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The Amelioration of the Impact of Physical Fatigue on Cognitive Performance by
Phytochemicals

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Olivia McKenzie

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Abstract

Fatigue is common in everyday life. It is experienced as either cognitive or physical fatigue, both of which are intertwined. Researchers are interested in investigating the ability of phytochemical supplementation to improve cognitive performance by diminishing the effects of physical fatigue. The results thus far have been highly inconsistent (Brisswalter & Arcelin, 1997). The present study examined the effects of phytochemical supplementation utilising a daily dose of 240 mg of blackcurrant extract, a berry fruit high in phytochemicals but under-researched compared to other berry fruits, such as blueberries. Fifty healthy participants completed two 3-hour trials, the first during Week 1 and the second 6 weeks later. Half of the participants were randomly assigned to the blackcurrant supplement group, the supplement being consumed each day over the 6-week period. Each trial consisted of five cognitive tests followed by a tailored HIIT cycle test. The purpose of the HIIT was to induce physical fatigue and took less than 10 min overall. Cognitive tasks and mood questionnaires were completed pre and post consumption of the supplement at both Week 1 and Week 6. Participants received the blackcurrant supplement 1 hour before post task measurements were completed. Analyses demonstrated that the blackcurrant supplementation had no influence on cognitive performance. However, it is questionable as to whether the degree of physical fatigue induced was sufficient to negatively influence cognitive performance, even though previous studies had found it to be so. Overall, it was concluded that blackcurrant supplementation taken across 6 weeks did not facilitate cognitive performance after physical fatigue. Possible explanations for these findings are discussed, including ways for future research to move forward.

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Chapter 1

Introduction

Cognition can be described as a group of mental processes that includes attention, language comprehension and production, learning, reasoning, decision making and problem solving (Pripis & Shukitt-Hale, 2014). Cognitive and physical health are both required for overall functioning and wellbeing. They can be detrimentally influenced by too much or too little cognitive stimulation, fatigue, and exercise. It is well known that the effects of exercise can influence individuals in different ways. It can leave some feeling disoriented, and others with increased clarity of thought (Brisswater, Collardeau, & Rene, 2002; Coles & Tomporowski, 2008; Tomporowski & Ganio, 2006). For example, studies have consistently found that maintaining a physical exercise regime, regardless of the type, can have positive effects on the long-term functioning of a person's cognition (Colcombe & Kramer, 2003). However, it is unclear what mechanisms are changed in order to produce improvements or decrements in cognitive performance through exercise. Coles and Tomporowski (2008) note that the effects of exercise on types of cognition, such as memory and executive functioning, are not well understood; neither is the relationship between exercise and cognitive performance (Brisswalter et al., 2002; Tomporowski, 2003). One of the primary problems that arise from excess physical or cognitive exertion is fatigue. Fatigue is a universal problem and a complex phenomenon. Fatigue can lead to accidents and lack of productivity and has been found to be the third most reported problem in a general practitioner's surgery (Zwarts, Bleijenberg, & van Engelen, 2008).

Physical and cognitive performance is of paramount importance to athletes and sports coaches. When athletes are involved in sports they are commonly engaged in great physical exertion as well as meticulous perceptual decision making (Brisswalter et al., 2002). Research over the years has sought to clarify the often contradictory findings of the effects of exercise on cognitive performance. Several theories have been proposed to explain why individuals may experience cognitive fatigue after an exhausting bout of physical exercise.

The first theory describes how exercise influences the way the brain functions (Colcombe & Kramer, 2003; Endres et al., 2003; Swain et al., 2003; Vaynman, Ying, & Gomez Pinilla, 2004). The U-effect explains how varying intensities of exercise can influence the ability of the brain to process and handle cognitive stimuli (Brisswalter et al., 2002). For example, moderate exercise has been found to improve cognitive performance (Brisswalter et al., 2002) and high intensity exercise has been found to have a negative effect on cognitive performance (Easterbrook, 1959). Brisswalter et al. (2002) observed that when individuals exercise, their level of arousal increases. The higher the intensity of the exercise, the lower the individual's attention becomes. Therefore, only highly relevant cues are important because attention is now limited to processing only immediately significant information. This theory highlights two important points. Firstly, if individuals participate in intense exercise it is highly likely that the fatigue they experience will influence their cognition. Secondly, individuals' level of fitness can affect their cognitive performance (Tomprowski, 2003). For example, if an individual is extremely fit then their cognitive performance will not be affected as much as someone who is unfit.

