cycles for each block for the m-LIST protocol. The insoles also recorded the five 3-min rest periods after blocks 1 to 5. Investigators pressed 'stop' on the web platform at the end of the test i.e. after 6 blocks and five 3-min rest periods at approximately 105 min since the start of the test. Participants removed the insoles, and the pods were placed onto the docking station to upload the data and generate a report. Different variables were assessed for performance, gait patterns, and load for our protocol.

5.2.10.2. Generating the run report

The manufacturer's software generated a full report for the 90-min running protocol. However, to compare and evaluate each block, the report was manually divided into each of the six individual blocks and extracted data to analyse the results for each trial for each participant (Figure 5.5).

5.2.11. Statistical analysis

Due to missing data points, we analysed data from only from 6 participants despite recruiting 12 participants for this study. The data was analysed using a two-way ANOVA with repeated measures (IBM SPSS Statistic (v. 28.0.1, IBM, USA) to examine main effects of i) treatment and ii) time and iii) interaction of treatment*time. Bonferroni's pairwise comparisons with 95% confidence were conducted to identify specific differences between groups and timepoints. Differences were considered significant at an alpha level of $p < 0.05$. To determine the effect size of responses, partial eta^2 (η^2) was also calculated using SPSS. The threshold values for effect size were interpreted as small (0.01), medium (0.06), and large effects (0.14). Data is presented as mean ± SD.

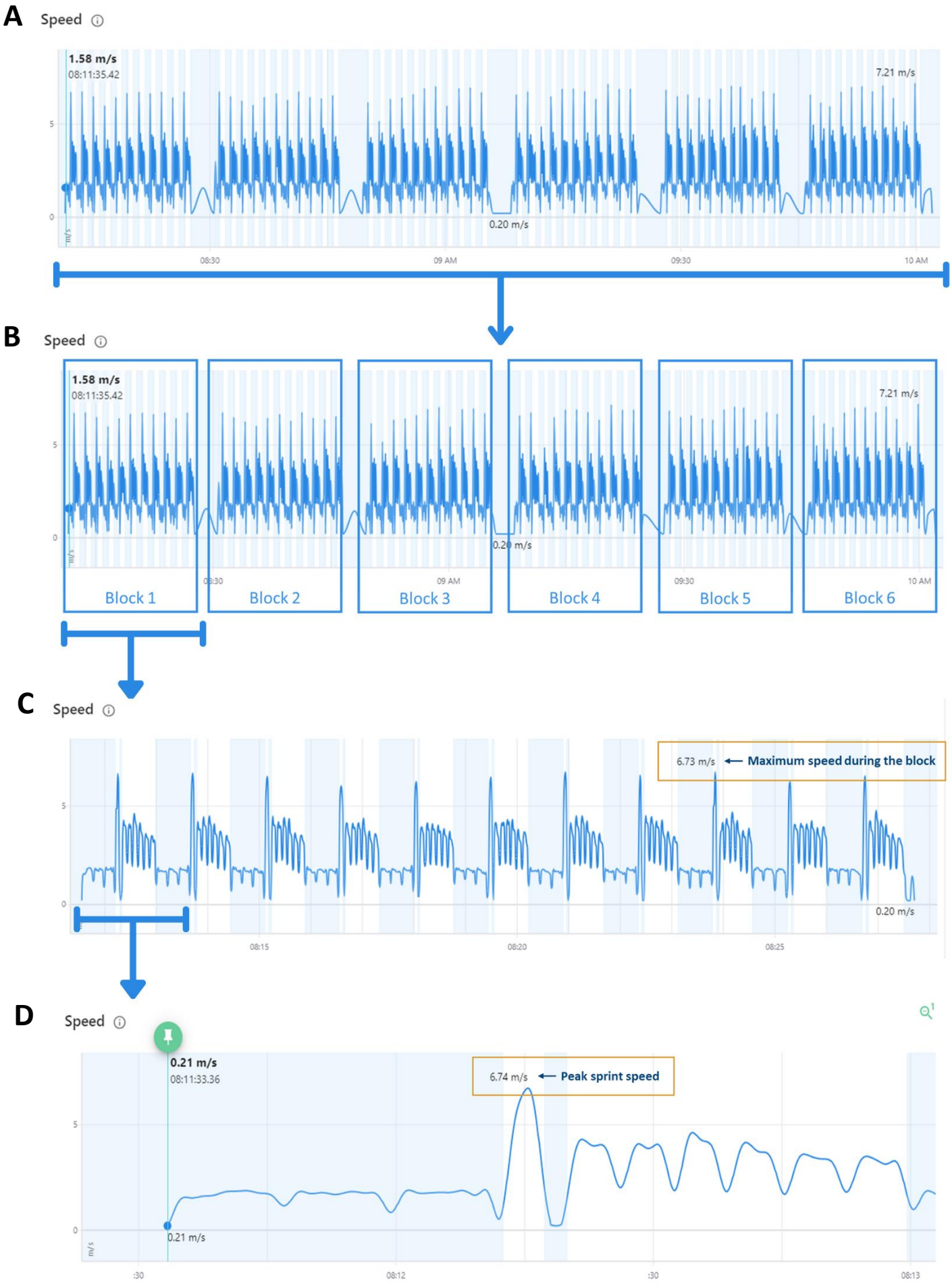

Figure 5.5 Dividing the participant report on the web platform to create individual data sets for each block: (A) data obtained from a full 90-min running trial (B) dividing the trial into 6 blocks (C) expanding a block to identify and evaluate a cycle (D) expanding a cycle to obtain the Peak Sprint Speed.

5.3. Results

5.3.1. Performance

Maximum speed and average peak sprint speed decreased as the trial progressed from blocks 1 to 6 for all treatments (p = 0.041, η^2 = 0.505 and p = 0.013, η^2 = 0.566), but were not different between treatments. There was an effect of treatment on peak deceleration (p = 0.027, η^2 = 0.448) with no effect of time. Pairwise comparison demonstrated that intake of CAFF had higher peak deceleration compared to NZBC intake (p = 0.038) during block 4 of the m-LIST protocol. Similarly, there was also trend for NZBC-CAFF to have higher peak deceleration compared to NZBC (p = 0.079) during block 4 of the m-LIST protocol. There was a trend for peak acceleration to decrease for all treatments as the trial progressed from blocks 1 to 6 with a large effect size (p = 0.055, η^2 = 0.521). However, there was no effect of treatment*time for all performance variables (Table 5.2).

The distance covered during the m-LIST protocol was inaccurately assessed by the IMUs reported. For the prescribed-pace blocks 1 to 4, participants covered 2.14 km of distance while alternating between walk, sprint, run, and jog, however the IMUs reported less than 1.8 km of distance covered for each block for all study visits. Hence, the results for distance covered have been excluded from this section (see appendix 4.2, supplementary material).

Table 5.2 Performance measures assessed using the smart IMU system during blocks 1 to 6 of the modified Loughborough Intermittent Shuttle-running Test.

	Treatment	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of Treatment (*p* value and effect size)	Effect of Time (*p* value and effect size)	Effect of Treatment *Time (*p* value and effect size)
Maximum speed (km/h)	PLA	25.9 ± 1.3	25.8 ± 1.7	25.6 ± 1.5	25.4 ± 1.7	25.5 ± 1.9	25.9 ± 2.1	*p* = 0.253	***p* = 0.041**	*p* = 0.285
	NZBC	25.8 ± 1.9	25.4 ± 1.7	24.5 ± 1.9	24.0 ± 1.9	24.6 ± 2.3	25.1 ± 2.7			
	CAFF	26.6 ± 1.2	26.4 ± 1.1	25.9 ± 1.4	25.6 ± 1.3	25.2 ± 1.7	25.5 ± 2.0	η^2 = 0.214	η^2 = 0.505	η^2 = 0.214
	NZBC-CAFF	26.2 ± 1.5	25.7 ± 1.2	25.4 ± 1.3	25.5 ± 1.8	25.1 ± 1.8	25.2 ± 2.4			
Average peak sprint speed (km/h)	PLA	24.9 ± 1.1	24.7 ± 1.5	24.4 ± 1.5	24.3 ± 1.7	24.6 ± 2.0	24.8 ± 1.4	*p* = 0.542	***p* = 0.013**	*p* = 0.191
	NZBC	24.6 ± 1.2	24.1 ± 2.1	23.3 ± 1.9	22.8 ± 2.0	23.4 ± 2.4	23.7 ± 2.3			
	CAFF	25.4 ± 1.1	25.3 ± 1.0	24.8 ± 1.5	24.2 ± 1.8	24.1 ± 2.0	24.5 ± 1.3	η^2 = 0.110	η^2 = 0.566	η^2 = 0.219
	NZBC-CAFF	25.1 ± 1.2	24.6 ± 1.2	24.1 ± 1.2	23.8 ± 2.0	23.8 ± 2.1	24.0 ± 1.6			
Average speed (km/h)	PLA	12.8 ± 0.7	12.8 ± 0.6	12.6 ± 0.6	12.6 ± 0.5	12.4 ± 1.0	12.4 ± 1.0	*p* = 0.709	*p* = 0.073	*p* = 0.631
	NZBC	12.4 ± 0.4	12.5 ± 0.5	12.3 ± 0.4	12.4 ± 0.6	12.1 ± 0.9	12.0 ± 1.0			
	CAFF	12.6 ± 0.7	12.6 ± 0.8	12.5 ± 0.9	12.5 ± 0.9	11.9 ± 1.0	12.0 ± 1.0	η^2 = 0.086	η^2 = 0.470	η^2 = 0.097
	NZBC-CAFF	12.8 ± 0.8	12.8 ± 0.9	12.7 ± 0.8	12.4 ± 0.8	12.1 ± 1.1	11.9 ± 0.9			
Peak acceleration (m/s^2)	PLA	3.7 ± 0.3	3.6 ± 0.3	3.5 ± 0.3	3.6 ± 0.4	3.4 ± 0.3	3.4 ± 0.5	*p* = 0.359	*p* = 0.055	*p* = 0.477
	NZBC	3.8 ± 0.3	3.6 ± 0.3	3.5 ± 0.3	3.5 ± 0.3	3.3 ± 0.4	3.3 ± 0.4			
	CAFF	3.9 ± 0.3	3.8 ± 0.3	3.5 ± 0.3	3.6 ± 0.2	3.4 ± 0.4	3.4 ± 0.4	η^2 = 0.176	η^2 = 0.521	η^2 = 0.155
	NZBC-CAFF	3.7 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	3.5 ± 0.4	3.4 ± 0.3	3.2 ± 0.4			
Peak deceleration (m/s^2)	PLA	-4.3 ± 0.3	-4.2 ± 0.4	-4.2 ± 0.3	-4.1 ± 0.3	-4.1 ± 0.4	-4.2 ± 0.4	***p* = 0.027**	*p* = 0.204	*p* = 0.400
	NZBC	-4.2 ± 0.4	-4.1 ± 0.4	-3.4 ± 0.4	-3.9 ± 0.3	-3.2 ± 0.5	-3.8 ± 0.7			
	CAFF	-4.4 ± 0.2	-4.4 ± 0.2	-4.2 ± 0.3	-4.1 ± 0.3	-4.1 ± 0.4	-4.1 ± 0.4	η^2 = 0.448	η^2 = 0.270	η^2 = 0.175
	NZBC-CAFF	-4.4 ± 0.2	-4.2 ± 0.4	-4.2 ± 0.2	-4.3 ± 0.4	-4.1 ± 0.4	-4.2 ± 0.6			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + caffeine. Values are mean ± SD.

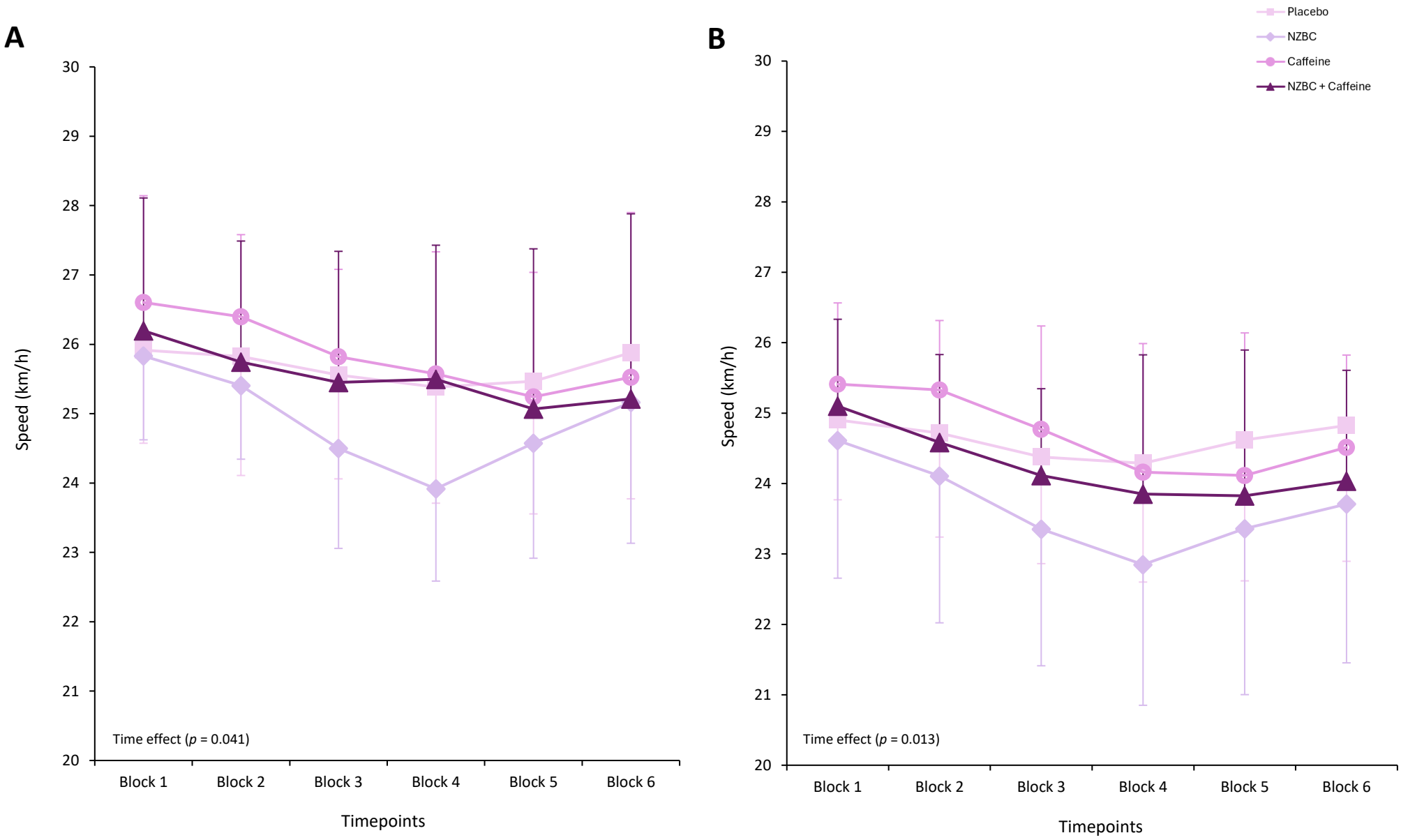

Figure 5.6 Changes in (A) maximum sprint speed and (B) average peak sprint speed (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF) trials from blocks 1 to 6 during modified Loughborough intermittent Shuttle Test. (A) Significantly higher in CAFF group compared to NZBC group and (B) significantly higher in CAFF and PLA group compared to NZBC group.

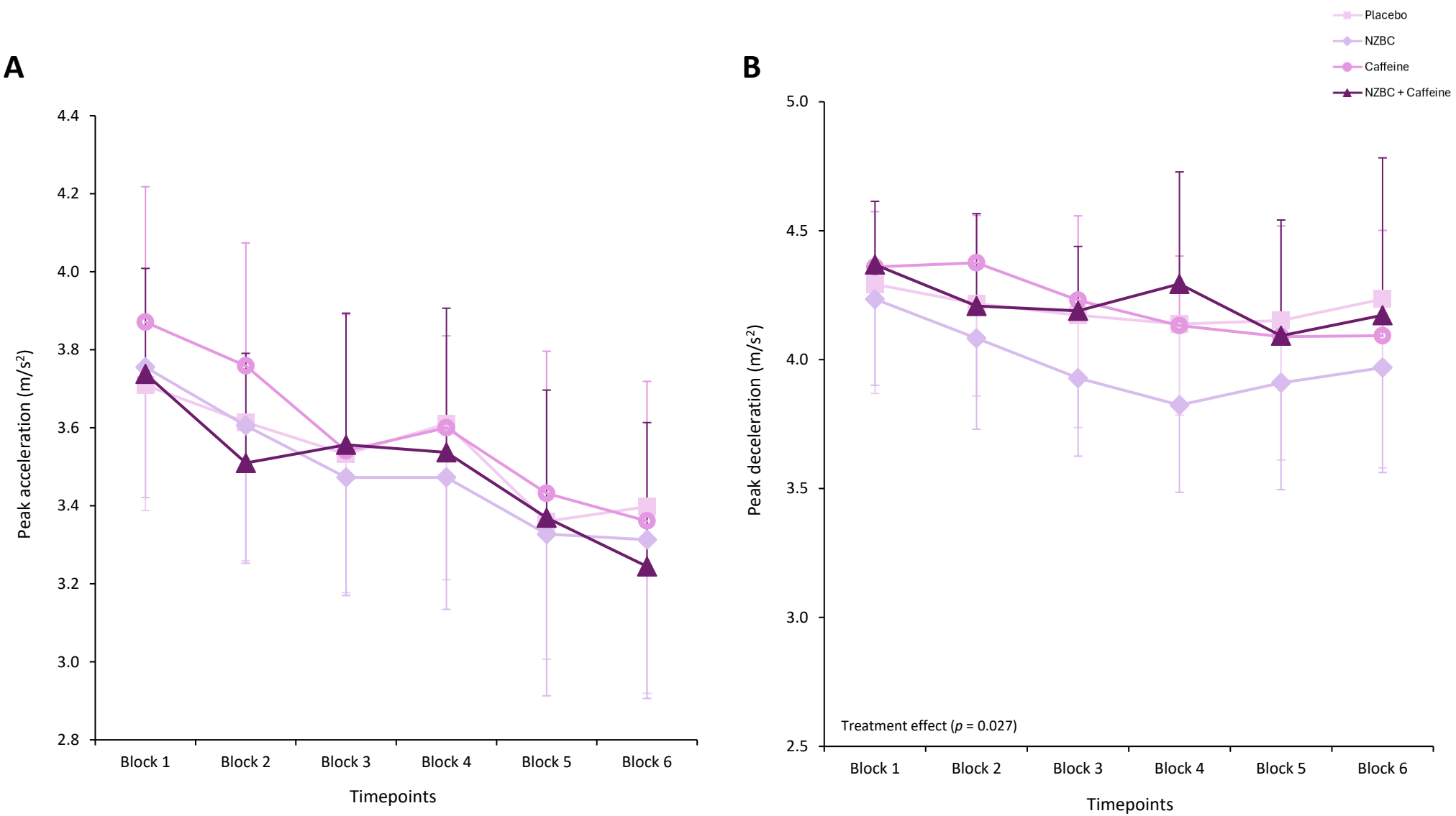

Figure 5.7 Changes in (A) acceleration and (B) deceleration (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF) trials from blocks 1 to 6 during modified Loughborough intermittent Shuttle Test. (B) significantly higher in NZBC-CAFF, CAFF, and PLA group compared to NZBC group

5.3.2. Gait patterns

There was no effect of treatment for all variables of gait pattern (Table 5.3). However, as the trial progressed from blocks 1 to 6 there was a decrease right leg stride length ($p = 0.038$, $\eta^2 = 0.542$) with a trend for an increase in average left leg ground contact time ($p = 0.070$, $\eta^2 = 0.476$) (Table 5.3).

Table 5.3 Gait patterns assessed using the smart IMU system during blocks 1 to 6 of the modified Loughborough Intermittent Shuttle Test.

		Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of Treatment (*p* value and effect size)	Effect of Time (*p* value and effect size)	Effect of Treatment *Time (*p* value and effect size)
Cadence (steps/min)	PLA	175.0 ± 10.3	175.5 ± 10.3	174.6 ± 9.6	174.2 ± 9.7	173.8 ± 11.2	173.8 ± 11.7	*p* = 0.237 η^2 = 0.255	*p* = 0.198 η^2 = 0.289	*p* = 0.456 η^2 = 0.155
	NZBC	175.3 ± 8.4	174.8 ± 7.7	173.5 ± 8.0	172.8 ± 8.1	173.1 ± 8.3	173.3 ± 8.1			
	CAFF	175.8 ± 6.7	175.6 ± 8.0	175.3 ± 7.2	175.1 ± 7.8	173.9 ± 7.8	174.5 ± 7.7			
	NZBC-CAFF	174.2 ± 7.6	174.7 ± 7.8	174.8 ± 8.0	174.5 ± 8.4	173.5 ± 8.1	173.9 ± 9.2			
Average Stride Length (m)	PLA	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.2	2.5 ± 0.2	*p* = 0.755 η^2 = 0.059	***p* = 0.037** η^2 = 0.546	*p* = 0.535 η^2 = 0.129
	NZBC	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.1	2.4 ± 0.2	2.4 ± 0.2			
	CAFF	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
	NZBC-CAFF	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
Left Leg Average Stride Length (m)	PLA	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.1	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	*p* = 0.751 η^2 = 0.059	***p* = 0.035** η^2 = 0.550	*p* = 0.504 η^2 = 0.137
	NZBC	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
	CAFF	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
	NZBC-CAFF	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
Right Leg Average Stride Length (m)	PLA	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	*p* = 0.750 η^2 = 0.058	***p* = 0.038** η^2 = 0.542	*p* = 0.551 η = 0.118
	NZBC	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
	CAFF	2.3 ± 0.6	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
	NZBC-CAFF	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	2.4 ± 0.2	2.4 ± 0.2			
Left Leg Stride Length Variability (m)	PLA	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	*p* = 0.260 η^2 = 0.239	***p* = 0.004** η^2 = 0.632	*p* = 0.699 η^2 = 0.098
	NZBC	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1			
	CAFF	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1			
	NZBC-CAFF	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1			

Right Leg Stride Length Variability (m)	PLA	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	$p = 0.057$ $\eta^2 = 0.395$	**$p = 0.003$** $\eta^2 = 0.727$	$p = 0.488$ $\eta^2 = 0.148$
	NZBC	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1			
	CAFF	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1			
	NZBC-CAFF	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1			
Ground Contact Time (ms)	PLA	217.2 ± 11.0	218.5 ± 12.9	221.6 ± 15.3	222.2 ± 15.0	225.0 ± 21.2	225.2 ± 23.2	$p = 0.430$ $\eta^2 = 0.163$	$p = 0.096$ $\eta^2 = 0.431$	$p = 0.456$ $\eta^2 = 0.156$
	NZBC	221.1 ± 14.3	221.7 ± 14.6	225.8 ± 15.9	227.1 ± 17.6	231.2 ± 20.8	230.0 ± 16.0			
	CAFF	218.0 ± 17.1	218.9 ± 18.5	220.4 ± 17.5	220.5 ± 17.7	230.0 ± 17.5	228.6 ± 17.9			
	NZBC-CAFF	215.3 ± 17.4	215.3 ± 17.7	217.4 ± 17.6	217.5 ± 18.7	224.5 ± 19.2	224.8 ± 21.4			
Left Leg Ground Contact Time (ms)	PLA	217.6 ± 11.9	218.6 ± 13.3	222.4 ± 15.7	222.3 ± 14.9	225.6 ± 20.8	226.2 ± 22.4	$p = 0.460$ $\eta^2 = 0.152$	$p = 0.070$ $\eta^2 = 0.476$	$p = 0.488$ $\eta^2 = 0.179$
	NZBC	221.0 ± 14.5	222.0 ± 15.4	226.2 ± 17.1	227.4 ± 17.8	231.4 ± 21.7	229.9 ± 16.3			
	CAFF	218.0 ± 17.3	219.4 ± 18.5	220.5 ± 17.5	221.2 ± 17.9	230.4 ± 18.0	229.6 ± 18.3			
	NZBC-CAFF	216.0 ± 17.4	216.0 ± 17.7	217.8 ± 17.4	218.5 ± 17.6	224.9 ± 18.5	225.7 ± 20.5			
Right Leg Ground Contact Time (ms)	PLA	217.1 ± 10.8	217.8 ± 12.5	220.7 ± 14.8	221.4 ± 15.3	224.1 ± 23.2	224.4 ± 24.2	$p = 0.990$ $\eta^2 = 0.007$	$p = 0.429$ $\eta^2 = 0.142$	$p = 0.553$ $\eta^2 = 0.099$
	NZBC	221.4 ± 14.1	222.0 ± 13.5	225.4 ± 14.9	226.7 ± 17.3	230.4 ± 20.9	216.6 ± 36.7			
	CAFF	217.4 ± 17.7	218.2 ± 18.5	218.9 ± 18.1	220.0 ± 18.0	228.8 ± 17.8	227.5 ± 18.1			
	NZBC-CAFF	215.0 ± 17.8	215.2 ± 17.9	216.2 ± 17.3	217.8 ± 18.3	223.4 ± 20.3	224.0 ± 22.8			
Flight Time (ms)	PLA	144.5 ± 41.6	141.4 ± 44.3	144.4 ± 38.8	140.7 ± 44.4	142.1 ± 45.2	144.6 ± 39.0	$p = 0.536$ $\eta^2 = 0.084$	$p = 0.428$ $\eta^2 = 0.150$	$p = 0.565$ $\eta^2 = 0.142$
	NZBC	131.3 ± 18.4	131.6 ± 19.5	130.8 ± 17.4	130.8 ± 19.8	128.4 ± 19.4	122.0 ± 31.3			
	CAFF	128.5 ± 21.8	131.4 ± 29.2	133.6 ± 27.1	135.4 ± 29.6	125.8 ± 30.8	123.1 ± 21.1			
	NZBC-CAFF	146.5 ± 35.4	130.7 ± 35.0	144.0 ± 36.7	128.7 ± 36.5	127.0 ± 38.1	132.9 ± 31.5			
Flight Ratio	PLA	0.4 ± 0.03	0.4 ± 0.04	0.4 ± 0.04	0.4 ± 0.04	0.4 ± 0.05	0.4 ± 0.05	$p = 0.655$ $\eta^2 = 0.070$	**$p = 0.049$** $\eta^2 = 0.505$	$p = 0.848$ $\eta^2 = 0.061$
	NZBC	0.4 ± 0.04	0.4 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.05	0.3 ± 0.05			
	CAFF	0.4 ± 0.05	0.4 ± 0.05	0.4 ± 0.05	0.4 ± 0.05	0.3 ± 0.05	0.3 ± 0.05			
	NZBC-CAFF	0.4 ± 0.05	0.4 ± 0.05	0.4 ± 0.05	0.4 ± 0.06	0.4 ± 0.06	0.4 ± 0.06			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

5.3.3. Assessment of mechanical load

There was no effect of time, treatment, and treatment*time for total, left leg, and right leg total load and intensity (Table 5.4).

Table 5.4 Load assessment using the smart IMU system during blocks 1 to 6 of the modified Loughborough Intermittent Shuttle-running Test.

		Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of Treatment (*p* value and effect size)	Effect of Time (*p* value and effect size)	Effect of Treatment *Time (*p* value and effect size)
Total Load (10^3·g-force)	PLA	34.9 ± 4.1	35.1 ± 4.7	36.0 ± 4.6	35.4 ± 4.4	32.9 ± 4.8	27.2 ± 12.2	*p* = 0.395 η^2 = 0.160	*p* = 0.129 η^2 = 0.393	*p* = 0.338 η^2 = 0.188
	NZBC	36.2 ± 3.6	36.1 ± 3.5	36.2 ± 3.7	36.0 ± 3.8	32.7 ± 4.9	31.6 ± 8.2			
	CAFF	35.7 ± 5.6	34.7 ± 6.5	36.0 ± 4.4	36.0 ± 4.1	32.5 ± 4.4	30.9 ± 8.0			
	NZBC-CAFF	36.4 ± 4.9	36.9 ± 4.6	36.8 ± 4.1	37.0 ± 4.4	33.9 ± 4.7	31.8 ± 9.1			
Left Leg Load (10^3·g-force)	PLA	17.4 ± 2.1	17.7 ± 2.4	17.6 ± 2.4	17.8 ± 2.3	16.5 ± 2.5	15.6 ± 4.5	*p* = 0.583 η^2 = 0.087	*p* = 0.206 η^2 = 0.295	*p* = 0.528 η^2 = 0.137
	NZBC	17.9 ± 2.0	18.1 ± 1.9	18.1 ± 1.9	18.1 ± 2.0	16.5 ± 2.5	16.0 ± 4.2			
	CAFF	17.9 ± 2.7	17.4 ± 3.2	18.0 ± 2.1	18.2 ± 2.0	16.4 ± 2.4	15.7 ± 4.2			
	NZBC-CAFF	18.3 ± 2.5	18.6 ± 2.4	18.6 ± 2.1	18.7 ± 2.3	17.1 ± 2.5	16.0 ± 4.7			
Right Leg Load (10^3·g-force)	PLA	17.4 ± 2.1	17.6 ± 2.4	17.8 ± 2.2	17.6 ± 2.2	16.4 ± 2.3	15.2 ± 4.2	*p* = 0.632 η^2 = 0.075	*p* = 0.187 η^2 = 0.317	*p* = 0.475 η^2 = 0.151
	NZBC	18.1 ± 1.8	17.9 ± 1.7	18.0 ± 1.9	18.0 ± 1.8	16.2 ± 2.4	15.6 ± 4.1			
	CAFF	17.7 ± 2.9	17.3 ± 3.4	18.0 ± 2.5	18.0 ± 2.2	16.1 ± 2.1	15.2 ± 3.9			
	NZBC-CAFF	18.1 ± 2.5	18.3 ± 2.37	18.2 ± 2.1	18.3 ± 2.3	16.8 ± 2.4	15.7 ± 4.5			
Intensity (10^3·gpm)	PLA	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	*p* = 0.628 η^2 = 0.065	*p* = 0.486 η^2 = 0.111	*p* = 0.321 η^2 = 0.203
	NZBC	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.3	2.3 ± 0.2			
	CAFF	2.4 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.4 ± 0.3	2.3 ± 0.3	2.4 ± 0.4			
	NZBC-CAFF	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.3 ± 0.3			
Left Leg Intensity (10^3·gpm)	PLA	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	*p* = 0.673 η^2 = 0.056	*p* = 0.599 η^2 = 0.065	*p* = 0.399 η^2 = 0.175
	NZBC	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.2			
	CAFF	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	1.2 ± 0.1			
	NZBC-CAFF	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.2			
Right Leg Intensity (10^3·gpm)	PLA	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	*p* = 0.632 η^2 = 0.072	*p* = 0.480 η^2 = 0.111	*p* = 0.444 η^2 = 0.161
	NZBC	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	1.2 ± 0.1			
	CAFF	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	1.1 ± 0.2	1.1 ± 0.1			
	NZBC-CAFF	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.2			

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

5.4. Discussion

This was the first study to incorporate the smart IMU system during a protocol that simulates the demands of football, such as the m-LIST, to assess biomechanical and kinematic sprint variables including acceleration, deceleration, stride length, and ground contact time. We found that using the IMUs could assess the biomechanical and kinematic during each block of the m-LIST protocol and identify differences in performance after the consumption of a single dose of NZBC with and without caffeine.

Laboratory-based protocols such as the LIST and m-LIST have been developed to understand the influence of fatigue on repeated-sprint and running performance during high-intensity intermittent sports. Fatigue manifests in the form of failure to maintain the intensity of a given effort over time, and thus, can be demonstrated by a decrease in sprint and running speed as well as acceleration. In previous studies, while speed is assessed as a split time during the LIST and the m-LIST, incorporating the IMUs could evaluate the changes in acceleration at the same time. Since we used the IMUs to evaluate the effect of NZBC and caffeine intake on running performance during the m-LIST, we observed that the intake of CAFF had higher peak deceleration compared to NZBC intake (p = 0.038) during the m-LIST protocol. We also observed that maximum speed and average peak sprint speed decreased as the trial progressed for all treatments (p = 0.041, η^2 = 0.505 and p = 0.013, η^2 = 0.566). There was a decrease right leg stride length (p = 0.038, η^2 = 0.542) with a trend for an increase in average left leg ground contact time (p = 0.070, η^2 = 0.476). This demonstrated that the smart insole system (i) could assess multiple biomechanical and kinematic variables in each individual block and (ii) was sufficiently sensitive to detect the differences in the expected decrease in speed and deceleration during m-LIST with NZBC and caffeine intake, both indicative of fatigue and a subsequent impairment in repeated-sprint performance.

Acceleration decreases with time during repeated-sprints tests and football matches, and hence, can be considered as marker of fatigue (Harper et al., 2019). In our study, we observed a trend for peak acceleration to decrease for all treatments as the trial progressed with a large effect size (p = 0.055, η^2 = 0.521). Since the m-LIST is a representation of the high-intensity intermittent running pattern during a football match, there is a potential for IMUs to be used during football matches for the assessment of fatigue.

The study by Magalhães et al. (2010) reported similar increases in creatine kinase and delayed-onset muscle soreness between a match and the LIST during recovery. This indicated that the number of turns and the repeated accelerations and decelerations of the LIST caused a similar extent of muscle damage as observed during a football match. Moreover, IMUs can also detect jumping, running

backwards/sideways, and sudden change in direction as seen during matches. These activities along with tackles, typically rely on the eccentric contractions that tend to increase the neuromuscular demands during matches compared to the LIST (Magalhães et al., 2010), making IMUs a useful tool to assess the mechanical load that leads to muscle damage during football matches.

Fatigue leads to a prominent decrease in hamstring strength and power output, and alters its activation patterns, thus elevating the risk of hamstring injuries during acceleration and other high-velocity actions (Harper et al., 2019; Huygaerts et al., 2020; Schwiete et al., 2023). Thus, incorporating methods such as an insole-embedded IMU system that captures the acceleration would improve the understanding of the onset of fatigue during repeated-sprint exercise and potentially highlight the risk for hamstring injury.

The smart insole units also assessed three performance variables that influence maximum speed – stride length, cadence, and ground contact time. There was no change in cadence across all groups as the trial progressed from blocks 1 to 6, but a decrease in average stride length with a trend for an increase in average left leg ground contact time ($p = 0.070$, $\eta^2 = 0.476$). Shorter stride length combined with longer ground contact time is associated with lower maximum sprint speeds (Korhonen et al., 2009). This was consistent with the observed decrease in maximum speed and average peak sprint speed in our study, which could be indicators of fatigue. Moreover, the smart insole system calculated total load as the cumulative mechanical load experienced during running. The mechanical load decreased as the trial progressed and was similar in all four groups, which further indicated the occurrence of fatigue with repeated-sprints.

5.5. Limitations and future directions

The IMUs underestimated the distance covered by ~16% during each individual block. Similarly, a study by Rawstorn et al. (2014) demonstrated that GPS underestimated the total distance covered during the LIST protocol, which indicated that GPS cannot accurately assess distance covered in protocols with rapid directional change. However, in our study, it could have been because of the selection of 'run' metric at the beginning on the study visit which could have led to the IMUs algorithm excluding the distance covered while walking (participants walked ~600-660 m per block for 6 blocks). Since, the m-LIST protocol can accurately assess distance covered through the sprint gates and the mobile application, the IMU system can be used to assess other sprint performance variables.

Currently, few studies have validated the use of IMUs for single-leg jump, gait analysis during walking, treadmill running, and differences in peak impact accelerations among foot strike patterns in recreational runners (Gaiesky et al., 2023; Napier et al., 2022). However, there are no studies that have tested reliability and validity for a high-intensity intermittent running protocol, and thus, future

research should focus on testing the reliability and validity of using IMUs to assess running performance during the m-LIST to improve the quality and quantity of data collected during a laboratory-based protocol.

5.6. Conclusions

We found that maximum speed and average peak sprint speed decreased as the trial progressed for all treatment. We also observed higher peak deceleration with CAFF treatment. This suggesting that incorporating IMUs during the m-LIST could provide insights to multiple biomechanical and kinematic sprint variables such as acceleration, deceleration, stride length, and ground contact time and identify differences in performance after the consumption of a single dose of NZBC with and without caffeine during repeated sprints intermittent exercise. More research is needed to test the reliability and validity of using IMUs to assess running performance during the m-LIST.

Chapter 6
General Discussion and Conclusions

6.1. Summary of key findings

A summary of the key findings, with their relevance to the three research questions determined for this PhD study (Chapter 1, Section 1.3) is outlined below:

1. **Can a consumption of NZBC drink containing 120 mg of anthocyanins influence substrate oxidation and serum free fatty acid (FFA) concentrations during cycling in recreationally active males?**

The findings from the cycling study (Chapter 3) showed that the intake of a single dose of NZBC had no effect on substrate oxidation and serum FFA concentration during mixed-intensity cycling in recreationally active males. There were no differences in oxygen consumption, energy expenditure, fat oxidation, carbohydrate oxidation, and respiratory exchange ratio between the placebo (PLA) and NZBC treatments during the trial. Furthermore, serum FFA concentration increased with time during cycling but did not different between NZBC and PLA treatments (p = 0.113).

2. **What is the effect of consumption of New Zealand blackcurrant drink (NZBC) containing 240 mg of anthocyanins, with and without 240 mg of caffeine on high-intensity intermittent running performance in fatigued recreationally active males? Does NZBC and caffeine intake affect serum FFA concentration before and after exercise?**

The findings from the running study (Chapter 3) showed that intake of New Zealand blackcurrant and caffeine drink (NZBC-CAFF) did not reduce slowing of sprint speed during blocks 5 and 6 of the modified-Loughborough Intermittent Shuttle Test (m-LIST). However, NZBC-CAFF treatment sustained higher average sprint speed compared to NZBC during the m-LIST protocol (p = 0.025). There was also a trend for CAFF (p = 0.059) and NZBC-CAFF (p = 0.061) treatments to sustain higher average sprint speeds compared to NZBC. Lastly, NZBC-CAFF was the only treatment in which serum FFA concentration kept increasing 1-h post exercise (p = 0.042, η^2= 0.214).

3. **Can we use a smart insole-embedded IMU system during the m-LIST to identify differences between biomechanical and kinematic sprint variables due to fatigue after the consumption of a single dose of NZBC with and without caffeine?**

The findings from Chapter 5 showed that incorporating the smart insole-embedded IMU system provided in-depth analysis of sprint performance. The IMUs were a feasible tool to assess multiple variables of sprint performance such as maximum speed, average peak sprint speed, acceleration, deceleration, cadence, ground contact time, stride length, and mechanical load during each block the m-LIST. The IMUs were also sufficiently sensitive to detect changes in running performance due to

fatigue as well as following ingestion of the intervention drinks (NZBC and caffeine) but did not accurately assess distance covered.

6.2. Discussion of main findings

Acute intake of the NZBC drink did not influence substrate oxidation during mixed-intensity cycling in recreationally active males. However, acute intake of NZBC with caffeine (NZBC-CAFF) and caffeine (CAFF) alone showed higher average sprint speeds compared to other treatments during the m-LIST protocol. New Zealand blackcurrant intake had no effect on serum FFA concentration during mixed-intensity cycling ($p = 0.113$). While the m-LIST protocol caused an increase in serum FFA concentration across all treatments ($p < 0.001$), NZBC-CAFF was the only treatment that showed a sustained increase in the serum FFA levels 1-h post m-LIST protocol ($p = 0.042$ and $\eta^2 = 0.214$).

6.2.1. Acute effect of NZBC intake on substrate oxidation

Our study found that the intake of a single dose of NZBC had no effect on substrate oxidation during cycling in recreationally active males. There were no differences in oxygen consumption, fat oxidation, carbohydrate oxidation, and energy expenditure with NZBC intake during exercise.

We also observed that the respiratory exchange ratio was higher at the beginning of the trial and stayed consistently higher throughout the trial with NZBC and PLA intake. This could have been due to two reasons: (i) CHO content of the meal consumed before the trial and the study drinks and (ii) the intensity of the cycling protocol. Intake of CHO elevates the blood insulin levels and subsequently inhibits lipolysis and FFA oxidation (Ormsbee et al., 2014; Rothschild et al., 2022). The inhibitory effect of insulin on fat oxidation persists even after the insulin levels return to basal values before exercise (Coyle et al., 1985). Thus, it is possible that the increase in insulin levels masked the effect of NZBC intake on fat oxidation and led to higher RER throughout the trial. The muscle glycogen depletion protocol used in our study could have been too anaerobically intense for our recreationally active participants, as they were football players and were unaccustomed to moderate-intensity intermittent cycling. Intense bouts of activity have been shown to increase the reliance on anaerobic metabolism which leads to acidosis and could have increased the RER values due to the formation of non-oxidative CO_2 by the HCO_3^- buffer system (Jeukendrup & Wallis, 2005).