More recent theories attempt to explain the improvement in cognitive performance when an individual participates in moderate exercise. For instance, an increase in cognitive performance due to prolonged periods of exercise has been attributed to increases in cerebral blood flow and/or increased neurotransmitter activity (Brisswalter et al., 2002). It is possible that these increases explain improvements in cognitive performance when prolonged exercise is performed. Different types of exercise can have different effects on cognitive performance emphasising the importance of defining the type and intensity of exercise performed in trials. Nonetheless, a wide range of types and intensities of exercise appear to affect cognitive performance.

Another theory that aims to explain an increase in cognitive performance that is often seen when it is tested after exercise is the cardiovascular fitness hypothesis. The cardiovascular hypothesis suggests that during exercise there is an increase in the ability of the heart to deliver oxygen to the muscles in the body (Etnier, Nowell, Landers, & Sibley, 2006). This increase can be attributed to changes in the pattern of brain activation (Colcombe & Kramer, 2003), including increased levels of cerebral blood flow (Endres et al., 2003; Swain et al., 2003) and brain-derived neurotrophic factor (Vaynman et al., 2004). These temporary changes in the body and the brain are deemed to be highly associated with cognitive performance.

Short bursts of acute strenuous exercise have been found to negatively affect cognitive processes. However, this decrease in cognitive performance improves after recovery from the exercise (Bue-Estes, Willer, Burton, Leddy, & Wilding, 2008). This was found earlier by Easterbrook (1959) who demonstrated that high exercise

intensity detrimentally influenced cognitive performance, whereas moderate exercise intensity improved cognitive functioning. In contrast, the results obtained by McMorris and Graydon (2000) showed that the accuracy of cognitive performance was not affected when participants exercised at high intensities.

The complexity of the relationship between exercise and cognition is demonstrated by the above contradictory findings. Consequently, research continues to be undertaken to understand the mechanisms of physical and cognitive fatigue and their interactions. Understanding the mechanisms and relationship between cognition, exercise, and fatigue will allow for research to investigate potential avenues to ameliorate fatigue within this relationship.

Establishing the link between exercise and cognition is integral to research that attempts to develop from this relationship. For example, there is an abundance of research investigating cognition and nutrition. Most of this research focuses on the healthy development of the brain or aiming to prevent cognitive decline, such as Parkinson's and Alzheimer's diseases, with the use of phytochemicals. There is little research that focuses specifically on fatigue and cognition and the possibility of mediating the detrimental effects of exercise on cognitive performance through the administration of nutritional supplements. In addition, one avenue that has not been widely researched is the potential of blackcurrant supplementation to ameliorate the effects of exercise fatigue on cognition.

Plant foods, such as berries, are thought to be beneficial for cognitive performance because they contain exogenous antioxidants and anti-inflammatory compounds

(Miller & Shukitt-Hale, 2012). Berries have also been found to benefit cognition though the ability of this so-called super food to mitigate oxidative stress (OS). The long-term effects of OS can be seen in cognitive disorders such as Parkinson's disease (Jenner, 1996) and Alzheimer's disease (Finch, & Cohen, 1997). Therefore, enhancing defences against oxidative stress with the use of antioxidants has been commonly reported as a way to prevent cognitive decline and aging. This is because plant foods contain secondary compounds that are not involved in primary metabolism. Among these secondary compounds are polyphenolics. Polyphenolics are believed to be the agents responsible for the beneficial effects of fruits and vegetables, such as protection against OS and solar irradiation (Schukitt-Hale et al., 2005).

Phytochemicals in plant foods include anthocyanins (Del Rio, Borges, & Crozier, 2010). Talavera et al. (2006) note that there is little research about the effects of anthocyanins on the brain. Currently, berries, such as blackcurrants, are recommended as an important component of an individual's diet due to the anthocyanins they contain. These anthocyanins are a subtype of flavonoid that are responsible for the colourful pigments of the fruit (Miller & Shukitt-Hale, 2012), also being found in a range of plants, fruits, vegetables, tea, wine, and fruit juices. They exert effects on the brain that have been proposed to enhance, stimulate, and protect neurons (Vauzour, Vafeiadou, Rodriguez-Mateos, Rendeiro, & Spencer, 2008) possibly resulting in improvements in memory, learning, and other aspects of cognitive performance (Vauzour et al., 2008).