In contrast, four studies have reported an increase in fat oxidation during moderate-intensity exercise in trained and recreationally active individuals following the consumption of 300, 600, and 900 mg/day NZBC for 7 days (Cook et al., 2015; Cook, Myers, Gault, Edwards, et al., 2017; Hiles et al., 2020; Strauss et al., 2018) in both fed and fasted states. Thus, it is possible that a 7-day intake of NZBC is necessary to increase fat oxidation duration moderate-intensity exercise.

Key takeaways: Acute intake of NZBC containing 120 mg anthocyanins did not influence fat oxidation during long duration moderate-intensity cycling in recreationally active males in a fed-state. As 7-day NZBC intake containing 105-315 mg anthocyanins has been shown to increase fat oxidation during cycling, future studies should focus on evaluating the dose-dependent effects to identify optimum (i) dose and (ii) duration of intake in both fed and fasted state.

6.2.2. Individual and additive effect of NZBC and caffeine intake on repeated-sprint performance

To the authors' best knowledge, the running trial (Chapter 4) was the first to examine the additive effect of NZBC and caffeine intake on repeated sprint performance during the m-LIST. Our study found that average sprint speed reduced significantly for all four treatments as the trial progressed from blocks 1 to 6 (p = 0.032, η^2 = 0.341). However, we observed an effect of treatment on average sprint speed (p = 0.049), with pairwise comparison showing that NZBC-CAFF treatment sustained higher average sprint speed compared to NZBC during the m-LIST protocol (p = 0.025). We also observed a trend for CAFF (p = 0.059) and NZBC-CAFF (p = 0.061) treatments to sustain higher average sprint speeds compared to NZBC, which indicated that caffeine may have helped sustain higher sprint speeds.

Our findings from the running trial (Chapter 4) are consistent with the study by Paton et al. (2022) that found no additive effect of a single dose of NZBC (155 mg anthocyanins) and caffeine (4 mg/kg body weight) intake on performance in trained male cyclists during repeated high-intensity cycling.

When consumed individually, New Zealand blackcurrant products and caffeine have shown varied results on sprint times during repeated-sprint tests. Acute intake of caffeine before exercise has been shown to improve the fastest sprint time by 0.06 s (1.4%) and reduce the total sprint time during repeated-sprint performance tests (Carr et al., 2008; Glaister et al., 2008). In contrast, intake of 300 mg/day NZBC (105 mg anthocyanins) for two days provided no benefit on critical speed and time to exhaustion during high intensity running in recreationally active males (Pastellidou et al., 2021). However, Willems et al. (2016) and Perkins et al. (2015) observed less slowing of the fastest maximal sprint during block 5 of the Loughborough Intermittent Shuttle Test (LIST) and a trend for reduced slowing of the sprint 5 out of 6 x 35-m sprints with the consumption of 300 mg/day (105 mg anthocyanins) of NZBC for 7 days, respectively. The observations by Willems et al. (2016) and Perkins et al. (2015) indicate that a 7-day intake could be necessary to show an improvement in repeated-sprint performance. On the other hand, based on the insights from the literature review (Chapter 2), acute NZBC intake resulted in faster 5 km time-trial in trained runners (Moss et al., 2023) and intake of NZBC for 7 days resulted in an increased time to exhaustion in recreationally active males during

incremental running test (Perkins et al., 2015), thus indicating that NZBC intake could improve endurance performance.

Exercising in a fatigued state could have also influenced the repeated-sprint performance outcome in our study. Faster 15-m sprint times during repeated-sprint performance have been observed with the intake of caffeine-electrolyte solution in previously fatigued males (Gant et al., 2010). On the other hand, there is no literature on the effect of NZBC on exercise performance in a fatigued state. Studies by Willems et al. (2016) and Perkins et al. (2015) that observed an effect of NZBC on repeated-sprint performance have been conducted in recreationally active males when in a rested state. Saltin (1973) suggested that intense training the day before a football game leads to a pronounced decrease in muscle glycogen stores which subsequently impairs sprint performance during the last 30 min of the game. As the participants in our study went through a muscle glycogen depletion protocol the day before the running trial and consumed less than the required amounts of carbohydrates for recovery, it is possible that they had poor glycogen stores at the start of the trial, which could have influenced the potential of NZBC-CAFF to reduce fatigue and improve repeated-sprint performance during the m-LSIT.

Key takeaways: Our findings demonstrate that intake of caffeine with and without NZBC helped sustain higher sprint speeds, whereas the intake of NZBC alone did not influence sprint performance in recreationally active males in an already fatigued state. As NZBC intake for 7 days has previously been shown to influence running performance, more research is needed to understand the dose-duration effect of NZBC intake with and without caffeine intake on repeated-sprint performance as well as exercise capacity. Future investigation should evaluate the effect of NZBC and caffeine consumption on exercise when in a rested state compared to fatigued state.

6.2.3. Individual and additive effect of NZBC and caffeine intake on free fatty acid concentration during and after exercise

Intake of a single dose of NZBC did not influence serum FFA levels before, during and after 90 min of mixed-intensity cycling (Chapter 3). Serum FFA concentrations increased with time during the m-LIST protocol ($p < 0.001$) but were not different between treatments. However, NZBC-CAFF was the only treatment in which serum FFA concentration kept increasing 1-h post exercise ($p = 0.042$, $\eta^2 = 0.214$, Chapter 4). This increase in FFA concentration 1-h post exercise with the intake of NZBC-CAFF in the running study indicates a potential effect on fat metabolism.

In the cycling trial, there was also no effect of NZBC intake on substrate partitioning during exercise. Intake of 600 mg of NZBC (210 mg anthocyanins) for 7 days has previously been shown to increase FFA and glycerol levels before exercising (Strauss et al., 2018). However, there were no differences in

the exercise-induced increase in serum FFA and glycerol levels with NZBC intake (Strauss et al., 2018). Strauss et al. (2018) also observed a 27% increase in fat oxidation rates and reported that serum FFA concentrations were moderately associated with mean average rates of fat oxidation during exercise. It is possible that intake of the NZBC extract may have increased FFA by influencing the genes involved in lipid metabolism (Tsuda et al., 2005). Several animal studies have shown an increase in fat metabolism following blackcurrant intake (Benn et al., 2014; Tsuda et al., 2005). For example, chronic intake of blackcurrant extract (0.1% BM for 12 weeks) in mice has been shown to increase the expression of genes involved with energy expenditure including peroxisome proliferator-activated receptor alpha (Benn et al., 2014). Similarly, the study by Tsuda et al. (2005) observed up-regulation of genes associated with lipid metabolism such as hormone sensitive lipase as well as increased lipolysis activity following treatment of adipocytes with cyanidin 3-glucoside or cyanidin. Further assessment of plasma glycerol, β-hydroxybutyrate, and catecholamines may provide insights into the effect of NZBC and caffeine consumption on serum FFA concentration before, during, and after exercise (following several days of supplementation).

Key takeaways: This was the first study to observe a sustained increase in serum FFA concentration 1-h post completion of a 90-min high-intensity intermittent exercise after an acute intake of NZBC-CAFF. However, the mechanism of action behind this effect remains unknown. Future studies should evaluate the acute as well as chronic effect of NZBC and caffeine intake on plasma glycerol, β-hydroxybutyrate, and catecholamines, to provide insights on lipolysis and fatty acid oxidation during and after exercise.

6.2.4. Use of IMUs for analysing repeated-sprint and running performance

The match play intensity in European football has significantly increased over the last 15 years due to the greater high-speed running (19.8 - 25.1 km/h) and sprinting (>25.1 km/h) demands, which now account for approximately 7% to 11% and 1% to 3% of the total distance covered during a match, respectively (Vanrenterghem et al., 2017). During football matches sprints are most frequent in the first 30 min and then from 75-90 min of the game, and therefore, it is crucial for players to sustain high-intensity efforts at the start and at the end the game (Oliva-Lozano et al., 2023). Acceleration and deceleration during high-intensity intermittent sports expose players to high levels of mechanical stress to musculoskeletal system and contribute to muscle soreness (Vanrenterghem et al., 2017). High-intensity decelerations in particular involve rapid decreases in momentum that generate high impact peaks and loading rates on the muscles and connective tissues (Harper & Kiely, 2018). Thus, being able to incorporate maximum speed, peak sprint speed, peak acceleration, peak deceleration,

gait patterns and load during the m-LIST protocol helps evaluate running performance as well as mechanical loading that contributes to tissue damage and neuromuscular fatigue.

Assessment of acceleration and deceleration can be considered crucial for team sports as frequency of high-intensity accelerations and decelerations has shown to have a significant impact on match performance outcomes in competitive football (Rhodes et al., 2021). According to the data collected using GPS, the highest number of high-intensity accelerations and decelerations were observed during winning games across a 45-game competitive season (Rhodes et al., 2021). Repeated-sprints performed during team sports are more complex than a single burst of acceleration and deceleration. A review by Haugen et al. (2014) reported that players perform 8 times as many accelerations compared to sprints per match during elite level football games. This could be because the absolute sprint values excluded short accelerations from analysis, primarily due to some periods of accelerations not crossing the high-intensity running and sprinting threshold. Thus, incorporating methods such as an insole embedded IMU system that capture the acceleration throughout the game would improve the understanding of repeated-sprint performance.

The m-LIST is a laboratory-based protocol to replicate demands of high-intensity intermittent running sports such as football in a controlled environment (Ali et al., 2014). We incorporated the IMUs in during the m-LIST protocol of our intervention study that evaluated the effect of NZBC and caffeine intake on repeated-sprint and running performance. We aimed to assess the use of IMUs to evaluate multiple variables such as maximum speed, peak sprint speed, peak acceleration, peak deceleration, gait patterns and mechanical load for in-depth analysis of sprint performance during each individual block of the m-LIST protocol. As the trial progressed from blocks 1 to 6 there was a decrease in maximum speed, average peak sprint speed, and peak deceleration for all four treatments, all markers of exercise-induced fatigue. However, CAFF treatment sustained higher peak deceleration values compared to other treatments. However, it underestimated the distance covered by ~16% during each individual block. The IMUs were also able to identify differences between experimental interventions. This shows that the smart insole system could (i) assess multiple biomechanical and kinematic variables in each individual block and (ii) was sufficiently sensitive to detect the differences in the expected decrease in speed, acceleration and deceleration during m-LIST with NZBC and caffeine intake, both indicative of fatigue and a subsequent impairment in repeated-sprint performance.

Key takeaways: The IMUs are a feasible tool to assess biomechanical and kinematic sprint variables including acceleration, deceleration, stride length, ground contact time, and mechanical load, that determine repeated-sprint and running performance and fatigue during laboratory-based protocols such as the m-LIST. Since the m-LIST protocol replicates the physical demands of football, there is the

potential for the IMUs to be used during football matches to assess fatigue and as a tool for the indirect measurement of muscle damage and injury risk.

6.3. Limitations

The limitations for the research conducted in this PhD project are discussed in the three individual experiment chapters. The key limitations are as follows:

- For Chapters 3 (running trial) and 5 (use of IMUs during the running trial), a common limitation was that the sample size was likely small to adequately evaluate the effect of NZBC intake with and without caffeine on sprint speed during the m-LIST protocol. Whilst a prospective power analysis was completed, the calculation compared sprint performance in caffeine and placebo trials during blocks 5 and 6 of the m-LIST protocol from previous research involving elite male football players. Since our participants were recreationally active males in a fatigued state and we were comparing NZBC intake with and without caffeine to placebo, it is possible more participants are needed to obtain significant results related to sprint performance, as we observed large effect sizes for treatment for some key variables during the high-intensity intermittent running protocol.
- There is a possibility that NZBC anthocyanin absorption was delayed and potentially reduced by the presence of other food sources. Participants in the running studies (Chapter 3 and 5) consumed the study drink along with a standardised breakfast containing muesli, yogurt, and milk before running which may have hindered absorption of anthocyanins. However, for the cycling study (Chapter 4), participants consumed the study drink after a 3-h fast without any other food. Since, we did not assess plasma anthocyanin levels before, during, and after exercise, the peak plasma anthocyanin levels as seen in other studies remains unknow.
- Indirect calorimetry could underestimate fat oxidation at exercise intensities above 70% $\dot{V}O_{2max}$ (Romijn et al., 1992; Rowlands & Jeukendrup, 2004), which are achieved during the m-LIST. Hence, for the running trial, we only assessed serum FFA concentration as a marker of fat metabolism. Further assessment of plasma glycerol and β-hydroxybutyrate could have provided insights into the acute effect of NZBC intake with and without caffeine on lipolysis and fatty acid oxidation.
- Lastly, our study involved cycling in the afternoon with a 3-h fast after their last meal. Moreover, the NZBC powder that we used for our study also contained 10 g of carbohydrates. Consumption of carbohydrates before exercise has been shown to reduce fat oxidation rates (Horowitz et al., 1997). Thus, it is possible that being in a fed-state and the carbohydrate

content of the study drink itself might have affected our observations on the effects of NZBC on substrate oxidation rates.

6.4. Future directions

The results found in this PhD study have highlighted several areas of future research into the individual and additive effect of NZBC and caffeine intake on running performance, as well as the acute intake of NZBC on substrate oxidation while cycling.

- After finding no acute effect of NZBC intake on running and cycling trials, it is possible that a loading phase combined with the last dose 2 h before exercise may be necessary to see the beneficial effects of NZBC intake on high-intensity intermittent running performance. Future studies should incorporate a loading-phase in their intervention as seen in older studies by Willems et al. (2016), Cook et al. (2015), and Montanari et al. (2021).
- Since our study showed no changes in speed and distance covered during the 90-min m-LIST protocol and the previous studies by Willems et al. (2016) and Perkins et al. (2015) have reported an increase time to exhaustion with 7 days of NZBC intake, future studies that evaluate the effect of NZBC on exercise should focus on time to exhaustion trials. More research is also needed to understand the dose-duration effect of NZBC with and without caffeine on repeated-sprint performance as well as endurance capacity.
- Presently, the intake of NZBC has been shown to increase blood flow at rest, during typing, and during isometric contractions, however its effect on blood flow during high-intensity intermittent exercise performance remains unknown. As increase in blood flow is one of the commonly cited mechanisms of action of blackcurrant anthocyanins to improve performance, future studies should evaluate the effect of NZBC intake on skeletal muscle blood perfusion during moderate-high intensity intermittent exercise.
- There is limited information available on the absorption of NZBC anthocyanins in healthy individuals before, during, and after exercise. Furthermore, there is growing interest in NZBC being a pro-oxidant before exercise and subsequently triggering an adaptive increase in the endogenous antioxidative capacity by activating the Nrf2 pathway. The increase in endogenous antioxidative capacity can be beneficial for athletes during recovery as it can help manage the exercise-induced inflammation. Hence, evaluating plasma Nrf2, oxidative stress, and anthocyanin concentration before and after exercise could be beneficial to understand the absorption of NZBC and its effect on recovery.
- Several animal studies have shown an increase in fat metabolism following blackcurrant supplementation (Benn et al., 2014; Tsuda et al., 2005). Several authors have speculated that

intake of NZBC for 7 days increases the expression of genes associated with fatty acid oxidation. Future studies should explore the effect of NZBC intake on the expression of key genes controlling glucose and fat metabolism in human skeletal muscle to understand the mechanism of action of blackcurrant anthocyanins on substrate oxidation.

- Incorporating the IMUs into the m-LIST protocol offered valuable insights into biomechanical and kinematic sprint variables such as acceleration, deceleration, stride length, and ground contact time that otherwise would have remained unknown. The IMUs could also detect the fatigue with repeated-sprint performance, as well as the differences between the study drinks, thus, indicating that it can be used for intervention trials. However, the validity and reliability using the IMUs for the m-LIST protocol have not been established until date and should be the focus of future research.
- The IMUs were able to measure different kinematic sprint variables as mentioned above during m-LIST. Since, the m-LIST has been developed to replicate the physical demands and running patterns of a football match, there is a possibility that the IMUs can be used during a football match to assess those same sprint performance variables and give insights into occurrence of fatigue, mechanical load, and injury risk.

6.5. Concluding remarks

In summary, the findings suggest that while caffeine intake improves sprint performance, intake of NZBC alone does not provide similar benefits when consumed acutely. Previous studies indicated that longer term NZBC intake may enhance performance, however, this study's focus on acute intake did not show ergogenic effects on sprint and exercise performance during high-intensity intermittent running. The lack of impact of acute NZBC extract consumption on substrate oxidation during cycling further highlights the need for investigating different dosing periods on substrate utilisation during moderate-intensity exercise.

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Appendices

Appendix 1: Publication, presentations, and awards list

Peer-reviewed journal publication
Nanavati, K., Rutherfurd-Markwick, K., Lee, S.J. et al. Effect of curcumin supplementation on exercise-induced muscle damage: a narrative review. European Journal of Nutrition (2022)
Poster and oral presentation
November 2023 | Nutrition Society of New Zealand and Nutrition Society Australia Conference, New Zealand (in-person) *Poster Presentation - Effect of Acute Supplementation with New Zealand Berry Anthocyanin-enriched Drink on Repeated Sprint Performance in Recreationally Active Males*
September 2023 | 2nd Makassar International Conference on Sport Science & Health (MICSSH), Indonesia (online) *Oral Presentation - Use of a Smart Insole System (Plantiga) for Gait Analysis During Free-Running Movement*
Awards
Winner of Poster Presentation at Nutrition Society of New Zealand Conference (2023) (NZD 500).
Winner of Massey University Visualize Your Thesis Competition (2020) (NZD 1000) and represented Massey University at the International level.

Appendix 2: Study documents

Appendix 2.1 - Ethics approval letter

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 22 March 2021

Dear Prof Aj Ali

Re: Ethics Notification - **SOA 21/09 - Individual and additive effects of blackcurrant juice and caffeine intake on high intensity intermittent exercise performance**

Thank you for the above application that was considered by the Massey University Human Ethics Committee: **Human Ethics Southern A Committee** at their meeting held on **Monday, 22 March, 2021**.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Professor Craig Johnson
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Research Ethics Office, Research and Enterprise
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Appendix 2.2 - Study recruitment poster

Appendix 2.3 - Participant information sheet

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Individual and additive effects of blackcurrant juice and caffeine intake on high intensity intermittent exercise performance

Researcher Introduction

We are researchers at Massey University and Plant & Food Research and are interested in examining whether blackcurrant juice and caffeine intake can enhance exercise performance.

Invitation to Participate in Research Study

Blackcurrant contains high levels of anthocyanins which have been shown to reduce cell damage, reduce inflammation and enhance blood flow. Exercise performance improves with blackcurrant supplementation in a variety of settings including running/sprinting, intermittent exercise, and rock climbing. Caffeine is one of the most widely used psychoactive drugs in the world due to its accessibility, evidenced ergogenic effects and few negative side effects. Both blackcurrant and caffeine seem to afford greater benefits to athletes when they are in a fatigued state; therefore, it would be reasonable to assume that adding caffeine to a blackcurrant solution will provide greater performance benefit. Therefore, we aim to examine the effect of caffeine and blackcurrant ingestion, provided before exercise to 15 previously fatigued male athletes, on various performance and metabolic parameters during a well-established exercise test that simulates a high-intensity team sport like football/soccer. We would like to investigate the individual and additive effects of blackcurrant and caffeine intake on performance and therefore we invite you to take part in four experimental trials.

Participant Recruitment

If you are male, aged 18-45 years, and well trained (regular training for high-intensity team sports like football, rugby and hockey) we'd like to invite you to participate in this study. However, if you have any of the conditions listed on the "health checklist" you should not volunteer to participate. If you are unsure about any of the listed conditions, then you should consult with the researchers. You will receive a $50 Westfield/MTA voucher per visit for travel expenses for participation in this study on a pro-rata basis upon completion of the study (9 visits = $450).

Project Procedures and Participant Involvement

Familiarisation trial

The first visit (90 min) will be for a familiarisation of the procedures and equipment to be used for the main trials. You will also be asked to complete a health-screening checklist and consent form. During this session we will collect a saliva sample to determine your caffeine metabolism genotypes (CYP1A2 and ADORA2A). You will also be asked to complete the 'beep' test to estimate your maximal oxygen uptake.

You will be shown how to complete the 24-h food record diary prior to each main trial. You will be asked to replicate the food intake prior to each testing session. We would also ask that you please refrain from consuming caffeine-containing foods and beverages (e.g. tea, coffee, energy drinks, and chocolate) for at least two days prior to each experimental session.