Over time, berries have become one of the most frequently researched fruits. In the past 20 years of research they have been marketed as a super food and associated with improved cardiovascular outcomes, improved immune functioning, and decreased recurrence of urinary tract infections. They have also been recognised as beneficial for limiting oxidative damage, inflammation, vascular reactivity, platelet aggregation, and improving immune functioning (Pribis & Shukitt-Hale, 2014). However, the current research on berry supplementation focuses predominantly on older adults with cognitive decline, or research conducted with animals. There is little research that focuses on a healthy population or a younger sample in terms of cognition and nutrition. This is understandable as declines in cognitive functioning, increased oxidative stress, and inflammation are at the centre of age-related neurodegenerative diseases (Pribis & Shukitt-Hale, 2014). Nevertheless, it is foreseeable that nutritional research on berries might focus on healthy younger populations to determine if various aspects of cognition might be improved in people with normal levels of cognitive performance.

The current research project is part of a larger research project being conducted by the New Zealand Institute of Plant and Food Research. The overall project aims to explore the effect of phytochemical supplementation on physical performance and recovery. The purpose of the present research is to investigate whether blackcurrant supplementation (*Ribes nigrum*) given over a number of weeks can ameliorate the effects of strenuous physical exercise on cognitive performance. As part of this overall research, Harold (2016) has already shown that a single dose of blackcurrant extract has only a minimal impact on reducing the effects of extreme physical exercise on cognitive performance. Her study will be discussed in detail below.

This chapter has provided a brief background on the current questions and issues in the area of physical exercise, cognition, and fruit supplementation. Before further examining the effects of phytochemical supplementation on physical and cognitive performance, it is important to describe how these two types of performance relate. It is far from clear how exercise fatigue might detrimentally affect aspects of cognitive performance. Therefore, Chapter 2 examines some of the literature that focuses on both physical and cognitive performance and how physical fatigue affects cognition. Chapter 3 discusses the effect of fatigue on different modalities of cognition and examines how fatigue is detrimental to overall cognitive functioning. Chapter 4 then presents a review of phytochemicals and the research that demonstrates their effect on both physical and cognitive performance. It also describes acute and long-term studies of phytochemical supplementation, as well as describing the influence mood has on cognitive and physical performance. Lastly, Chapter 5 discusses the present study and its position in the current literature.

Chapter 2

The Relationship between Exercise and Cognitive Performance

In the current study the ability of exhausting physical exercise to induce cognitive fatigue was investigated. Results in this area have been mixed, largely due to lack of consistency in the methods chosen by different researchers (Brisswalter, Durand, Delingieres, & Legros, 1995). The present chapter describes some issues that need to be considered when conducting research with exercise and cognition. The relationship between physical fatigue and cognition is addressed by discussing the ability of exercise to negatively and positively affect cognition, the influence of the type of exercise used to alter cognition, the required intensity of the exercise, the fitness of the participants, and how different aspects of cognition are affected by exercise.

In order to compare the literature on exercise and cognition researchers have to break the studies down into different categories of exercise. For example, maximal intensity exercise, submaximal intensity exercise, and exercise in conjunction with hydration status (Chang, Labban, Gapin, & Etnier, 2012). The literature has previously been criticised as having a lack of consistency in methodology and failure to follow theory-based approaches to research. Reviews of the literature are therefore limited in their ability to compare studies because of multiple differences in protocols and the need to moderate different exercise factors. Moderating variables that are commonly required to be controlled for include, exercise intensity and duration, timing of the cognitive tests, exercise mode, type of cognitive task, and study design. Chang et al. (2012) states that due to all of these moderating variables, studies are often left out of meta-

analyses because they do not meet the specific focus. It is also apparent that the theories researchers have previously focused on have changed. For example, the U-hypothesis dominated the early literature on exercise and cognition and the cognitive tasks used primarily assessed reaction time and visual recognition. Recently, there has been a focus on cognitive tasks that test executive functioning and frontal lobe-dependent measures, a move from the U-hypothesis to theories such as the transient hypofrontality hypothesis (Change et al., 2012). Certain relationships cannot be explored within meta-analyses because they are not able to control for all of the moderators. This demonstrates the importance of testing specific moderators systematically and empirically, instead of meta-analytically (Chang et al., 2012). Furthermore, specific exercise tasks and intensities influence cognition in different ways, making it clear that the relationship between exercise and cognition is very complex.

The Relationship between Physical and Cognitive Performance: Does Exercise Enhance or Harm Cognition?

Examination of the literature on the relationship between exercise and cognition shows that it is complex and disorganised (Hotting & Roder, 2013; Pontiflex & Hillman, 2007). The majority of the research describes some kind of change in cognition following exercise (Brisswalter et al., 2002; Tomporowski, 2003), but it is the direction of cognitive change that is highly disputed. Is cognitive performance improved or hindered by exercise? Tomporowski and Ellis (1986) grouped the findings from exercise and cognition literature into four different categories: studies that found a beneficial relationship, studies that found a detrimental relationship, studies that found both a beneficial and a detrimental relationship, and studies that

found no relationship. Of particular interest to the current study is the detrimental effect physical fatigue can have on cognitive performance. During our daily lives, individuals carry out a number of difficult tasks even when they are fatigued, as demonstrated by shift workers and athletes. For instance, when athletes are engaging in decision making on the field they are likely to also be affected by fatigue. Despite the numerous scenarios that accompany our everyday lives and the extreme cases observed in shift workers and athletes, there is little research that examines the effects of strenuous exercise on cognitive functioning.

Numerous hypotheses have been developed in an attempt to explain the fluctuating relationship between cognition and exercise. The majority of the early research began with predictions drawn from arousal theories such as the U-hypothesis. The U-hypothesis was first discussed by Yerkes and Dodson in 1908. According to this hypothesis, cognitive performance increased as physiological arousal increased with exercise, and then began to decrease again as arousal reached maximal levels (Lambourne & Tomporowski, 2010). For instance, Brisswalter et al. (2002) note that, despite variations in the literature on effect of exercise on performance, exercise that lasts more than 20 mins consistently results in a positive effect on cognitive processing, explained by an increase of metabolic load that is associated with exercise over a long period of time. Increases in metabolic load are associated with increased sweating, enhanced heart rate, and a change in noradrenaline and adrenaline levels. This suggests that improvements in cognitive performance are associated with increased metabolic load. However, the duration of the exercise is only one factor of many that play a role in inducing physical fatigue.

Other research examining the link between exercise and cognitive performance has looked at the effects of mental fatigue on physical performance. For example, the perception of exercise plays an important role in cognitive performance. Marcora, Staniano, and Manning (2009) investigated the effect of mental fatigue on physical performance. The participants who were mentally fatigued rated their perceptions of effort during exercise to be significantly higher than participants who were not mentally fatigued (Marcora et al., 2009). The participants were required to watch either a 90-min emotionally neutral or cognitively demanding documentary before cycling to exhaustion at 80% of their peak power output. The mentally fatigued participants reached their maximum perceived level of exertion in the physical task faster than the participants in the control condition who were not mentally fatigued. Furthermore, participants who were mentally fatigued rated their perceptions of effort during exercise significantly higher than participants who were not mentally fatigued. These results demonstrate that mental fatigue influences physical performance whereby participants' higher perceptions of effort affected their ability to perform physically. It appears that while physical exercise can influence cognitive performance, cognitive fatigue can influence physical performance.

The Influence of Exercise Type

The type of exercise can greatly influence cognitive performance. For example, the transient hypofrontality hypothesis predicts how different sports influence cognitive functioning. This hypothesis states that changes in consciousness as a result of exercise are due to transient prefrontal cortex deregulation (Dietrich, 2003). It was initially proposed to enable predictions to be made about the benefits of exercise on mental health, and thereby explain improvements in cognition from exercise and

reported changes in emotion (Dietrich, 2003). Endurance athletes would commonly recount experiences such as endless peacefulness, elation, or pure happiness. The transient hypofrontality hypothesis explains this altered sense of consciousness by arguing for the prolonged disengagement of higher cognitive controls in the prefrontal cortex. It predicts that the type of sport or exercise may have unique effects on cognitive functioning. For example, strategic team sports require a variety of frontal-dependent cognitive processes, whereas running down a familiar street does not. The hypothesis allows for the prediction that a minimum level of exercise intensity is required to redistribute the resources in the brain and that skill level required in a sport is also an important factor. If a skill is practiced, then an individual can deregulate the prefrontal cortex. In other words, the individual has more capacity to process cognitive tasks because the exercise task does not require as much concentration.