For all trials you will be asked to wear apparel comfortable enough such as trainers, shorts and a shirt or cycling gear if you have it. Please bring a towel as showering facilities will be available.

Main trials

You will be asked to come into the lab for four main trials, with each trial taking place over two days. The table below explains the four conditions:

Condition	Day 1	Fatiguing Exercise	Day 2	Performance Metrics / Exercise
Blackcurrant only	Blackcurrant juice + 0mg caffeine	90 min cycling	Blackcurrant juice + 0mg caffeine	90 min LIST
Placebo	Placebo + 0mg caffeine	90 min cycling	Placebo + 0mg caffeine	90 min LIST
Caffeine only	Placebo + 0mg caffeine	90 min cycling	Placebo + 240mg caffeine	90 min LIST
Blackcurrant + Caffeine	Blackcurrant juice + 0mg caffeine	90 min cycling	Blackcurrant juice + 240mg caffeine	90 min LIST

LIST = Loughborough Intermittent Shuttle Test (high-intensity shuttle running; designed to simulate demands of a football match)

The test beverages will contain blackcurrant juice (2Before) or placebo (same colour and taste but without blackcurrant). You will also be asked to consume a capsule containing either 240mg of caffeine or a placebo (0mg caffeine).

On the evening of day 1 you will be asked to come to the lab after observing at least a 3-hour fast. We will ask you to provide a mid-stream urine sample for determining hydration status (and not used for any other purpose). We will then undertake baseline tests (including perceptual tests, putting on heart rate monitor, resting oxygen uptake) and then provide the test beverage. One hour after beverage intake you will be asked to complete a 90-minute cycling exercise to induce fatigue. Water will be provided at regular intervals to keep you hydrated. A 10-ml blood sample (venepuncture) will be taken before and after the cycling exercise by a trained phlebotomist. We will feed you a standardised meal after the exercise and then you will be able to head home (you will be asked not to consume any more food or drinks; only water will be allowed).

You will be asked to arrive back to the lab on the morning of day 2 in an overnight-fasted state. After resting measures have been taken (mid-stream urine sample, 10-ml blood sample, weight, heart rate, blood flow), we will provide a standardised breakfast as well as the beverage and a capsule to ingest. Sixty minutes after ingestion we will ask you to complete the 90-min high intensity shuttle running test (the LIST). We will take 7 finger prick blood samples (0.2ml each time; approx. 15 min apart) to measure blood lactate, as well as various perceptual tests (exertion, arousal, pleasure-displeasure) during the 90-min exercise test. Following the running exercise, you will be asked to undertake post-exercise measures (same as pre-exercise measures) and then you will be able to take shower and then sit quietly in the lab. We will take a 10-ml blood sample (venepuncture) before (0min), during (45min), post (90min) and 1-hour after the LIST to monitor oxidative markers, caffeine metabolism and energy metabolism. Therefore, total blood collected will be 61.4ml per trial (2 x 10-ml day 1; 4 x 10-ml day 2; plus 7 x 0.2ml for blood lactate during the LIST).

The above procedures will be repeated for the second, third and fourth trials. Each trial will be separated by 7 days (1 week). The total time commitment for the 9 visits will be approx. 33.5 hours (1.5 hours for familiarisation; 3 hours for day 1 of each trial; 5 hours for day 2 of each trial).

Individuals trained in resuscitation (NZ Red Cross First Aid, Level 2) and the use of a defibrillator will be present for all exercise sessions. In addition, the researchers will be constantly monitoring physiological and perceptual variables that will aid in identifying any issues.

Participant's Rights

You are under no obligation to accept this invitation. Should you choose to participate, you have the right to:

• decline to answer any particular question

• withdraw from the study at any time, even after signing a consent form (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed)

• ask any questions about the study at any time during participation

• provide information on the understanding that your name will not be used unless you give permission to the researcher

• be given access to a summary of the project findings when it is concluded

Note: As a participant you can agree to receive your genetic information. Before agreeing to this you should be aware that under New Zealand law an insurance company could ask you to disclose such information should you apply for life or health related insurance – such as medical cover. You could be obliged to disclose it even if the insurer does not ask for it expressly. Not disclosing it could result in the insurer not having to pay out under the policy.

Good Practice and Cultural Safety for Massey University Research

The study was discussed with the Student Recruitment Adviser - Māori Academic Support. We have considered the inclusion of Māori and indigenous values and concepts, allowing for the use of whānau support and appropriate Māori protocols. We acknowledge the concept of manaakitanga, respecting the participant`s inherent dignity and acting in a caring manner towards them by way of:

- Taking full responsibility to perform research in a safe and ethical manner (aroha)
- Providing the participant with all of the critical information regarding the study in a clear way, so they can make informed decisions (tūmanako and whakapono)

- An awareness of the cultural significance and sensitivity for a culturally safe implementation of the study (māhaki)
- Respect for the privacy and confidentiality of Māori participants
- Acknowledging the tapu (sacred) nature of blood/human tissue by offering remaining blood samples (if appropriate) back to the donor and keeping human samples secured and separated from other biological material, to ensure that the tapu māheuheu is not mixed with or contaminated by other tapu or noa (profane) substances.

Confidentiality

All data collected will be used solely for research purposes and has the possibility of being presented in a professional journal. All personal information will be kept confidential by assigning numbers to each participant. No names will be visible on any papers on which you provide information. All data/information will be dealt with confidentiality and will be stored in a secure location for five years on the Massey University Albany Campus. After this time, it will be disposed of by an appropriate staff member from the School of Sport and Exercise.

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact either of the following people for assistance:

Principal researchers

Prof Ajmol Ali (School of Sport, Exercise and Nutrition, Massey University)
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Roger.Hurst@plantandfood.co.nz

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Dan Gordon (PhD student)
D.B.Gordon@massey.ac.nz

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 21/09. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 356 9099 x 85094, email humanethicsoutha@massey.ac.nz

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Appendix 2.4 - Health screening questionnaire and consent form

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

INDIVIDUAL AND ADDITIVE EFFECTS OF BLACKCURRANT JUICE AND CAFFEINE INTAKE ON HIGH INTENSITY INTERMITTENT EXERCISE PERFORMANCE

Health Screening Questionnaire and Consent Form

Name:_____

Address: _____

Phone: _____

Age: _____

Gender:_____

Please read the following questions carefully. If you have any difficulty, please advise the medical practitioner, nurse or exercise specialist who is conducting the exercise test.

Please answer all of the following questions by ticking only one box for each question:

The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Q 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

Q 2. Do you feel a pain in your chest when you do physical activity?

Yes ☐ No ☐

Q 3. In the past month have you had chest pain when you were not doing physical activity?

Yes ☐ No ☐

Q 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

Q 5. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Yes ☐ No ☐

Q 6. Have you been hospitalised in the past few months?

Yes ☐ No ☐

Qu 7. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

Yes ☐ No ☐

Qu 8. Have any immediate family had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 9. Do you have any issues with having your blood taken?

Yes ☐ No ☐

Qu 10. Do you have any infectious disease that may be transmitted in blood?

Yes ☐ No ☐

Qu 11. Do you have any issues wearing a face mask while sitting at rest?

Yes ☐ No ☐

Qu 12. Do you have any allergic reactions, or issues consuming berry or blackcurrant products?

Yes ☐ No ☐

Qu 13. Do you have any allergic reactions, or issues consuming caffeine?

Yes ☐ No ☐

I have read, understood and completed this questionnaire.

Signature ***(Participant):*** _____ Date: _____

References

1. Thomas S, Reading J and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.
2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med Sci Sports Exerc* 28(4): 468-472

CONSENT FORM

2Before Study -Individual and additive effects of blackcurrant juice and caffeine intake on high intensity intermittent exercise performance

Please tick to indicate you consent to the following

I have read and I understand the Participant Information Sheet.	o	
I have been given sufficient time to consider whether or not to participate in this study.	o	
I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.	o	
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	o	
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	o	
I consent to the research staff collecting and processing my information, including information about my health.	o	
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes o	No o
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethics Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	o	
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	o	
I understand the compensation provisions in case of injury during the study.	o	
I know who to contact if I have any questions about the study in general.	o	
I understand my responsibilities as a study participant.	o	
I wish to receive a summary of the results from the study.	Yes o	No o

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

Appendix 2.5 - List of foods to avoid before the trial

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Individual and additive effects of blackcurrant juice and caffeine intake on high intensity intermittent exercise performance

List of foods to avoid for at least 2 days before the experimental sessions

Here is a list of common foods that are high in caffeine, or nutritional polyphenolic compounds and antioxidants. We ask you to avoid eating these foods for at least 2 days before and during each experimental session of the study.

Fruit and fruit juice:

Blackcurrants, Plums, Blackberries, Blueberries, Cherries, Kiwifruit, Cranberries, Figs, Avocado, Raspberry, Strawberry, Grapes (black & red), Apples (red & green), Citrus fruit (oranges, grapefruit, lemons)

Vegetables and vegetable juice:

Aubergine, Beans (red, kidney), Beetroot, Onion (red), Cabbage (red), Olives (black), Broccoli (purple), Corn (purple), Potato/sweet potato (red, purple), Rice (red, black/purple)

Miscellaneous:

Coffee, Energy drinks, Wine (red), Tea (black & green), Chocolate (dark)

Dietary supplements:

Please avoid all supplements especially those claiming antioxidant or anti-inflammatory activity and containing berries, green tea extract, quercetin, resveratrol, vitamin C and vitamin E

If you have any queries about other foods that you may eat as part of your normal diet please ask.

Appendix 2.6 - Two-day food dairy

Two-Day Food Record

Name: _____

Dates of recorded intake: _____

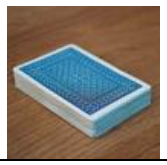
Instructions for Keeping Your Two-Day Food Record

- Please keep your two-day food record for the two consecutive days prior to the Cycle trial and 3 main trials.
- Try to replicate the same diet and lifestyle factors prior to the second main trial.
- Each time you eat or drink anything (meals, snacks, etc.) during the two days, write down what was eaten.
- To measure how much was eaten; use a set of **measuring cups and spoons** to help estimate amounts. Also see the examples below to estimate portion sizes, as this is much more practical and easier.
- Note if food choices are homemade or purchased. Please include brand names whenever possible.

Amounts and Conversions

1/4 cup = 50 ml or 4 Tablespoons
1/3 cup = 75 ml or 5 1/2 Tablespoons
1/2 cup = 125 ml or 8 Tablespoons
2/3 cup = 150 ml or 10 1/2 Tablespoons
3/4 cup = 175 ml or 12 Tablespoons
1 cup = 250 ml or 16 Tablespoons
1 oz = 1 slice of processed cheese or lunchmeat

How to Estimate Your Portion Size

Meat Three (3) ounces of meat are about the size and thickness of a deck of playing cards or an audiotape cassette.	
Fruit A medium apple or peach is about the size of a tennis ball.	
Grains One cup of rice or pasta is about the size of your fist.	
Cheese One ounce of cheese is about the size of four dice.	

Two-Day Food Record Checklist

Beverages	What kind of milk? homogenised, 2%, 1%, skim, other. Was it fruit juice or fruit beverage or drink?
Breads	Did you spread on butter or margarine?
Cereal	Did you add milk? Did you add sugar or fruit?
Dairy	What brand or kind of yogurt? What brand or kind of cheese?
Vegetables	Was it raw or cooked? Was it fresh, frozen or canned? Did you add any butter, margarine or sauce?
Fruit	Was it a small, medium or large fruit? Was it fresh, frozen or canned?
Grains	Did you add any butter, margarine, peanut butter, jam or honey? Was it a half or whole sandwich? Was it a small or large muffin or bagel?
Fish	Was your canned fish packed in water or oil How did you cook your fish?
Meats	How did you cook your meat?

	What kind of cut was it e.g. chicken leg or chicken breast?
Soups	Was your soup prepared with milk, water or cream?
Restaurants	What restaurant was it?
Packaged food	What brand was it?

Sample Menu

Day 1: Tuesday, May 14, 2018				
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** (e.g. amount of food served, too tired to eat)
Breakfast	Cereal	½ cup	Honey Nut Cheerios	
	Milk 2%	½ cup		On cereal
	Banana	½ med		
AM Snack	Animal Crackers	10	Christie	
	Apple juice	4 oz	Allen's pure apple juice-canned	
Lunch	Grilled cheese sandwich			
	Whole wheat bread	1 slice	Dempsters	No crusts
	Cheese slice	1 slice	Kraft slices	
	Butter on bread	1 Tbsp		
	Yogurt – strawberry	75 ml	Mini-go	
	Milk	½ cup	2%	
PM Snack	Granola bar	1 bar – 35 g	Quaker Chewy, Trail Mix – tropical fruit	Ate half of it
Dinner	Chicken fingers	1 ½	President's Choice	
	French fries	10	McCain regular	
	Honey	2 Tbsp		For dipping
	Ketchup	2 Tbsp	Heinz	
	Carrots	½ medium	Raw, cut in sticks	
	Milk	½ cup	2%	
Evening Snack	Ice cream	1 cup	Chocolate Nestle	

Was this day's intake considered: [ ] Poor [X] Average [ ] Very Good

Trial 1

Day 1	**Date:**			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** **(e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 2	Date:			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments (e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Trial 2

Day 1	**Date:**			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** **(e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 2	Date:			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments (e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Trial 3

Day 1	**Date:**			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** **(e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 2	Date:			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments (e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Trial 4

Day 1	**Date:**			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** **(e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 2	Date:			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments (e.g. amount of food served, too tired to eat)**
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Appendix 2.7 - Data collection sheet for cycling and running trials

Cycling Recording Sheet

START TIME:_____
Date: _____ _____ Trial: _____
Participant's name: _____ ID number: _____
Temp: _____ Humidity: _____

Baseline measurements:

Baseline	Weight	USG	BP (mmHg)	HR	BP (mmHg)	HR	SV	SVR

Pre-exercise: 2ml/kg
Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Pre: 10ml blood sample

Resting	FS	FAS	BP (mmHg)	Resting HR	BP (mmHg)	Resting HR	USCOM - SV	Resting O2

2 min Warm Up (~80 watts)

RPE

First 30 min	FS	FAS	RPE

4-6 mins☐ 10-16 mins☐ 24-30 mins☐

During: 10ml blood sample (before sprints begin)

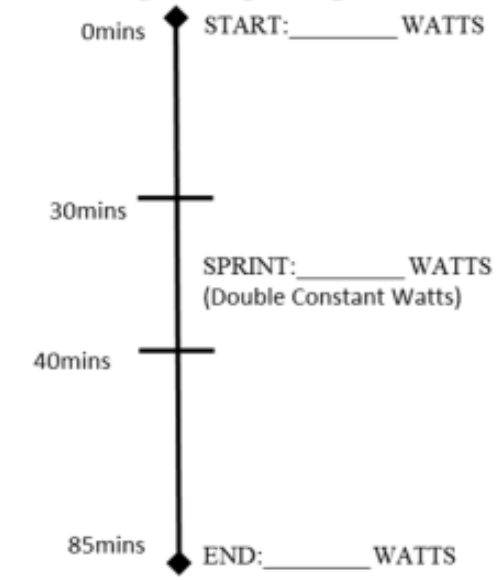
Post 'sprints'	FS	FAS	RPE

Last 45 min	FS	FAS	RPE

0-6 mins☐ 17-23 mins☐ 39-45 mins☐

Post: 10ml blood sample

Watts Changes During Testing



WATER

☐ 0 mins
☐ 15 mins
☐ 30mins
☐ 0 mins
☐ 15 mins
☐ 30 mins

End measurements:

Post	Weight

Appendix 2.8 - LIST main trial recording sheet

Date: _____ Trial: _____

Participant's name: _____ ID number: _____

Temp: _____ Humidity: _____

Baseline measurements (pre-supplementation): Collect 10ml blood sample (-60 mins)

Weight	USG	BP (mmHg)	BP (mmHg)	Resting HR	USCOM - SVR	USCOM - SV	FS	FAS

Pre: Collect 10ml blood sample ☐

Measurements (post-supplement) (0 mins)

BP (mmHg)	HR	BP (mmHg)	HR	USCOM - SVR	USCOM - SV	CJM

Block 1	FS	FAS	RPE	Blood lactate	CJM

Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Block 2	FS	FAS	RPE	Blood lactate	CJM

Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Block 3	FS	FAS	RPE	Blood lactate	CJM

Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Block 4	FS	FAS	RPE	Blood lactate	CJM

Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Block 5	FS	FAS	RPE	Blood lactate	CJM

Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Block 6	FS	FAS	RPE	Blood lactate	CJM

Provided with 2 $ml.kg^{-1}$ fluid every 15 minutes: _____

Notes:

_____

_____

_____

_____

Post: Collect 10ml blood sample (90 mins)

Weight	BP (mmHg)	HR	BP (mmHg)	HR	USCOM - SVR	USCOM - SV	FS	FAS

1 h Post LIST: Collect 10ml blood sample ☐

Snack bag given to participant ☐

Next Trial Booking: _____

Appendix 2.9 - Rating scale for perceptual measures

Appendix 2.9.1. - Feeling scale ratings

+5	**Very good**
+4	
+3	**Good**
+2	
+1	**Fairly good**
0	**Neutral**
-1	**Fairly bad**
-2	
-3	**Bad**
-4	
-5	**Very bad**

While participating in exercise it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasurable.

Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise.

Scientists have developed this scale to measure such response

Appendix 2.9.2. - Felt arousal scale ratings

FELT AROUSAL SCALE (FAS)
(Svebak & Murgatroyd, 1985)

Estimate here how aroused you actually feel. Do this by circling the appropriate number. By "arousal" we meant how "worked-up" you feel. You might experience high arousal in one of a variety of ways, for example as excitement or anxiety or anger. Low arousal might also be experienced by you in one of a number of different ways, for example as relaxation or boredom or calmness.

1 LOW AROUSAL

2

3

4

5

6 HIGH AROUSAL

Appendix 2.9.3. - Ratings of perceived exertion

Borg RPE Scale®

Use this scale to tell how strenuous and tiring the work feels to you. The exertion is mainly felt as fatigue in your muscles and as breathlessness or possibly aches. When the exercise is hard it also becomes difficult to talk. It is your own feeling of exertion that is important. Don't underestimate it, but don't overestimate it either. For common exercise, such as cycling, running or walking, 11-15 is a good level. For strength and high-intensity interval training (HIIT), 15-19 is good. If you are sick follow your doctor's advice. Look at the scale and the descriptions and then choose a number. Use whatever numbers you want, even numbers between the descriptions.

6	**No exertion at all**	No muscle fatigue, breathlessness or difficulty in breathing.
7	**Extremely light**	Very, very light.
8		
9	**Very light**	Like walking slowly for a short while. Very easy to talk.
10		
11	**Light**	Like a light exercise at your own pace.
12	**Moderate**	
13	**Somewhat hard**	Fairly strenuous and breathless. Not so easy to talk.
14		
15	**Hard**	Heavy and strenuous. An upper limit for fitness training, as when running or walking fast.
16		
17	**Very hard**	Very strenuous. You are very tired and breathless. Very difficult to talk.
18		
19	**Extremely hard**	The most strenuous effort you have ever experienced.
20	**Maximal exertion**	Maximal heaviness.