Sabzi (2012) demonstrated how the type of exercise could influence cognitive performance. Sabzi conducted a study to examine the effect of different fatigue protocols on choice reaction time (CRT). Fifteen males participated in five different types of exercise including aerobic exercise, anaerobic exercise, mixed exercise, prolonged exercise, and supra maximal intermittent exercise. Sabzi found that CRT performance increased on all forms of exercise. However, CRT was most disrupted when assessed after anaerobic exercise, mixed exercise, and supra maximal intermittent exercise. Supra maximal exercise required the participants to run a 60 m sprint for 6 s and then have 40 s of slow walking recovery before continuing with the running. This sequence continued until they could not run at the predetermined speed. Anaerobic exercise was measured at 110% of heart rate at lactate threshold (lactate

threshold can be defined as the point in which lactate production exceeds the rate it can be broken down by the body and begins to accumulate in the muscles, causing fatigue). The participants then ran at an average speed until they could not match the pace. The mixed exercise group consisted of increasing bouts of exercise without rest until the participants could not continue. Overall, the results of the study showed that anaerobic exercise, mixed exercise, and supra maximal intermittent exercise, were most detrimental to CRT. However, regardless of the type of exercise when CRT was measured after exercise there was some decrease in performance.

The Influence of Fitness on Cognition

The individual's level of fitness can also mediate the effect of exercise on cognitive performance. Weingarten and Alexander (1970) conducted an experiment with highly fit men and men with average fitness. Tests of short-term memory and paired associate learning were administered. Each participant completed these tasks during the last 5 mins of a 12-min bicycle exercise. The results demonstrated that the participants with average fitness performed more poorly on the memory and paired associate tasks than the highly fit participants. This indicates that individuals who are highly fit are more resistant to the detrimental effects of physical exercise than individuals with lower levels of fitness. The level of fitness influences the effect exercise has on cognitive functioning demonstrating the importance of tailoring the exercise intensity to each participant so that they are sufficiently fatigued (Tomporowski, 2003).

Etnier et al. (2006) conducted a meta-analysis to investigate the relationship between aerobic exercise and cognitive performance. The authors found that, while regular

exercise is known to be beneficial for cognition, the underlying mediators of the relationship are yet to be completely understood. They discuss how the cardiovascular fitness hypothesis is often used to explain how fitness is a physiological mediator, which can account for the benefits of physical activity. When this hypothesis is applied to cognitive performance, the advances in cardiovascular fitness achieved through regular physical activity mediate cognitive processing resulting in improved performance.

On a similar vein, a longitudinal study across six years demonstrated that cardiorespiratory fitness is associated with the maintenance of cognitive functioning (Barnes, Yaffe, Santariano, & Tager, 2003). Barnes et al. (2003) found that baseline measures of cardiorespiratory fitness were associated with the preservation of cognitive functioning. Three measures of cardiorespiratory fitness and a broad range of cognitive tests were used. Global cognitive function and attention/executive function were found to have the strongest association with cardiorespiratory fitness and cognition. In summary, fitness has been shown to mediate the effects of physical fatigue on cognitive performance and should always be considered when conducting trials of this nature.

The Effect of Exercise Intensity

Exercise intensity also plays an important role in the relationship between exercise and cognitive performance. For example, Chang et al. (2012) found that the intensity of the exercise was a major factor influencing cognition. Very light exercise was found to have a negative effect on cognitive performance, whereas all other intensity levels resulted in a positive effect on cognition. However, a study conducted by

Brisswalter et al. (1995) found that intense exercise resulted in a decrease in cognitive performance. Brisswalter et al. found that cycling at 50 rpms/min resulted in increased cognitive performance, but when the participants increased to 70 rpms/min their cognitive performance decreased. This study supports the U-hypothesis as baseline reaction time assessed at 30 rpm decreased at 50 rpm, before increasing again at 80 rpm. Physiologically, the central nervous system is affected by the intensity of the exercise, which in turn, affects cognitive performance (Brisswalter et al., 2002).

Hogervorst, Riedel, Jeukendrup, and Jolles (1996) found that endurance exercise did not have a detrimental effect on complex cognitive tasks. The participants in their study were required to cycle at 75% of their maximal work capacity. Participants' workload increased by 25 watts every 2.5 mins until their heart rate reached 160 beats/min. The results showed an improvement on complex reaction time with no increase in the number of errors. Hogervorst et al. (1996) demonstrates how the relationship between exercise and cognition can be changed contingent upon the intensity of the exercise performed. In general, moderate exercise tends to improve cognitive performance on a number of different tasks, whereas strenuous exercise may result in decrements in cognitive performance.

How Different Aspects of Cognition are Affected by Exercise

Many studies have found beneficial effects of moderate exercise on cognition.