Borg RPE Scale®
Ratings (R) of Perceived (P) Exertion (E).
© Gunnar Borg, 1970, 1998, 2017
English

Appendix 2.10 - Warm-up protocol for list

Takes approx. 10 min

1 length = 20 m

32 x 20 = 640 m

10 x lengths jogging

2 x lengths sideways (facing wall until halfway, then switching over)

2 x lengths jogging/shuffling backwards

2 x lengths knees-up

2 x lengths heel-flicks

1 x length kicking out (straight kicks)

1 x length kicking out (kicking across body)

1 x length groin (out)

1 x length groin (in)

1 x length lunges

1 x length 'lawn bowls' hamstring stretch

2 x skipping

2 x lengths faster running (75%)

2 x lengths sprints

2 x lengths jogging

Stretches: Hamstrings - 30 s each leg

Quads – 30 s each leg

Calf – 30 s each leg

Appendix 2.11 - Study Randomisation

	Trial 1		Trial 2		Trial 3		Trial 4	
	Cycling	**Running**	**Cycling**	**Running**	**Cycling**	**Running**	**Cycling**	**Running**
85001	PLA	PLA	NZBC	NZBC	PLA	CAFF	NZBC	NZBC-CAFF
85002	PLA	PLA	NZBC	NZBC-CAFF	NZBC	NZBC	PLA	CAFF
85003	NZBC	NZBC	PLA	CAFF	PLA	PLA	NZBC	NZBC-CAFF
85004	PLA	CAFF	NZBC	NZBC-CAFF	PLA	PLA	NZBC	NZBC
85005	NZBC	NZBC-CAFF	PLA	PLA	PLA	CAFF	NZBC	NZBC
85006	PLA	PLA	PLA	CAFF	NZBC	NZBC	NZBC	NZBC-CAFF
85008	NZBC	NZBC-CAFF	PLA	PLA	NZBC	NZBC	PLA	CAFF
85009	NZBC	NZBC-CAFF	PLA	CAFF	NZBC	NZBC	PLA	PLA
85011	PLA	CAFF	NZBC	NZBC	NZBC	NZBC-CAFF	PLA	PLA
85013	NZBC	NZBC	NZBC	NZBC-CAFF	PLA	CAFF	PLA	PLA
85014	PLA	PLA	NZBC	NZBC	NZBC	NZBC-CAFF	PLA	CAFF
85016	NZBC	NZBC-CAFF	NZBC	NZBC	PLA	PLA	PLA	CAFF
85017	NZBC	NZBC	PLA	PLA	NZBC	NZBC-CAFF	PLA	CAFF
85019	PLA	PLA	PLA	CAFF	NZBC	NZBC-CAFF	NZBC	NZBC

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine

Appendix 3: HPLC method for assessment of plasma caffeine and its metabolites

Plasma caffeine and caffeine metabolites (paraxanthine and theophylline) were analysed by reversed-phase HPLC using a LC-20 liquid chromatograph equipped with an SPD-M20A photodiode array detector (Shimadzu Inc., Kyoto, Japan) (Holland et al., 1998). Frozen plasma samples were thawed and deproteinised by adding 400 µl of 0.8 M perchloric acid to 400 µl of the sample. Samples were then vortexed for 10 s and then centrifuged for 10 min at 9900 x g. The supernatant was removed and put in a glass vial and used for reversed-phase HPLC. The mobile phase A was 0.1% trifluoroacetic acid ($C_2HF_3O_2$) in milliQ water (H_2O), mobile phase B was 0.1% trifluoroacetic acid ($C_2HF_3O_2$) in 40% acetonitrile (C_2H_3N), and stationary phase was a Phenomenex Luna (Synergi 4 µm Hydro-RP 80 Å, LC Column 150 x 4.6 mm, part number: 00F-4375-E0). Flow rate was set at 0.75 ml/min with an oven temperature of 22°C and a gradient from 100% A to 100% B was run as described in Table 3.3. Injection volume was set at 100 µl and the samples were analysed at a wavelength of 274 nm. Paraxanthine (D5385), theophylline (PHR1023), and caffeine (PHR1009) (all from Sigma-Aldrich, USA) were used for the preparation of a 10-point linear standard curve. The peak for each metabolite was identified at 20.8 min, 21.0 min, and at 27.9 min of the run, respectively and the peak area was used to calculate the concentration of the metabolites.

Table A3.1 HPLC gradient method of mobile phase concentrations A and B for analysis of caffeine metabolites

Time (min)	A Concentration (%)	B Concentration (%)
0	100	0
15	65	35
20	65	35
28	50	50
30	0	100
35	0	100
41	100	0

Appendix 4: Supplementary material – graphs and results

Appendix 4.1 Chapter 3, Section 3.3.1: Sprint speed during the modified Loughborough Intermittent Shuttle Test

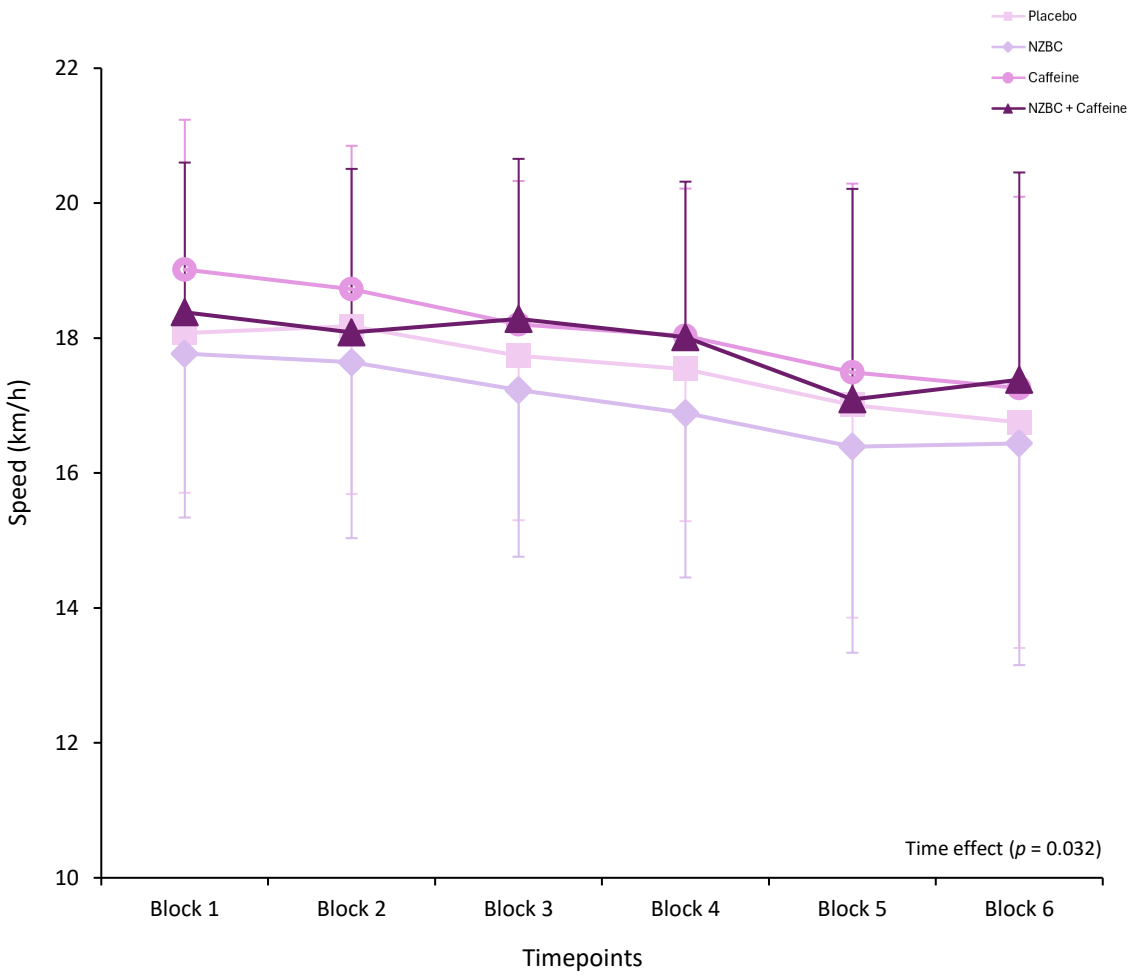


Figure A4.1 Changes in sprint speed (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF) trials from blocks 1 to 6 during modified Loughborough intermittent Shuttle Test.

Appendix 4.2 Chapter 3, Section 3.3.4: Blood lactate levels during the modified Loughborough Intermittent Shuttle Test

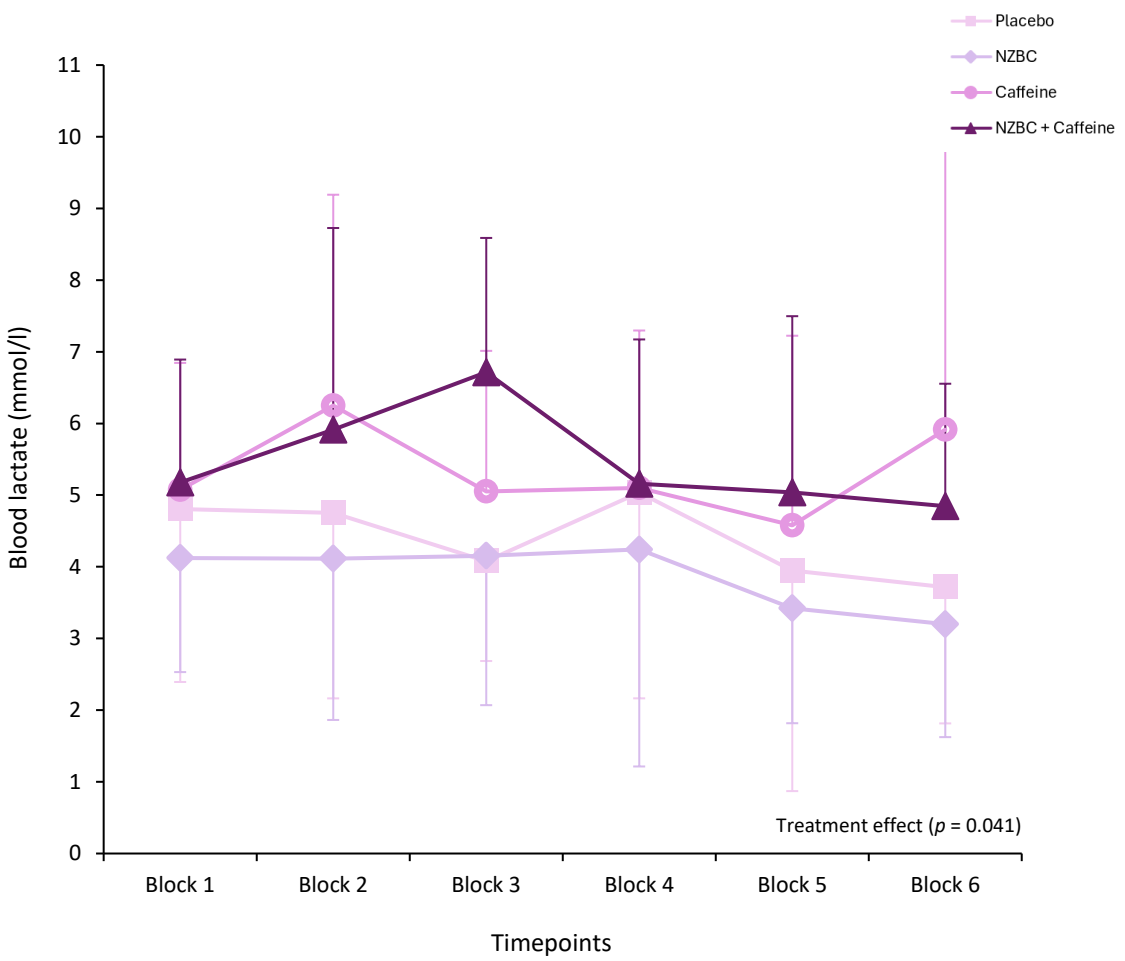


Figure A4.2 Changes in blood lactate concentration (mean ± SD) for placebo (PLA), NZBC, caffeine (CAFF), and NZBC + caffeine (NZBC-CAFF) trials from blocks 1 to 6 during modified Loughborough intermittent Shuttle Test.

Appendix 4.3 Chapter 3, Section 3.4 Fatigue index calculation

We calculated the fatigue index to evaluate the rate of decline in sprint and running performance measures using percentage decrement score (Girard et al., 2011). The calculation used compared actual performance to an imagined 'ideal performance' (i.e. where the best effort would be replicated in each sprint). In our study, the average sprint performance during block 1 was considered as 'ideal performance' and the number of sprints used in the formula was considered as 5 (for the average sprint speed from blocks 1 to 5).

Table A4.1 Fatigue index for sprint and running performance measures during the modified Loughborough Intermittent Shuttle Test

	PLA	NZBC	CAFF	NZBC-CAFF	Effect of condition (*p* value and effect size)
Walk speed	0.6 ± 1.9	-1.3 ± 4.2	0.6 ± 1.8	0.2 ± 1.8	$p = 0.183$ $\eta^2 = 0.128$
Sprint speed	-3.3 ± 3.8	-4.1 ± 4.3	-5.2 ± 2.7	-2.6 ± 4.6	***p* = 0.048** $\eta^2 = 0.253$
Run speed	-2.3 ± 3.1	-2.6 ± 2.6	-2.0 ± 3.3	-2.0 ± 2.6	$p = 0.542$ $\eta^2 = 0.048$
Jog speed	-0.6 ± 3.0	-0.8 ± 2.4	-0.5 ± 2.9	-0.5 ± 2.1	$p = 0.947$ $\eta^2 = 0.005$
Reaction time	49.3 ± 23.3	38.3 ± 37.5	51.3 ± 39.6	45.6 ± 22.3	$p = 0.915$ $\eta^2 = 0.011$
Movement time	10.7 ± 7.6	9.9 ± 7.9	8.1 ± 9.8	8.9 ± 8.0	$p = 0.615$ $\eta^2 = 0.045$

PLA, Placebo; NZBC, New Zealand blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand blackcurrant + Caffeine. Values are mean ± SD.

Girard, O., Mendez-Villanueva, A., & Bishop, D. (2011). Repeated-sprint ability - part I: factors contributing to fatigue. Sports Med, 41(8), 673-694. https://doi.org/10.2165/11590550-000000000-00000

Appendix 4.4 Chapter 5, Section 5.3.1: Distance covered reported with the IMUs during the Loughborough Intermittent Shuttle Test

Table A4.2 Distance covered reported with the IMUs during the modified Loughborough Intermittent Shuttle Test

		Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Effect of Treatment (*p* value and effect size)	Effect of Time (*p* value and effect size)	Effect of Treatment *Time (*p* value and effect size)
Distance covered (km)	PLA	1.6 ± 0.1	1.6 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.3			
	NZBC	1.7 ± 0.0	1.6 ± 0.8	1.7 ± 0.0	1.6 ± 0.0	1.5 ± 0.1	1.4 ± 0.3	***p* = 0.359**	***p* < 0.001**	*p* = 1.000
	CAFF	1.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.1	1.7 ± 0.0	1.5 ± 0.1	1.4 ± 0.3	$\eta^2 = 0.251$	$\eta^2 = 0.511$	$\eta^2 = 0.257$
	NZBC-CAFF	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.4			

PLA, Placebo; NZBC, New Zealand Blackcurrant, CAFF, Caffeine; NZBC-CAFF, New Zealand Blackcurrant + Caffeine. Values are means ± SD.

Appendix 5: DRC 16 forms

Appendix 5.1 DRC 16 form for Chapter 2

DRC 16

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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Krutika Manoj Nanavati
Name/title of Primary Supervisor:	Prof Ajmol Ali
In which chapter is the manuscript /published work:	Chapter 2

Please select one of the following three options:

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Candidate's Signature:	
Date:	04-Sep-2024
Primary Supervisor's Signature:	
Date:	4-Sep-2024

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Appendix 5.2 DRC 16 form for Chapter 3

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Appendix 5.3 DRC 16 form for Chapter 4

DRC 16

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Appendix 5.4 DRC 16 form for Chapter 5

DRC 16

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○ The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

◉ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

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Date:	04-Sep-2024
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Appendix 6: The Influence of a Protein-rich Dairy Beverage Fortified With Curcumin On Health And Wellness In Older Adults - PhD topic from April 2020 to December 2022

Appendix 6.1 - Peer-reviewed journal publication

European Journal of Nutrition (2022) 61:3835–3855
https://doi.org/10.1007/s00394-022-02943-7

REVIEW

Effect of curcumin supplementation on exercise-induced muscle damage: a narrative review

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Abstract
Curcumin, a natural polyphenol extracted from turmeric, is a potent antioxidant and anti-inflammatory agent. In the past few decades, curcumin's ability to impact chronic inflammatory conditions such as metabolic syndrome, arthritis, and cancer has been widely researched, along with growing interest in understanding its role in exercise-induced muscle damage (EIMD). EIMD impacts individuals differently depending on the type (resistance exercise, high-intensity interval training, and running), intensity, and duration of the exercise. Exercise disrupts the muscles' ultrastructure, raises inflammatory cytokine levels, and can cause swelling in the affected limb, a reduction in range of motion (ROM), and a reduction in muscular force-producing capacity. This review focuses on the metabolism, pharmacokinetics of various brands of curcumin supplements, and the effect of curcumin supplementation on EIMD regarding muscle soreness, activity of creatine kinase (CK), and production of inflammatory markers. Curcumin supplementation in the dose range of 90–5000 mg/day can decrease the subjective perception of muscle pain intensity, increase antioxidant capacity, and reduce CK activity, which reduces muscle damage when consumed close to exercise. Consumption of curcumin also improves muscle performance and has an anti-inflammatory effect, downregulating the production of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-8. Curcumin may also improve oxidative capacity without hampering training adaptations in untrained and recreationally active individuals. The optimal curcumin dose to ameliorate EIMD is challenging to assess as its effect depends on the curcumin concentration in the supplement and its bioavailability.

Keywords Curcumin · Pharmacokinetics · Inflammation · Muscle soreness · Oxidative stress · Antioxidant

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Introduction

Curcumin, chemically known as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or diferuloylmethane [1], is isolated from the plant *Curcuma longa* [2]. Over the past few decades, curcumin has been widely researched for its antioxidant and anti-inflammatory properties in numerous chronic and malignant diseases such as metabolic syndrome [3], arthritis [4, 5], and cancer [6–8]. However, there has been a growing interest in understanding the effect of curcumin on exercise-induced muscle damage (EIMD) in recent years. EIMD affects all individuals depending upon the type, intensity, and duration of the exercise they undertake and training status of the individual [9, 10]. Resistance training [11], high-intensity interval training [12], trail running [13, 14], and downhill running [15] contribute to EIMD, leading to ultrastructural muscular disruption and an increase in inflammatory cytokine levels. Swelling of the affected limb, decreased range of motion (ROM),

and impaired muscle force-producing capacity which can result from EIMD are undesirable [16–18].

Curcumin has been shown to attenuate muscle soreness, improve performance, reduce blood levels of inflammatory markers, and enhance endogenous oxidative capacity post-exercise [19–26]. Muscle damage is prominent post-exercise due to the release of prostaglandins under the influence of cyclooxygenase (COX-1 and COX-2), which contributes to redness, swelling, and pain at the site of damage [27, 28]. Curcumin downregulates the expression of COX-2 and thus decreases the release of prostaglandins [29] which in turn reduces muscle damage [19–23]. Exercise, and production of prostaglandins under the influence of COX-2, leads to increased membrane permeability [30, 31] and releases creatine kinase (CK) into the interstitial fluid and then circulation via the lymphatic system [32] and indicates muscle damage. Although research on the chronic effects of curcumin supplementation is limited, data so far have not demonstrated any ergolytic effects as observed following supplementation with other natural antioxidants such as vitamin C and E supplementation. A detailed review [36] on the effects of vitamin C and vitamin E supplementation on exercise performance suggests that these antioxidant supplementations may impair neuromuscular adaptation by affecting muscle mitochondrial biogenesis and muscle hypertrophy [33].

Clinical trials indicate consumption of curcumin close to exercise downregulates cyclooxygenase that influences membrane permeability [31] and offers a membrane-protective effect by altering the structure of the cell membrane to improve its integrity [34]. Curcumin also helps in reducing inflammation by hindering the activation of nuclear factor-kappa B (NF-κB), suppressing the activation and phosphorylation of Janus kinase/signal transducers and activators of transcription (JAK/STAT) proteins, and inhibiting mitogen-activated protein kinase (MAPK) signalling that releases inflammatory markers such as tumour necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), and interleukin-6 (IL-6) at the site of damage [35]. In addition, the downregulation of NF-κB can also lead to the elevation of antioxidant responses by activation of nuclear factor erythroid 2-related factor 2 (NRF2) [36]. NRF2 regulates the synthesis of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation [37].

Curcumin can be tolerated without any associated toxicity at 8000 mg/day [38]. However, poor aqueous solubility [39] and low bioavailability [40] of curcumin have led to the development of different curcumin formulations such as nanoparticles [41], phytosomes [42], micelles [43], and phospholipid complexes [44]. Each formulation contains varying levels of curcuminoids and has a different rate of absorption, making it difficult to conclude a single recommended dose. Nevertheless, different curcumin formulations have been shown to effectively reduce EIMD and inflammation in doses varying from 90 mg/day to 5000 mg/day [21, 25]. This narrative review evaluates the different curcumin formulations and their effect on EIMD, inflammation, and oxidative markers.