Davranche and McMorris (2009) note that past research has recognized exercise can enhance some cognitive functions. However, the effect of exercise on cognition has not been clearly established due to inconsistencies on the effect of exercise on higher

cognitive functions such as problem solving, decision making, and critical thinking (Zoller & Tsapralis, 1997). Continuing investigation testing the transient hypofrontality hypothesis has demonstrated that endurance exercise affects particular prefrontal-dependent executive functions. These include working memory, sustained attention, and the ability to inhibit habitual responses (Dietrich & Sparling, 2004). Dietrich (2003) concluded that cognitive processes that required little prefrontal-dependent cognition are unaffected by exercise.

Similar to the transient hypofrontality hypothesis is the dual-mode theory proposed by Ekkekakis (2009). This theory states that during exercise the brain is forced to choose where to focus its resources. Therefore, both theories suggest that because of the limited resources in the brain, during exercise, brain activity is redistributed, shifting activity away from areas that are not involved in the exercise task that is being completed. For instance, regions that are not involved with planning or executing motor commands. In addition, some types of exercise have been found to increase top-down cognitive control. For example, Pontiflex and Hillman (2007) found that response accuracy on the Stroop test was decreased on the incongruent subtest of attention, whereas response accuracy on the congruent subtest was not affected by exercise. Therefore, the brain delegates attentional resources to most efficiently solve the task at hand. Studies such as the one conducted by Pontiflex and Hillman are in line with hypotheses that propose that the delegation of resources can also result in changes in cognitive performance from focused attention.

Cognitive performance can also vary contingent on the time after exercise that it is assessed. Some studies that administered cognitive tests within 0-10 mins of exercise

completion found that exercise negatively affected cognition (Chang et al., 2012). However, studies that administered cognitive tests within 11-20 mins of exercise completion generally found that exercise positively influenced cognitive performance (Chang et al., 2012). The duration of the exercise was also found to have an influence. Chang et al.'s (2012) meta-analysis found that short exercise sessions resulted in negligible effects on cognitive performance, whereas exercise sessions that lasted longer than 11 mins resulted in positive effects on cognition.

Physical Fatigue and Performance

Fatigue is a complex and multidimensional concept (Schwid et al., 2003). It results in an inability to sustain or complete a previously easy task (Nozaki et al., 2009) and therefore, the individual's physical and mental abilities are negatively affected. It is well known that fatigue is associated with mistakes, errors (Moore, Romine, O'connor, & Tomporowski, 2012) and suboptimal functioning (Lorist, Boksem, & Ridderinkhof, 2005). For this reason, it is important to define the concept of fatigue and the process that an individual follows to reach the point of physical and cognitive exhaustion.

There are two types of fatigue, peripheral fatigue, and fatigue that is generated in the central nervous system (CNS). CNS fatigue can be defined as fatigue that is produced by changes at the neuromuscular junctions (Ament & Verkerke, 2009). Prolonged and intense exercise may cause qualitative changes in the ability of the CNS to control movement. Using EMG, researchers have found changes in the neuronal activity of the motor cortex associated with CNS fatigue. This shows that individuals are experiencing fatigue of the muscles when they are engaging in exercise that is

directed by the motor cortex which is responsible for signalling to the muscles. On the other hand, peripheral fatigue is described as an alteration in the mechanical response of the muscles due to electrical stimulation of the nerves to the muscles. This develops progressively, contingent upon the duration and intensity of the exercise (Froyd, Millet, & Noakes, 2013). In basic terms Gandevia (2001) described physical fatigue as any exercise-induced reduction in the ability of the individual to generate force. Although peripheral and CNS fatigue can be clearly defined, the underlying mechanisms and relationship between the two currently precludes a clear explanation (Kent-Braun, 1999). This may be due to the fact that only a few studies exist that have investigated CNS and peripheral fatigue simultaneously (Schillings, Hoefsloot, Stegeman, & Zwarts, 2003).

Muscular exercise is contingent upon the limit and the contour of the oxygen uptake response profile. The intensity of the exercise can be measured using the lactate threshold. This is the point at which lactate production exceeds the rate it can be broken down by the body and begins to accumulate in the muscles. Moderate intensity workouts that are below the lactate threshold are highly sustainable and the individual will not experience fatigue (Noakes, 2000). Cardiovascular measures and measures of maximum oxygen consumption (VO₂ max) and anaerobic threshold have been found to be incomplete measures and can vary between individuals with the same abilities who have completed similar exercise (Noakes, 2000). Therefore, it is important to consider more than one measure when attempting to ascertain the level of fatigue an individual is experiencing. A number of variables can influence the body at any one time, and thus affect subsequent performance (Noakes, 2000). Close monitoring to assess heart rate, lactate threshold, and VO₂max are important.

However, it is also important to include subjective scales that aim to ascertain the perceived level of exertion of the individual.

Physical exercise consumes energy and if an individual does not reduce or stop the physical exercise they are participating in they will experience physical exhaustion (Ament & Verkerke, 2009). Physical and biochemical changes are described as physiological changes as a result of fatigue and exhaustion. Discontinuation or reduction in exercise before fatigue is often the result of psychological processes. For example, the sensations of fatigue and exhaustion are important because they prompt changes in behaviour when exercising. It is important to recognise both the physiological and psychological changes experienced because both play a role in shaping how fatigue and exhaustion are experienced, (Paul, Beatty, Schneider, Blanco, & Hames, 1998), and subsequently how it is understood scientifically.

A number of measures can be used to monitor the exertion and fatigue of an individual. Firstly, maximal oxygen uptake (VO_2 max) is used to establish the workload of the exercise (Kashihara, Maruyama, Murota, & Nakahara, 2009). VO_2 max can also be used as an indicator of functional capacity of respiratory, circulatory, and metabolic systems, such as lactate threshold. Heart rate is often monitored as well and can be used as an alternative to VO_2 max. Heart rate and VO_2 max should both increase with increased exercise and exertion (Kashihara et al., 2009). Moderate intensity exercise has been characterised as 64-76% of maximal heart rate, compared to high intensity exercise characterised by 77-95% of maximal heart rate (Gillen & Gibala, 2014). High intensity interval training (HIIT) is known for its short and repeated bursts of intense exercise that are separated by short periods of rest. If HIIT

consists of less than 10 mins of exercise it is considered a low volume training session (Gillen & Gibala, 2014). Athletes frequently use HIIT types of exercise for short training sessions that will have maximum beneficial effects. Past research shows that studies use different definitions of fatigue and protocols to induce fatigue making it difficult to compare results. HIIT has a clear definition relating to VO₂ max, intensity, duration, and protocols. Using HIIT as a benchmark could alleviate problems comparing studies that used different methods to induce fatigue.

Hancock and McNaughton (1986) previously defined fatigue as a state in which their participants were exercising above anabolic threshold. In their study they found that participants' lower level cognitive skills, such as short-term memory and estimation, improved when they were fatigued, while higher-level cognitive skills, such as interpretation, deteriorated. Teichner (1954) found that the cognitive performance of their participants decreased at only 20% of maximal power output (P_{max}). This decrease was seen in both fit and unfit groups. However, when the participants were exercising at 40% P_{max} their cognitive performance levelled off for fit participants but decreased again in unfit participants. This demonstrates the importance of taking into account individuals' current level of fitness when participating in studies measuring exercise-induced fatigue. It also demonstrates the possibility of a participant not experiencing fatigue due to incorrect exercise measures. For example, if one participant has a higher level of fitness than the other participants, they may not experience the same level of fatigue as the others.

Other research has shown that exercising at a high intensity will negatively affect cognitive performance. Moore et al. (2012) examined the influence of exercise-induced fatigue on cognitive function. They found significant differences in cognitive

performance when comparing exercise and rest groups. The participants in the exercise group cycled for 60 mins on a stationary bicycle at 90% ventilatory threshold. They showed declines in performance on perceptual-discrimination tasks compared to the control group, demonstrating a negative effect on cognition via exercise.

Therefore, physical exercise will not induce cognitive fatigue if the intensity of exercise is under the fatiguing threshold of the participants. The intensity of exercise needs to be between 77-95% of maximal heart rate to affect cognitive performance. High intensity exercise training has become a popular method of inducing fatigue in restricted time settings such as lab-based scientific studies.

High Intensity Exercise and Cycle Tests

Contradictory findings in past research on exercise and fatigue have led to the importance of controlling the intensity and duration of the exercise for the study. High intensity exercise is normally achieved through the use of interval exercise. Repeated bouts of exercise in short or moderate durations are completed at a specified intensity that is greater than the anaerobic threshold. The short rest breaks between bouts of exercise allow for only partial recovery. This leads to repeated stress on the physiological systems similar to endurance-type exercise (Laursen & Jenkin, 2002).