Methods

The databases SCOPUS, Medline (PubMed), and Web of Science (WOS) were searched using a mix of Medical Subject Headings (MeSH) and free words for key concepts related to curcumin, muscle, exercise, inflammation, recovery, along with bioavailability of curcumin as follows: ("curcumin" OR "turmeric") AND ("muscle damage" OR "delay onset muscle soreness" OR "DOMS" OR "inflammation" OR "inflammatory" OR "inflammatory markers" OR "oxidative stress") AND ("exercise"). For articles on other natural antioxidants and their effect on exercise performance, search terms included ("antioxidants"), ("vitamin E and C"), ("tart cherry juice"), ("natural extracts") AND ("exercise" OR "exercise-induced muscle damage" OR "exercise performance''), between December 2020 and March 2021. Only full-text articles (written in English) describing human trials were included for review.

Curcumin metabolism

The bioavailability of curcumin is low due to its water insolubility and poor metabolism in the small intestine and liver, where it undergoes extensive reductive and conjugative metabolism and is finally eliminated through the gall bladder [45]. The metabolism of curcumin can be divided into two phases (Fig. 1). Phase I comprises the reduction of its double bonds to dihydrocurcumin, tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin by reductases in enterocytes and hepatocytes [40]. For phase II, both curcumin and its metabolites from phase I undergo conjugation with sulphate at its phenolic site in the hepatic and intestinal cytosol. In addition, metabolites also undergo glucuronidation via UDP-glucuronosyltransferase in the intestinal and hepatic microsomes [40]. Alternatively, curcumin can be metabolised by intestinal microbiota, such as *Escherichia coli* to tetrahydrocurcumin with the help of an NADPH-dependent reductase and by *Blautia* sp. MRG-PMF1 (anaerobic bacterial strain) that facilitates curcumin demethylation to form demethylcurcumin and bis-demethylcurcumin [46].

Pharmacokinetics of curcumin supplements

Absorption, distribution, hepatic and intestinal metabolism, and excretion regulate the bioavailability of ingested curcumin [45]. In addition, the physicochemical properties of each curcumin formulation and the dose, along with its rate

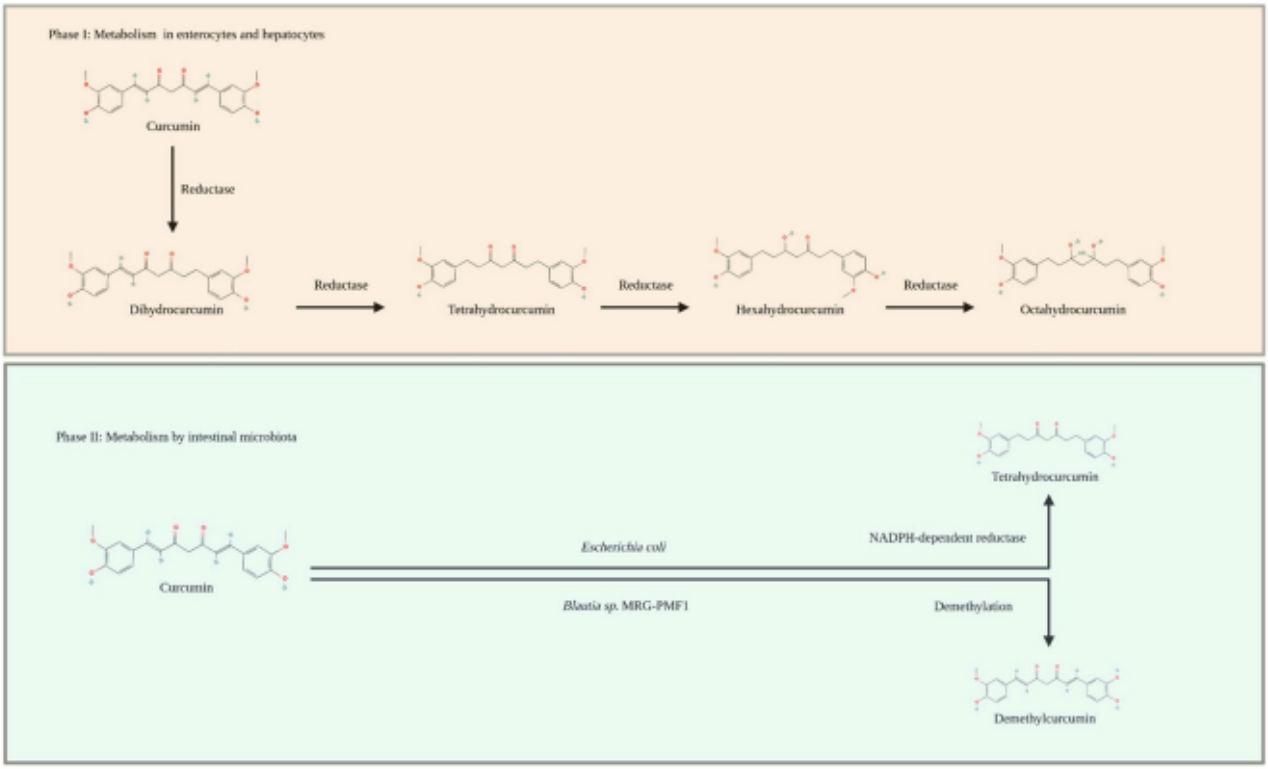

Fig. 1 Metabolic pathways of curcumin

of degradation in the lumen, lipophilicity, and gastric emptying time, help determine the body's pharmacokinetics [47].

Some studies have assessed plasma curcumin levels after hydrolysis of blood plasma samples, however, such hydrolysis prior to extraction masks the amount of free, bioactive curcumin and total curcuminoids as compared to non-hydrolysed samples [50]. When treated with the enzymes β-glucuronidase and sulfatase, curcumin generates glucuronide curcumin and curcumin sulphate, which are the primary circulating forms of curcumin but are physiologically inactive conjugates. This obscures the free bioactive curcumin and overestimates the amount of curcumin detected, thus providing incorrect and misleading results regarding the bioavailability of the formulation [48]. This highlights the importance of reporting free curcumin in the plasma without hydrolysis of the sample. Plasma samples obtained from studies involving Theracurmin [41], Meriva [42], and NovaSol [43] were all hydrolysed using β-glucuronidase/sulfatase before analysis and thus the bioavailability results should be viewed with caution.

Overview of bioavailability of different curcumin formulations

Information on dosage, study type, and study population of six different formulations, namely Theracurmin [41], Meriva [42], NovaSol [43], CurQfen [49], Longvida [44], and Curcumin C3 Complex [50], along with their pharmacokinetic parameters are presented in Tables 1 and 2 [47]. The evidence from human trials suggests that formulating curcumin improves systemic exposure and increases area under curve (AUC) and maximum blood concentration of curcumin, and thus, increases the bioavailability of curcumin (C_{max}) [41–44, 49, 50].

The use of gum ghatti in a water-soluble formulation called Theracurmin [41] led to a preparation of a stable water-soluble complex that contributed to colloidal dispersion and enhanced gastrointestinal absorption [41]. A dose of 30 mg of Theracurmin containing 3.6 mg curcuminoids showed a 27-fold improvement in its $AUC_{(0–6\ h)}$, with a T_{max} of 1 h. Another water-soluble curcumin: NovaSol [43] demonstrated the highest C_{max} along with 185-fold better bioavailability compared to its native form, with a single dose of 500-mg of curcuminoids. The improved relative bioavailability was contributed by the micellar-based curcumin formulation with Tween 80 (non-ionic surfactant and

Table 1 Composition of different curcumin formulations

Formulation name	Formulation		
	Technology	Curcuminoid concentration	Ingredients
Theracurmin [41]	Colloidal nanoparticles	12%	12% curcuminoids, 46% glycerine, 4% gum ghatti, 38% water
Meriva® [42]	Phytosome	18–20%	Curcumin, soy lecithin, microcrystalline cellulose
NovaSol [43]	Liquid micelles	6%	93% Tween 80, and 7% curcumin powder
CurQfen [49]	Soluble fibre blend	Not defined	Fenugreek soluble fibre blend, and 40% curcumin
Longvida® [44]	Solid lipid curcumin particle	20–30%	Solid lipid curcumin particle lipids, phosphatidylcholine, and 20% curcumin
Curcumin C3 Complex® + Bioperine [50]	Not applicable	Not defined	Bioperine and curcuminoids

Table 2 Pharmacokinetic parameters of curcumin from the different curcumin-based formulations and reference (unformulated curcumin) in human studies

Formulation	Clinical study design	Population	Intervention	Dose	Sample hydrolysis	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng h/mL)
Theracurmin [41]	Randomised, crossover	Asian 14 (8 males, 6 females) 44.1 ± 8.5 years	Formulation[a]	30 mg theracurmin	Hydrolysed	29.5 ± 12.9	1	113 ± 61[*]
			Control[a]	30 mg curcumin powder		1.8 ± 2.0	6	4.1 ± 7.0[*]
Meriva® [42]	Randomised, double-blind, crossover	Caucasian 9 (8 males, 1 female)	Formulation[b]	376 mg curcumin	Hydrolysed	206.9 ± 54.9	2.7 ± 0.3	1336.0 ± 357.1[*]
			Control[b]	1799 mg curcumin		14.4 ± 4.2	6.9 ± 2.2	202.8 ± 53.8[*]
NovaSol [43]	Randomised, single-blind crossover	Caucasian 23 (10 males, 13 females) 23 ± 3 years	Formulation[a]	500 mg curcuminoids	Hydrolysed	1189.1 ± 518.7	1.1 ± 0.4	4474.7 ± 1675.2[‡]
			Control[a]	500 mg curcuminoids		2.6 ± 4.9	7.5 ± 8.2	24.1 ± 42.6[‡]
CurQfen [49]	Crossover	Indian 8 (males) 25–50 years	Formulation[a]	600 mg curcumin	Not hydrolysed	0.4 ± 0.2 (μg/g)	1	8100 ± 287[*] (μg h/g)
			Control[a]	1000 mg curcumin		0.02 ± 0.01 (μg/g)	0.5	510 ± 123[*] (μg h/g)
Longvida® [44]	Randomised, double-blind, crossover	Indian 6 (males) 18–40 years	Formulation[b]	650 mg curcuminoids	Not hydrolysed	22.4 ± 1.9	2.4 ± 0.4	95.3 ± 4.6[*]
			Control[b]	650 mg curcuminoids		< 1	ND	ND
Curcumin C3 Complex® + Bioperine [50]	Randomised, crossover	Indian 8 (males) 20–26 years	Formulation[b]	2000 mg curcumin with bioperine	Not hydrolysed	180 ± 30	0.69 ± 0.07	80 ± 10[†]
			Control[b]	2000 mg curcumin		6 ± 5	1	4[†]

[a] Mean ± standard deviation

[b] Mean ± standard error of mean

[*] AUC_{0-24}

[‡] AUC_{0-12}

[†] AUC_{0-6}

Control: unformulated curcumin, *AUC* area under the drug concentration–time curve, C_{max} maximum drug concentration, T_{max} time at maximum drug concentration, *ND* not defined

emulsifier) that could deliver most of the curcumin to the intestinal wall for absorption by escaping the phase separation in the gastrointestinal tract [43]. Schiborr et al. [43] also observed that less than 0.2% of the oral dose of Novasol curcumin was excreted in urine within 24 h, and concluded that the remaining >98.8% of the ingested curcumin was either excreted via the bile and faeces or may have been distributed to body tissues where it may potentially exert biological activities. Meriva [42], a formulation using natural curcuminoids and lecithin (phosphatidylcholine phytosome complex of soy) in the ratio of 2:1 along with two parts of microcrystalline cellulose yielded the highest T_{max} at 2.7 ± 1 h for a dose of 376 mg of curcuminoids, and 29-fold higher curcuminoid absorption compared to the unformulated curcumin. Interestingly, the authors [42] concluded that lecithin favoured the bioavailability of demethoxycurcumin as its plasma content was found to be higher than curcumin itself, despite its low concentration in the formulation.

The formulation of fenugreek fibre and 40% curcumin called CurQfen [51] yielded the highest AUC over 24 h at a dose of 1500 mg (equivalent to 600 mg curcumin). The use of soluble fibre in the formulation produced a non-digestible gel hydrocolloid that could ferment in the colon, prevent curcumin degradation in the gastrointestinal tract, and retard curcumin release resulting in a lag time of more than 5 h and less than 30% the total release after 24 h [51]. Thus, the fibre–curcumin complex contributed to improved and delayed curcumin absorption [51].

The curcumin formulation named Longvida [44] incorporated solid lipid curcumin particles (SLCP; 650 mg) in their formulation. A single dose of 130–195 mg of curcumin showed a T_{max} of 2.4 h. The SLCP is a proprietary formula and comprises of curcumin mixed with soy lecithin containing purified phospholipids, docosahexaenoic acid (DHA), and/or vegetable stearic acid, ascorbyl (vitamin C) esters, and inert ingredients. The improved bioavailability of SLCP compared to unformulated curcumin is linked to key parameters such as curcumin/lipid/antioxidant ratio, globule-size distribution, and stability [51].

Consumption of a combination of curcumin and piperine in the Curcumin C3 Complex + Bioperine resulted in a 20-fold increase in plasma curcumin concentrations compared to the control formulation with the lowest T_{max} of 0.69 h. Piperine is a P-glycoprotein and a uridine diphosphate-glucuronosyltransferase (UGT) inhibitor, and is suggested to improve absorption of curcumin by decreasing the efflux in the intestine and increasing the freely available curcumin in the systemic circulation [50]. Although curcumin was not observed in the plasma from 3 to 6 h, the bioavailability improved by 1.5 times compared to that of unformulated curcumin [50].

Plasma curcumin concentration in exercise trials

Several studies [22, 23, 25, 52, 53] that investigated the effect of curcumin on exercise-induced inflammatory and oxidative stress markers also evaluated plasma curcumin concentration post-supplementation. All studies [22, 23, 25, 52, 53] observed an increase in plasma curcumin concentration post-supplementation at time points ranging from 2 h to 1–4 days (Table 3). The studies also concluded that supplementation with curcumin resulted in an increase in oxidative capacity [25], improvements in visual analogue scale for muscle soreness [23] and daily analysis of life demands questionnaire [52], and a decrease in CK levels [22, 53] (Table 4).

It is difficult to compare and evaluate the plasma curcumin concentration as studies by Tanabe et al. [22, 53] did not describe the sample preparation process and studies by Takahashi et al. [25], Sciberras et al. [52], and Tanabe et al. [23] hydrolysed their plasma samples and, therefore, the amount of curcumin in the plasma [48], may have been overestimated.

Difficulties in comparing the effects of different pharmacokinetic characteristics of curcumin supplements on muscle damage markers

It is challenging to understand how the measured differences in pharmacokinetic characteristics (C_{max}, T_{max}, and AUC) of the specific supplements may relate to changes in inflammatory markers and attenuation of muscle damage. One key contributing factor is that although pharmacokinetic and exercise trials have both been carried out using the same formulations (Theracurmin [41] and Meriva [42]) different doses have been used for the exercise [23, 25, 43] versus the pharmacokinetic studies [41, 42]. In addition, some researchers have not measured the plasma curcumin concentrations post-supplementation, or do not clearly state the curcumin concentrations in the supplement, also making it challenging to compare results [24]. Others have hydrolysed the plasma samples before analysis [23, 25, 43], thus overestimating the true concentration of curcumin in the blood and making it difficult to accurately correlate any observed changes in levels of inflammatory markers to plasma curcumin levels. Finally, although some researchers [54] have reported that curcumin supplementation increased working capacity at the fatigue threshold and delayed the onset of neuromuscular fatigue, they did not analyse common inflammatory markers such as IL-6, TNF-α, and CK, also hindering comparisons between studies [54].

Table 3 Summary of plasma curcumin concentration in studies examining the effect of curcumin intake on exercise-induced muscle damage (EIMD)

Author and year	Population	Duration	Curcumin supplement	Dosage	Plasma curcumin concentration	Sample hydrolysis
Takahashi et al., 2013 [25]	26 males (26.8 ± 2.0 years) (recreationally active)	1 day	Theracurmin	Control 90 mg/day 2 h before exercise 180 mg/day 2 h before and immediately after exercise	Plasma curcumin concentrations in the double curcumin supplementation trial 2 h after exercise were significantly higher than those in the single curcumin supplementation trial	Hydrolysis
Tanabe et al., 2018 [22]	10 males (28.5 ± 3.4 years) (untrained)	7 days	Theracurmin	Experiment 1—180 mg (90 mg twice a day—at breakfast and dinner) consumed for 7 days before exercise	Plasma curcumin concentrations significantly decreased from baseline (38.8 ± 17.8 ng mL^{-1}) to 1, 3, 5, and 7 days after exercise (10.2 ± 5.4, 5.0 ± 2.7, 0.1 ± 0.4, and 0.1 ± 0.2 ng mL^{-1}, respectively)	No mention of the process used
	10 males (29.0 ± 3.9 years) (untrained)			Experiment 2—180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 7 days after exercise	Plasma curcumin significantly increased from baseline (5.3 ± 6.1 ng mL^{-1}) to 1 day (50.6 ± 25.6 ng mL^{-1}), and then maintained a high plasma curcumin concentration through 3, 5, and 7 days after exercise (58.5 ± 37.1, 42.2 ± 46.0, and 41.1 ± 29.2 ng mL^{-1}, respectively)	
Tanabe et al., 2019 [23]	8 males (28.0 ± 3.2 years) (untrained)	7 days	Theracurmin CR-033P	Control	–	Hydrolysis
	8 males (28.8 ± 3.6 years) (untrained)			PRE—180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 7 days before exercise	At baseline and at 1–3 d after exercise, the plasma curcumin concentration of the PRE group was significantly higher than that in the control and POST groups	
	8 males (29.8 ± 3.4 years) (untrained)			POST—180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 4 days after exercise	At 1–4 d after exercise, the plasma curcumin concentration of the POST group was significantly higher than that of the control and PRE groups	

Table 3 (continued)

Author and year	Population	Duration	Curcumin supplement	Dosage	Plasma curcumin concentration	Sample hydrolysis
Tanabe et al., 2015 [53]	14 males (23.5 ± 2.3 years) (UNTRAINED)	Single dose	Theracurmin	300 mg 150 mg—1 h before exercise 150 mg—12 h after exercise	Plasma curcumin concentration increased from baseline (0.04 ± 0.07 ng/mL) to 127.7 ± 144.6, 85.7 ± 51.6, 8.6 ± 5.5, 2.2 ± 1.4 and 0.9 ± 0.6 ng/mL at 0, 24, 48, 72 and 96 h after exercise, respectively	No mention of the process used
Sciberras et al., 2015 [52]	11 males (35.5 ± 5.7 years) (trained)	4 days	Meriva®	500 mg with midday meal for 3 days and 500 mg just before exercise	Mean ± SD (range) curcumin concentration obtained was 79.7 ± 26.3 ng/ml (50.7 ng/mL to 125.5 ng/mL)	Hydrolysis

Effect of curcumin on exercise-induced muscle damage

Intense training can lead to EIMD and can cause swelling, reduced ROM, and loss of muscle strength in the affected limb [16–18]. EIMD is characterised by muscular ultrastructural disruption that increases the release of inflammatory cytokines from myofibers and consequently increases their circulating levels. Muscle soreness increases from about 24 to 48 h post-exercise and decreases gradually from 72 h post-exercise. The activity of CK, a marker of EIMD, increases from 24 h onwards post-exercise and is sustained over a period of 7 days post-exercise [30].

Curcumin ingestion results in the attenuation of the release of inflammatory and oxidative markers, muscle pain, muscle performance, and CK levels by modulating inflammatory signalling cascades. Table 4 contains information from studies investigating the effect of curcumin on EIMD. Out of the 15 studies discussed below, the majority of participants in the trials were young physically active males 20–40 years of age. In addition, studies investigating the effect of curcumin supplementation on EIMD employed exercise protocols that led to different levels of muscle damage and assessed a variety of parameters, thus making it difficult to directly compare results and provide definitive conclusions as to whether curcumin supplementation is effective.

Muscle soreness

EIMD leads to delayed onset muscle soreness, associated with muscle pain, resulting in reductions in muscle strength and function and impairing physical function for several days post-exercise [55]. Damage to skeletal muscle activates phospholipase A_2, which leads to the removal of arachidonic acid from the cell membrane [56]. Arachidonic acid is converted to prostaglandin G_2 (PGG_2) under the influence of cyclooxygenase (COX-1 and COX-2) and then to prostaglandin H_2 (a common precursor to all prostaglandins) [27]. Prostaglandins are pro-inflammatory and cause redness, swelling (due to increased membrane permeability), and pain at the site of muscle damage [28]. Curcumin down-regulates the expression of COX-2 and thus, decreases the release of prostaglandins [29] which in turn reduces muscle soreness [19–23].

Studies [19–23] have shown significantly lower levels of muscle soreness when curcumin was consumed approximately 24 h before or after exercise. Consumption of 150 mg of curcumin (Theracurmin) immediately post-exercise resulted in a lower visual analogue scale (VAS) score for perceived muscle soreness compared to placebo at 48 and 72 h after unaccustomed squat exercises in untrained males [19]. Similarly, consumption of 180 mg of Theracurmin

Table 4 Summary of studies examining the effect of curcumin intake on exercise-induced muscle damage (EIMD)

Author and year	Population	Study design	Duration	Curcumin supplement	Dosage	Curcuminoids content	Activity type	Key findings
Takahashi et al., 2013 [25]	26 males (26.8 ± 2.0 years) (recreationally active)	Double-blind, placebo-controlled, counterbalanced crossover design	1 day	Theracurmin	Placebo	NA	Walking or running at 65% VO_2 max on a treadmill for 60 min	↑ GPX ↑ d-ROM's
					90 mg/day 2 h before exercise	10% curcumin, 2% curcuminoids without curcumin		↑ Plasma curcumin ↑ BAP ↑ GSH † TBARS † GSSH † SOD
					180 mg/day 2 h before and immediately after exercise			
Nakhostin-Roohi et al., 2016 [19]	10 males (25.0 ± 1.6 years) (untrained)	Randomised controlled trial—double-blind crossover	Single dose	Theracurmin	150 mg Immediately post-exercise	10% curcumin, 2% curcuminoids without curcumin	Unaccustomed squat exercises	↓ CK ↓ VAS for pain ↑ TAC
Tanabe et al., 2018 [22]	10 males (28.5 ± 3.4 years) (untrained)	Double-blind crossover design	7 days	Theracurmin	180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 7 days before exercise	Not defined	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	↓ Plasma curcumin ↓IL-8
	10 males (29.0 ± 3.9 years) (untrained)				180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 7 days after exercise			↑ Plasma curcumin ↓ VAS for muscle soreness ↓ CK
Tanabe et al., 2019 [23]	8 males (28.8 ± 3.6 years) (untrained)	Randomised, controlled, single-blind, parallel design study	7 days	Theracurmin CR-033P	PRE—180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 7 days before exercise	30% curcumin, 6% other curcuminoids	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	↑ Plasma curcumin at baseline † CK
	8 males (29.8 ± 3.4 years) (untrained)		4 days		POST—180 mg (90 mg twice a day—at breakfast and dinner) Consumed for 4 days after exercise			↓ VAS for muscle soreness † CK

Table 4 (continued)

Author and year	Population	Study design	Duration	Curcumin supplement	Dosage	Curcuminoids content	Activity type	Key findings
Tanabe et al., 2015 [53]	14 males (23.5 ± 2.3 years) (untrained)	Randomised, crossover design	Single dose	Theracurmin	300 mg 150 mg—1 h before exercise 150 mg—12 h after exercise	Not defined	50 maximal eccentric contractions of the elbow flexors at an angular velocity 120°/s	↑ Plasma curcumin ↓ MVC torque ↓ CK † IL-6 and TNF-α
McFarlin et al., 2016 [24]	16 (5 M, 11 F) (20 ± 1 years) (untrained)	Randomised controlled	6 days	Longvida	400 mg 48 h before exercise and for 72 h after	Not defined	6 sets of 10 repetitions of the leg press exercise with a beginning load set at 110% of their estimated 1RM	† Subjective quadriceps pain †ADL ↓CK ↓TNF-α ↓IL-8 † IL-10 † IL-6
Sciberras et al., 2015 [52]	11 males (35.5 ± 5.7 years) (trained)	Double-blind randomised crossover	4 days	Meriva®	500 mg with midday meal for 3 days and 500 mg just before exercise	Not defined	Participants exercised for 2 h at a power output equivalent to 95% of their lactate threshold	† IL-6, IL-10 ↑ DALDA Questionnaire (better than usual)
F. Drobnic et al., 2014 [70]	C—9 males (32.7 ± 12.3 years) (trained) P—10 males (38.1 ± 11.1 years) (trained)	Randomised, placebo-controlled, single-centre, single-blind pilot trial	4 days	Meriva®	1 g twice a day 48 h prior to exercise and continued for 24 h after exercise	200 mg/dose	Modified downhill running test—running at a constant speed for 45 min after a 10-min warm-up on treadmill	†CRP † hsCRP † MCP-1 † FRAP † GPx † CK ↓ IL-8 † Intensity of pain
Jäger et al., 2019 [57]	63 (31 M, 32 F) (21 ± 2 years) (trained)	Double-blind, randomised, placebo-controlled parallel design	8 weeks	CurcuWIN®	Low-dose group 250 mg thrice a day (breakfast/lunch/dinner) High-dose group 1000 mg thrice a day (breakfast/lunch/dinner) Placebo	50 mg of curcuminoids 200 mg of curcuminoids NA	45-min downhill run at a – 15% grade and speed equivalent to 65% VO_2Max after 5-min warm up	↑ Subjective muscle pain (anterior, posterior) all groups † Subjective (total) muscle pain in high-dose group 1 h and 24 h post-exercise † Maximum bending torque and bending power in low-dose group

Table 4 (continued)

Author and year	Population	Study design	Duration	Curcumin supplement	Dosage	Curcuminoids content	Activity type	Key findings
McAllister et al., 2020 [66]	14 males (21–30 years) (trained)	Double-blinded, randomised, crossover design	4 days	CurcuFresh	1500 mg/day 1000 mg at breakfast and 500 mg at dinner for days 1, 2 and 3 and 45 min before testing on day 4	69 mg of curcuminoids	Dual stress challenges task that consisted of 35 min of steady-state exercise at a workload corresponding to 60% VO_2 peak with mental stress challenges	† GHS ↓SOD ↓ AOPP ↓ H2O2
S. Basham et al., 2019 [26]	20 males (21.7 ± 2.9 years) (trained)	Randomised, double-blinded, placebo-controlled, crossover	28 days	CurcuFresh	1500 mg/day 1000 mg at breakfast and 500 mg at dinner for 28 days	69 mg of curcuminoids	225 repetitions of sit and stand using an aerobic step bench in 15 min	↓ CK ↓ VAS † TAC † MDA † TNF-α
Amalraj et al., 2020 [20]	30 (12 M, 18 F) (36 ± 11 years) (trained)	Randomised, placebo-controlled, double-blind	4 days	Cureit	500 mg/day (consumed on day 2, 3 and 4 of study)	Not defined	Downhill running for 45 min	† CK ↓ VAS for muscle pain
Delecroix et al., 2017 [61]	16 males (20.7 ± 1.4 years) (trained)	Randomised, placebo-controlled, balanced crossover design	4 days	MGD nature	2 g curcumin + 20 mg Piperine consumed three times a day every 6 h between 8 am and 10 pm On exercise day—45 min before, immediately post- and 6 h post-exercise	Not defined	25 repetitions over 25 m of one-leg jumps on an 8% downhill slope	† Isometric peak torque † Concentric peak torque † Jump performance † CK † Muscle soreness at any time point
Herrick et al., 2020 [54]	47 (25 M, 22 F) (21.0 ± 2.6 years) (untrained) G1: 18 (C + FEN) G2: 14 (FEN) G3: 15 (P)	Randomised, double-blind, placebo-controlled, parallel design	28 days	CurQfen	500 mg/day curcumin + 300 mg fenugreek dietary fibre (galactomannans) Consumed in the morning before eating	190 mg of curcumin	Maximal graded exercise test on a cycle ergometer	† Physical working capacity at the fatigue threshold † delay the onset of neuromuscular fatigue † VO_2peak †Time to exhaustion

Table 4 (continued)

Author and year	Population	Study design	Duration	Curcumin supplement	Dosage	Curcuminoids content	Activity type	Key findings
Nicol et al., 2015 [21]	17 males (33.8 ± 5.4 years) (trained)	Double-blind randomised-controlled crossover	5 days	Eurofins scientific inc.	5000 mg/day 5 capsules twice daily for 2.5 days prior to exercise, then 5 capsules twice daily for 2.5 days after exercise	Not defined	7 sets of 10 eccentric single-leg press repetitions on a leg press machine	[a] ↓ VAS for muscle pain ↓ CK ↓ IL-6 (at 24 h relative to baseline) ‡TNF-α

↑ Statistically significant increase

↓ Statistically significant decrease

† Change without statistical significance

‡ No impact

[a] moderate to large effect

C Curcumin Group, *P* Placebo Group, *M* Males, *F* Females, *GPX* glutathione peroxidase, *d-ROM*'s reactive oxygen metabolites, *BAP* biological antioxidant potential, *GSH* and *GSSH* reduced and oxidised glutathione, *TBARS* thiobarbituric acid-reactive substances, *SOD* superoxide dismutase, *CK* creatine kinase, *VAS* visual analogue scale, *TAC* total antioxidant capacity, *IL-8* Interleukin-8, *MVC* Maximal voluntary contraction, *ADL* Activities of daily living soreness, *TNF-α* Tumour Necrosis Factor-α, *IL-10* Interleukin-10, *IL-6* Interleukin-6, *DALDA* Questionnaire Daily Analysis of Life Demands Questionnaire, *CRP* C-Reactive Protein, *MCP-1* monocyte chemoattractant protein-1, *FRAP* Ferric Reducing Antioxidant Power, *AOPP* advanced oxidation protein products, H_2O_2 hydrogen peroxide, *MDA* malondialdehyde, *FEN* Fenugreek

(90 mg twice a day at breakfast and dinner) for 7 days after exercise resulted in a significant reduction in muscle soreness 3–6 days after eccentric exercise in untrained males compared to the placebo group [22].

A study on untrained males reported a significantly lower perceived muscle soreness VAS score for the upper arm and elbow joint at 3 and 4 days after consumption of 180 mg Theracurmin CR-033P (90 mg twice a day at breakfast and dinner) for 4 days after eccentric elbow flexion [23]. Intake of 500 mg/day (Cureit; consumed on days 2, 3, and 4 after exercise) by trained participants resulted in a decreased pain VAS score from 2.90 to 1.17 in the curcumin group compared to a smaller decrease in the placebo group [20]. Moreover, administration of 2500 mg twice daily of curcumin for 2.5 days before exercise, and then 2500 mg twice daily for 2.5 days after exercise, resulted in moderate to large effect size reductions in muscle pain during single-leg squat and gluteal stretch at 24 and 48 h [21].

Not all studies show clear benefits of curcumin supplementation in reducing muscle soreness. For example, one study implementing 56 days of supplementation with 200 mg of curcuminoids (1000 mg curcumin by CurcuWIN) in trained individuals indicated not statistically significant reductions in muscle soreness. Compared to the placebo and low-dose (50 mg) curcumin groups, the treatment group showed non-significant reductions of 26, 20, and 8% lower muscle soreness immediately, 24, and 48 h post-exercise (conducted on 57 ± 3 days), respectively. Further research would be required to determine if the non-significant recovery benefits from curcumin may have been due to the final supplementation dose being administered 24 h prior to the exercise and the lack of supplementation after exercise [57].

A wide range of curcumin doses were administered across these studies with differences in formulation, frequency, and timing of ingestion relative to exercise, along with the training status of the participants and the exercise protocol used that may have influenced the extent of muscle soreness post-completion of exercise. In addition, VAS for soreness is a subjective measure, varying from individual to individual, thus potentially influencing the results. Nonetheless, studies have reported lower levels of soreness when 180–2500 mg/day curcumin was consumed immediately after exercise and/or within at least 24 h before and/or after exercise [21, 23].

Muscle performance

NF-κB is the transcriptional control for myokines (cytokines synthesised and released during muscular contractions) that are involved in post-exercise inflammatory responses [58]. Overuse of joints contributes to high mechanical stress and generates bone and cartilage extracellular matrix fragments that are recognised by receptors expressed by innate immune cells. Cell activation mediated by this process stimulates the

activation of NF-κB, resulting in secretion of inflammatory cytokines such as IL-1 and TNF-α, which contribute to tissue damage [59]. Curcumin blocks the signalling pathway of NF-κB, reducing the inflammatory response, decreasing swelling, while improving joint mobility and stiffness (assessed via maximum voluntary contraction (MVC) force and ROM) [22, 23, 53].

Intake of 90 mg curcumin (Theracurmin) twice a day (breakfast and dinner) for 7 days in untrained males resulted in significant improvements in MVC torque and ROM compared to the placebo group 3–7 days after eccentric exercise [22]. In a similar study, consumption of 90 mg curcumin (TheracurminCR-033P) twice a day (breakfast and dinner) for 4 days after eccentric exercise resulted in improvements in ROM of the elbow joint at 3–4 days in untrained males compared to the placebo group. The increases in the degree of ROM coincided with improvements in muscle soreness, indicating that the two could be related. However, another study [23] involving 30 maximal eccentric contractions of elbow flexors showed no significant differences in MVC torque between curcumin and placebo groups at all time points [23]. Muscle regeneration begins on day 3 after exercise [60], and since the follow-up of the recovery period was only 4 days, this time period may have been insufficient to establish the effect of curcumin on MVC torque [23].

One study examining the effect of 50 mg of curcumin (in the form of 250 mg of CurcuWIN®) or 200 mg of curcumin (in the form of 1000 mg of CurcuWIN®) over 56 days in physically active men and women reported that curcumin could prevent decreases in peak extension torque values observed at 1 and 24 h after muscle-damaging exercise (downhill running) [57]. However, changes in isokinetic peak and average flexion torque, peak extension and flexion power, peak and average peak torque failed to yield statistical significance at 1, 24, 48, and 72 h post-exercise. One of the limitations of the supplementation protocol used was conducting the exercise protocol after discontinuing curcumin supplementation and it is possible that continuing supplementation may have prevented a decrease in the performance measures [57].

Ingestion of 150 mg of curcumin (Theracurmin) immediately and 12 h after eccentric exercise in untrained males led to a decrease in MVC from baseline [53]. However, the decrease in MVC was significantly smaller (33.0 ± 8.0%) in the curcumin group than in the placebo group (40.0 ± 9.1%) immediately after exercise and 48–96 h after exercise. The smaller decrease in MVC indicates that intake of curcumin leads to a lower level of muscle damage than the placebo group. However, ROM significantly decreased through all time points from the baseline in the curcumin group and there was no significant interaction effect for the changes compared to the placebo groups [53]. This suggests that supplementation with 150 mg Theracurmin twice a day for only one day is inadequate to improve ROM post-exercise.

In a randomised crossover trial, elite rugby players, consuming 6 g of curcumin and 60 mg of piperine (MGD Nature, Brandérion, France) each day starting 48 h pre-exercise and continuing until 48 h post-exercise experienced a moderately smaller loss of mean power output during the 6-s sprint compared to the control group 24 h after exercise [61]. However, this result was counterbalanced by the absence of effect from curcumin supplementation on isometric peak torque, concentric peak torque, and jump performance at all time points. This was possibly because the EIMD protocol consisted of 25 repetitions over 25 m of one-leg jumps on an 8% downhill slope, which required a greater neuromuscular recruitment pattern compared to isokinetic and isometric tests [61]. Furthermore, a possible limitation of this study could be the quick muscle damage recovery kinetics (training adaptations) which could be a result of the population tested, i.e. elite rugby players [61].

Another study examining the effects of curcumin in combination with fenugreek soluble fibre (CUR + FEN) or fenugreek soluble fibre alone (FEN) on the physical working capacity at the fatigue threshold (PW_{CFT}), peak oxygen consumption (VO_2 peak), and time to exhaustion (T_{lim}) on a graded exercise test in untrained subjects for 28 days [54], showed no effect of curcumin supplementation on VO_2 peak or T_{lim}. However, the PW_{CFT} was greater after combined supplementation with curcumin and FEN compared with a placebo in ~20% of subjects [54].

Thus, based on the results obtained by Tanabe et al. [23], improvements in both MVC force and ROM in untrained males could be a result of consumption of 180 mg of curcumin in divided doses (two times a day) across 4–7 days post-eccentric exercise [23].

Creatine kinase

Muscle creatine kinase (CK-MM), a marker of EIMD, is one of the three isoforms of CK, and is present at places within the muscle fibre where ATP consumption is high [62]. Eccentric muscle contractions exceeding the muscle's resistance result in perforations in the sarcolemma and damage to the sarcomeres, leading to increased membrane permeability [30, 62, 63], and release of CK into the interstitial fluid that then enters circulation via the lymphatic system [32]. In addition, the production of prostaglandins under the influence of COX-2 leads to vascular hyperpermeability, which could further aid release of CK [31].

Curcumin supplementation indirectly lowers plasma CK activity in several ways. First, curcumin in the blood offers a membrane-protective effect by altering the structure of the membrane [34] and improving membrane integrity, thus reducing CK release into the blood [64]. Second, curcumin

can suppress the regulation of the COX-2 pathway, reducing prostaglandin release and hence influence vascular permeability, ultimately decreasing the intracellular–intravascular flow of CK [31]. Lastly, curcumin's antioxidant properties can suppress the activity of ROS generated during muscle contractions that would ordinarily contribute to muscle damage via CK release [25, 65].

Several studies [19, 21, 22, 24, 26, 53] have observed significantly lower CK activity in the curcumin supplemented group at doses of 150–5000 mg/day in both trained [21] and untrained individuals [24, 53]. Single-dose investigations with an intake of 150 mg curcumin (Theracurmin) post-eccentric exercise showed a lower rise in CK activity immediately 0, 24, 48, and 72 h post-exercise compared to the placebo group [19]. In addition, CK activity was significantly lower in the curcumin group compared to the placebo group at 24 h [19]. Intake of 180 mg of curcumin (Theracurmin) for 7 days (90 mg twice a day, at breakfast and dinner) after exercise in untrained men also resulted in lower CK activity compared to the placebo group [22]. Consumption of 300 mg curcumin (Theracurmin) in divided doses (150 mg 1 h before exercise and 150 mg 12 h after exercise) showed a significantly smaller peak CK activity for the curcumin group (3398 ± 3562 IU/L) compared to placebo (7684 ± 8959 IU/L) and lower activity at 48, 72 and 96 h compared to baseline CK levels [53]. Similarly, 1500 mg/day (CurcuFresh) of curcumin resulted in significantly lower CK activity (199.62 U/L in the curcumin group compared to 287.03 U/L in the placebo group) [66]. Supplementation with 5000 mg/day of curcumin (Eurofins Scientific Inc.) for 5 days (5 capsules twice daily for 2.5 days before exercise, then 5 capsules twice daily for 2.5 days after exercise) in trained men showed a small reduction in CK activity at 24 and 48 h (−22 to 29%; ±21 to –22%) compared to the baseline values [21]. Lower CK activity in the curcumin groups across the studies may suggest that myofibril damage due to exercise was attenuated by curcumin ingestion [53].

Curcumin supplementation before and/or after exercise can decrease CK activity post-exercise. However, the magnitude of the reduction varies from study to study. Several factors may have contributed to the differing outcomes, including the training status of the participants, exercise protocol, duration of the study, the timing of ingestion of the curcumin supplement, and the formulation of the curcumin supplement itself (including treatments and other ingredients that affect the rate of absorption).

Inflammatory markers

Curcumin exerts anti-inflammatory actions by hindering the activation of NF-κB, suppressing the activation and phosphorylation of JAK/STAT proteins, and inhibiting MAPK signalling that contribute to the production of inflammatory markers such as TNF-α, IL-6, and IL-8 at the site of muscle damage [35].

TNF-α: The effect of curcumin supplementation on reducing TNF-α levels in the blood has been evaluated in several studies [21, 22, 24, 26, 53], with equivocal observations reported. No significant differences in plasma TNF-α levels were found between the curcumin and placebo trials when 150 mg [53] and 180 mg of curcumin [22] were consumed by untrained males following elbow flexor eccentric exercise [22, 53]. This could be because the exercise protocol involved small muscle mass and was short in duration, and, therefore, did not affect the inflammatory cytokine levels in the blood [67]. In addition, as TNF-α has a very short half-life (15–30 min), plasma concentrations do not always reflect those produced by myocytes [68]. Moreover, the TNF-α levels were measured in the blood and not the muscle tissue. As concentrations of inflammatory markers and oxidative stress markers after exercise are different between muscle tissue and blood [69], the observation on the effect of curcumin on TNF-α levels post-exercise may be limited. In the study by McFarlin et al. [24], untrained subjects completed 6 sets of 10 repetitions of leg press exercise and consumed 400 mg/day of curcumin 48 h before exercise, up until 72 h after exercise. They observed that TNF-α levels were significantly lower with curcumin at 1 day (− 25%), 2 days (− 23%), and 4 days (− 23%) compared to placebo and concluded that a minimum dose of 400 mg curcumin could be effective in decreasing circulating levels of TNF-α [24].

However, studies involving 1500 mg/day of curcumin supplementation (69 mg of curcuminoids) for 28 days (CurcuFresh) [26] and 5000 mg/day for 5 days (Eurofins Scientific Inc.) [21] reported no significant decrease in plasma TNF-α levels after exercise in trained males. Curcumin supplementation in both the studies [21, 26] was likely ineffective because physically active individuals were recruited and the participants' aerobic training status (150 min of moderate-intensity aerobic activity or 30 min of vigorous-intensity aerobic activity per week) offered the stimulus for adaptations that contributed to lower resting levels of TNF-α (1.2 pg/mL), thus negating any potential anti-inflammatory benefits of curcumin supplementation [26]. In addition, the authors proposed that no significant decrease in TNF-α levels was observed in the trained individuals because the exercise protocol they used (7 sets of 10 eccentric single-leg press repetitions) failed to cause sufficient muscle damage due to muscle adaptations from prior physical activity [21].

In most studies [21, 22, 53], the exercise protocol used led to only minor increases in plasma TNF-α concentrations and reported no significant differences in plasma TNF-α levels in curcumin groups compared to placebo groups. Nonetheless, as observed for untrained individuals, a minimum of 400 mg curcumin supplementation before and after eccentric

exercise may result in lower increases in TNF-α levels post-eccentric exercise [24].

IL-6 and IL-8 The effect of curcumin supplementation on IL-6 and IL-8 levels before and after exercise in trained and untrained individuals has been evaluated in several studies [21, 22, 24, 53, 70]. No significant decrease in post-exercise IL-6 levels was observed in the curcumin group when compared to placebo groups when supplemented with 300 mg (Theracurmin) [53] or 400 mg of curcumin (Longvida), respectively [24]. However, the exercise protocol chosen (6 sets of 10 repetitions of the leg press exercise with a beginning load set at 110% of their estimated 1-repetition maximum) may not have been sufficient to increase the pro-inflammatory cytokines in the body due to the muscle mass involved and the short duration of the activity [24]. In addition, blood samples were not taken immediately after exercise but were taken at 24, 48, 72, and 96 h post-exercise; therefore, the researchers may have missed observing changes in IL-6 levels which typically increase from 8 to 12 h post-eccentric exercise, and return to baseline levels by 24 h post-exercise [53, 71]. In contrast, Nicol et al. [21] observed a decrease in IL-6 values in the curcumin supplemented group (5000 mg curcumin; Eurofins Scientific Inc.) in trained participants after 7 sets of 10 eccentric single-leg press repetitions on a leg press machine at 24 h relative to immediately post-exercise [21]. However, they also observed an increase in IL-6 levels (small standardised differences) immediately post-exercise (31%; ±29%) and again at 48-h post-exercise (32%; ±29%) relative to baseline, thus, making the overall effect of curcumin supplementation unclear [21].

Supplementation with 180 mg of curcumin for 7 days prior to exercise (30 maximal eccentric contractions of the elbow flexors) in untrained males was associated with significantly lower plasma IL-8 levels 12 h after exercise than the placebo group [22]. This decrease in plasma IL-8 levels was associated with high concentrations of curcumin in the blood during and after exercise that suppressed the exercise-induced inflammatory effect [22]. However, no significant differences were observed when curcumin was ingested after exercise [22]. Conversely, intake of 400 mg/day of curcumin (Longvida) (2 days before exercise and for 3 days after exercise) resulted in a significantly lower IL-8 concentrations at day 1 (−21%) and day 2 (−18%) post-exercise (6 sets of 10 repetitions of leg press) compared to placebo in untrained individuals [24]. Furthermore, intake of 1 g of curcumin supplement (Meriva) twice a day 2 days before exercise and for 1 day after exercise (modified downhill running) in trained individuals also resulted in a significantly lower increase in plasma IL-8 levels 2 h post-exercise [70]. Thus, although the effect of curcumin supplementation on IL-6 levels remains unclear, curcumin intake before and after exercise may lower serum IL-8 levels in both trained and untrained individuals.

Oxidative markers

Reactive oxygen species are produced by a variety of extracellular and intracellular agents such as electron leakage from the mitochondrial respiratory chain and NADPH oxidases [72]. Exercise can induce oxidative stress by increasing oxygen utilisation up to 200-fold in active muscles and contributing to excessive amounts of ROS [73, 74], which can damage DNA, proteins, and lipids [75, 76] and affect exercise performance [77].

Reactive oxygen species contribute to oxidative stress and maintain inflammation by promoting the activation of NF-κB. During a sustained inflammatory response, accumulation of neutrophils within tissues provides a growth medium for producing oxidative enzymes, cytokines, and chemokines [78–80]. Curcumin can suppress the activation of NF-κB and potentially lead to elevated antioxidant responses by activating NRF2 [36], which upregulates the synthesis of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation [37]. Thus, activation of NRF2 can improve the total antioxidant capacity of the body and reduce the harmful effects of ROS [36]. In addition, the phenolic OH group of curcumin has the potential to act as a ROS scavenger and a quencher of the lipid peroxidative side chain, thus reducing the activity of lipid hydroperoxides [81].

Curcumin supplementation (Theracurmin) with 90 mg/day (2 h before endurance exercise) and 180 mg/day (90 mg 2 h before and immediately after endurance exercise) in healthy men attenuated exercise-induced increases in the serum concentrations of derivatives of reactive oxygen metabolites (d-ROMs) and serum biological antioxidant potential (BAP), and also reduced plasma glutathione levels (GSH) post-exercise [25]. However, there were no significant increase observed in superoxide dismutase (SOD), and glutathione peroxidase (GPx) concentrations immediately after and post-2 h of exercise compared to the pre-values in both single and double curcumin supplementation groups [25]. In addition, supplementation with 150 mg curcumin (Theracurmin) after squat exercises in untrained males resulted in improved total antioxidant capacity (TAC) at 24 and 48 h post-exercise compared to the placebo group [19].

In a study [22] where curcumin (180 mg; Theracurmin) was supplemented for 7 days before and after exercise in a double-blind crossover study, no statistically significant improvements in serum concentrations of d-ROMs and BAP were observed in the curcumin and placebo groups as well as between groups over time [17]. It is possible that the exercise protocol used in this study (30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s) was insufficient to result in significant oxidative stress [22]. Similarly, curcumin supplementation (1500 mg/day (1000 mg breakfast, 500 mg dinner); CurcuFresh) for

28 days in trained males showed no significant improvements in TAC post-exercise (225 repetitions of sit and stand using aerobic step bench over 15 min) compared to the placebo. According to the authors, the lack of significant changes in TAC may be because the assay did not quantify changes in enzymatic antioxidants and had poor sensitivity [26].

In a trial comparing the effects of curcumin (1 g, Meriva, 2× per day, 2 days before, and 1 day after exercise) versus placebo on catalase (CAT) and GPx levels in aerobically trained males who completed a 2 h downhill run, levels of both enzymes tended to increase 2 h after exercise and returned towards baseline values 24 h after exercise in both curcumin and placebo groups [70]. The recruitment of aerobically trained participants may have limited muscle damage with the exercise protocol used, thus possibly explaining limited changes in CAT and GPx activity [65].

Thus, results from two studies indicate that supplementation with 90–180 mg curcumin 2 h before exercise or immediately after exercise may improve the antioxidant capacity of the body [19, 25]. However, more quality research is needed to clarify the optimal dose.

Based on the studies discussed in this review, curcumin is beneficial in alleviating exercised-induced muscle damage. However, due to the vast differences in the formulations of curcumin supplements and study protocols, it is difficult to determine a single dose that would be effective in reducing various inflammatory markers and increase the antioxidant enzymes such as SOD and GPx. Table 5 summarises the timing and dose of curcumin supplementation required to improve muscle soreness and performance, inflammatory markers, and oxidative markers associated with EIMD in trained and untrained participants after eccentric and endurance exercise.

Limitations of research and future directions

The curcumin formulations discussed in this review have been developed with varying amounts of curcuminoids and different ingredients that impact their bioavailability, thus making it complicated to suggest a single optimal dose for reducing inflammation post-exercise. However, formulation-specific doses can be suggested based on the scientific research for each particular product.

Most of the studies discussed in this review recruited young healthy participants and there are no data available for the effect of curcumin on EIMD in older adults. Older adults are of special interest as they experience sarcopenia: generalised loss of skeletal muscle mass and muscle strength with age. As a result, older individuals have low muscle mass, low muscle strength, and an increased body fat percentage that contributes to chronic inflammation and oxidative stress compared to young individuals [82]. Moreover, the body composition of older individuals with sarcopenia is significantly altered compared to that of a young population. Therefore, the results from the studies based on the effect of curcumin on EIMD in young trained and untrained individuals should not be extrapolated to a sarcopenic older population. In addition, research on the chronic effects of curcumin consumption based on specific formulations, in association with an appropriate exercise protocol are required to evaluate their effects on recovery from EIMD.

Conclusion

Curcumin is a challenging ergogenic aid to study due to its poor bioavailability and poor metabolism in the intestine and liver. However, new formulations using nanoparticles [41], phytosomes [42], micelles [43], and phospholipid complexes [44] have been developed which demonstrate improved bioavailability and their effects on EIMD have been investigated in humans. Overall, curcumin supplementation is most effective in reducing EIMD when consumed by untrained individuals. Supplementation immediately after exercise and/or within at least 24 h before and/or after exercise is highly recommended. Though the optimum amount of curcumin required to decrease serum IL-6 levels is still unclear, supplementation with 400–1000 mg of curcumin 1–2 times a day could be considered to aid in improving muscle performance and lowering circulating IL-8 levels. To date, one study in untrained males has shown decreases in TNF-α levels post-exercise following consumption of 400 mg/day of curcumin for a period of 6 days [24]; however, more studies are needed to confirm this effect. In addition, curcumin supplementation in the range of 150–5000 mg/day has been effective in decreasing CK levels in both untrained and trained individuals when consumed pre- and/or post-exercise. To improve the antioxidant capacity of the body post-exercise, curcumin supplementation with 90–180 mg curcumin 2 h before exercise or immediately after exercise may be effective [19, 25].

Curcumin impacts the production of several exercise-induced inflammatory markers. However, the amount of curcumin required to elicit changes in these inflammatory markers vary significantly. In addition, as no two studies follow the same protocol along with the same curcumin formulation, it is challenging to conclude the amount of curcumin required to reduce EIMD.

Author contributions KN reviewed papers, wrote, and revised the article and AA, KRM, SJL and NCB suggested improvements and reviewed the article.

Table 5 Summary of the timing and dose of curcumin supplementation required to improve muscle soreness and performance, inflammatory markers, and oxidative markers associated with exercise-induced muscle damage (EIMD)

EIMD markers and oxidative markers	Author	Training status of participants	Exercise protocol	Formulation	Curcuminoids content	Duration	Dosage	Timing of dose
Muscle soreness	Nakhostin-Roohi et al., 2016 [19]	Untrained	Unaccustomed squat exercises	Theracurmin	10% curcumin, 2% curcuminoids without curcumin	Single dose	150 mg	Immediately post-exercise
	Tanabe et al., 2018 [22]	Untrained	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	Theracurmin	Not defined	7 days	180 mg	90 mg twice a day at breakfast and dinner, consumed for 7 days after exercise
	Tanabe et al., 2019 [23]	Untrained	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	Theracurmin	30% curcumin, 6% other curcuminoids	4 days	180 mg	90 mg twice a day at breakfast and dinner, consumed for 4 days after exercise
	Amalraj et al., 2020 [20]	Trained	Downhill running for 45 min	Cureit	Not defined	4 days	500 mg/day	Consumed on day 2, 3 and 4 of study
	Nicol et al., 2015 [21]	Trained	7 sets of 10 eccentric single-leg press repetitions on a leg press machine	Eurofins scientific Inc	Not defined	5 days	5000 mg/day	5 capsules (2.5 g curcumin) twice daily for 2.5 days prior to exercise, then 5 capsules twice daily for 2.5 days after exercise
Muscle performance	Tanabe et al., 2018 [22]	Untrained	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	Theracurmin	Not defined	7 days	180 mg	90 mg twice a day at breakfast and dinner, consumed for 7 days after exercise
	Tanabe et al., 2019 [23]	Untrained	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	Theracurmin	30% curcumin, 6% other curcuminoids	4 days	180 mg	90 mg twice a day at breakfast and dinner, consumed for 4 days after exercise
	Tanabe et al., 2015 [53]	Untrained	50 maximal eccentric contractions of the elbow flexors at an angular velocity 120°/s	Theracurmin	Not defined	Single dose	300 mg	150 mg 1 h before exercise and 150 mg 12 h after exercise

Table 5 (continued)

EIMD markers and oxidative markers	Author	Training status of participants	Exercise protocol	Formulation	Curcuminoids content	Duration	Dosage	Timing of dose
Creatine kinase	Nakhostin-Roohi et al., 2016 [19]	Untrained	Unaccustomed squat exercises	Theracurmin	10% curcumin, 2% curcuminoids without curcumin	Single dose	150 mg	Immediately post-exercise
	Tanabe et al., 2018 [22]	Untrained	30 maximal eccentric contractions of the elbow flexors at an angular velocity of 120°/s	Theracurmin	Not defined	7 days	180 mg	90 mg twice a day at breakfast and dinner, consumed for 7 days after exercise
	McFarlin et al., 2016 [24]	Untrained	6 sets of 10 repetitions of the leg press exercise with a beginning load set at 110% of their estimated 1RM	Longvida	Not defined	6 days	400 mg	48 h before exercise and for 72 h after
	Tanabe et al., 2015 [53]	Untrained	50 maximal eccentric contractions of the elbow flexors at an angular velocity 120°/s	Theracurmin	Not defined	Single dose	300 mg	150 mg 1 h before exercise and 150 mg 12 h after exercise
	S. Basham et al., 2019 [26]	Trained	225 repetitions of sit and stand using an aerobic step bench in 15 min	CurcuFresh	69 mg of curcuminoids	28 days	1500 mg/day	1000 mg at breakfast and 500 mg at dinner
	Nicol et al., 2015 [21]	Trained	7 sets of 10 eccentric single-leg press repetitions on a leg press machine	Eurofins scientific Inc	Not defined	5 days	5000 mg/day	5 capsules (2.5 g curcumin) twice daily for 2.5 days prior to exercise, then 5 capsules twice daily for 2.5 days after exercise
Tumour necrosis factor-α	McFarlin et al., 2016 [24]	Untrained	6 sets of 10 repetitions of the leg press exercise with a beginning load set at 110% of their estimated 1RM	Longvida	Not defined	6 days	400 mg	48 h before exercise and for 72 h after

Table 5 (continued)

EIMD markers and oxidative markers	Author	Training status of participants	Exercise protocol	Formulation	Curcuminoids content	Duration	Dosage	Timing of dose
Interleukin-6	Nicol et al., 2015 [21]	Trained	7 sets of 10 eccentric single-leg press repetitions on a leg press machine	Eurofins scientific Inc	Not defined	5 days	5000 mg/day	5 capsules (2.5 g curcumin) twice daily for 2.5 days prior to exercise, then 5 capsules twice daily for 2.5 days after exercise
Interleukin-8	McFarlin et al., 2016 [24]	Untrained	6 sets of 10 repetitions of the leg press exercise with a beginning load set at 110% of their estimated 1RM	Longvida	Not defined	6 days	400 mg	48 h before exercise and for 72 h after
	F. Drobnic et al., 2014 [70]	Trained	Modified downhill running test—running at a constant speed for 45 min after a 10-min warm-up on treadmill	Meriva®	200 mg/dose	4 days	1 g twice a day	48 h prior to exercise and continued for 24 h after exercise
Biological antioxidant potential and glutathione	Takahashi et al., 2013 [25]	Recreationally active	Walking or running at 65% VO_2 max on a treadmill for 60 min	Theracurmin	10% curcumin, 2% curcuminoids without curcumin	1 day	180 mg/day	2 h before and immediately after exercise

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Declarations

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Ethics approval Not applicable.

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Appendix 6.2 – Ethics approval letter (acute study)

Health and Disability Ethics Committees

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011
hdecs@health.govt.nz

Ethics reference: 2022 EXP 11720

31 August 2022

Miss Krutika Nanavati

Massey University - Albany College of Sport, Exercise, and Nutrition, SNW Extension Building
Private Bag 102 904, North Shore, Auckland 0745
Albany, Auckland
0632
New Zealand

Tēnā koe Miss Nanavati

APPROVAL OF APPLICATION

Study title: Effect of Curcumin-Fortified Whey Protein Beverage on Antioxidant Capacity and Exercise-Induced Muscle Damage in Older Adults

I am pleased to advise that your application was **approved** by the Southern Health and Disability Ethics Committee (the Committee) with non-standard conditions. This decision was made through the EXP pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Southern Health and Disability Ethics Committee is required.

Standard conditions:

- Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
- Before the study commences at *any* locality in New Zealand, it must be registered in a clinical trials registry. This should be a registry approved by the World Health Organization (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au or https://clinicaltrials.gov/).
- Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Ethics RM. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

1. Please explain EIMD in full the first time the acronym is used.
2. Please explain what sarcopenia is in lay language.
3. Please delete yes/no tick-box from blood sample consent clause if this is a mandatory component of study participation.
4. Please delete yes/no tick-box from indefinite data storage if this is a mandatory component of study participation.

Non-standard conditions must be completed before commencing your study, however, they do not need to be submitted to or reviewed by HDECs.

If you would like an acknowledgement of completion of your non-standard conditions you may submit a post approval form amendment through the Ethics Review Manager. Please clearly identify in the amendment form that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see paragraphs 125 and 126 of the *Standard Operating Procedures for Health and Disability Ethics Committees (SOPs)*.

After HDEC review

Please refer to the *SOPs* for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 31 August 2023.

Participant access to compensation

The Southern Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialed. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation.

Further information and assistance

Please contact the HDECs Secretariat at hdecs@health.govt.nz or visit our website at www.ethics.health.govt.nz for more information, as well as our General FAQ and Ethics RM user manual.

Nāku noa, nā

Mr Anthony Fallon

Chair

Southern Health and Disability Ethics Committee

Encl: Appendix A: documents submitted

Appendix 6.2 – Ethics approval letter (chronic study)

Health and Disability Ethics Committees

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011
hdecs@health.govt.nz

Ethics reference: 2022 EXP 12905

26 July 2022

Miss Krutika Nanavati

Massey University - Albany College of Sport, Exercise, and Nutrition, SNW Extension Building
Albany, Auckland
0632
New Zealand

Tēnā koe Miss Nanavati

APPROVAL OF APPLICATION

Study title: Effect of Curcumin-Fortified Whey Protein Beverage and Strength Training on Physical Performance in Older Adults

I am pleased to advise that your application was **approved** by the Southern Health and Disability Ethics Committee (the Committee) with non-standard conditions. This decision was made through the EXP pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Southern Health and Disability Ethics Committee is required.

Standard conditions:

- Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
- Before the study commences at *any* locality in New Zealand, it must be registered in a clinical trials registry. This should be a registry approved by the World Health Organization (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au or https://clinicaltrials.gov/).
- Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Ethics RM. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

- It is noted that an updated study flyer has not been submitted. Please ensure that this document is also amended to address point 4 of the HDEC Provisional Approval Letter (Protocol assessments / interventions should not be framed as a benefit of study participation, nor should these be described as 'FREE' - there should never be an expectation for participants to pay for protocol assessments or study treatments). Please retain a tracked changes copy of the amended flyer for submission to HDEC with the annual progress report.

Non-standard conditions must be completed before commencing your study, however, they do not need to be submitted to or reviewed by HDECs.

If you would like an acknowledgement of completion of your non-standard conditions you may submit a post approval form amendment through the Ethics Review Manager. Please clearly identify in the amendment form that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see paragraphs 125 and 126 of the *Standard Operating Procedures for Health and Disability Ethics Committees (SOPs)*.

After HDEC review

Please refer to the *SOPs* for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 26 July 2023.

Participant access to compensation

The Southern Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialed. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation.

Further information and assistance

Please contact the HDECs Secretariat at hdecs@health.govt.nz or visit our website at www.ethics.health.govt.nz for more information, as well as our General FAQ and Ethics RM user manual.

Nāku noa, nā

Mr Anthony Fallon

Chair

Southern Health and Disability Ethics Committee

Encl: Appendix A: documents submitted