Moderate intensity represents an exercise intensity that is below the lactate threshold, whereas intense exercise represents exercise intensity that is above the lactate threshold (Brisswalter et al., 2002). The level of intensity of the exercise in turn affects the CNS. Arousal level corresponds to subsequent cognitive performance thus suggesting that optimal cognitive performance is associated with a moderate level of

arousal. When arousal is increased due to increased exercise intensity, the individual's attention becomes narrowed and an optimal level of cognitive functioning cannot be reached due to the inability to adequately process relevant cues.

The ability of low volume, high intensity interval training can produce similar results to endurance and high intensity exercise. Low volume, high intensity interval training is appealing due to the short amount of time required to achieve fatigue, as well as the unsuitability of endurance exercise for some individuals (Gibala, Little, MacDonald, & Hawley, 2012). Gibala et al. (2012) designed a practical low volume, high intensity regime consisting of 10 x 60 s work bouts and constant load intensity, giving the participants a 60 s rest in between bouts. This work rate elicits around 90% of maximal heart rate and is considered low volume, high intensity due to its higher intensity of work bouts but shorter duration of exercise and rest. Usually the participants will exercise for less than 10 mins when using this programme.

With increasing evidence, HIIT is being used more frequently than endurance training to alter cardiorespiratory fitness (Astorino, Allen, Roberson, & Juranchich, 2012).

HIT is being used frequently due to its high intensity that leads to the individual working at maximal or near supramaximal work rates. HIIT stresses many physiological and biochemical systems that are used in aerobic exercise. It also affects skeletal muscle due to reductions in muscle glycogen and pH, and increases in blood lactate, body carbohydrate, and fat oxidation (Bayati, Farzad, Gharakhanlou, & Agha-Alinejad, 2011).

A meta-analysis conducted by Gist, Fedewa, Dishman, and Cureton (2014) concluded that sprint interval training was beneficial to participants and improved VO₂ max in healthy young people. This type of short, high intensity interval exercise is just as beneficial as endurance training but with reduced time commitment. Saanijoki et al. (2015) note that with the effectiveness of HIIT, Wingate cycle tests have emerged as one of the main forms of exercise to improve and test aerobic fitness. Although high intensity exercise can induce feelings of fatigue, dizziness, and nausea, Wingate cycle HIIT is tolerated well by active men and women. Studies using cycle HIIT typically consist of four to six, 30 s “all out” maximal sprints performed on a cycle ergometer (Saanijoki et al., 2015). When using this type of exercise and equipment it is beneficial to familiarise the participants with the exercise, as well as to determine their fatigue threshold before the main study begins. Saanijoki et al. (2015) found that participants were considerably more stressed when participating in HIIT exercise than endurance exercise resulting in high rates of fatigue. This can be explained by the high intensity and short rest periods in HIIT.

HIIT is now recognised as one of the most efficient and effective forms of training. In sedentary and recreationally active individuals it is well known that to achieve the VO₂max of a trained athlete it will take several years (Laursen & Jenkins, 2002). However, when using a HIIT program VO₂max can be noticeably increased in less than 10 weeks (Hickson, Bomze, & Holloszy, 1977). The effects of exercise-induced muscle fatigue from HIIT on maximal performance have been studied over the past 30 years. The overall research suggests that HIIT is an effective form of exercise to induce fatigue in a time-restricted setting. HIIT using a Wingate cycle was the exercise programme used in the present investigation.

Summary

In summary, Chapter 2 has described a wide range of different exercise regimes, exercise durations, and intensities. Many studies, including meta-analytic studies, are sometimes comparing apples with pears because these variables are not being held constant. This is also contributing to the complex nature of fatigue and the ambiguous relationship between exercise and cognition. However, recent research has demonstrated that HIIT is an effective and efficient way to induce physical fatigue in healthy participants.

Chapter 3

Cognition and Fatigue

Four models have been proposed to explain cognitive fatigue (Leavitt & DeLuca, 2010). Firstly, cognitive fatigue can be seen after a long day of prolonged cognitive activity. Secondly, cognitive fatigue may be experienced after sustained cognitive effort, even after a short period of time. Thirdly, the experience of effortful cognitive exertion can result in cognitive fatigue. Lastly, cognitive fatigue can be experienced after effortful physical activity (Leavitt & DeLuca, 2010). Despite this knowledge, there is no consensus regarding the mechanisms that underlie cognitive fatigue, or research that confirms the type of exercise intensity that affects specific regions of the brain (Brummer, Schneider, Abel, Vogt, & Struder, 2011). The current chapter examines the effects of exercise on brain activity and cognitive fatigue by describing the neurocognitive mechanisms and neurotransmitters thought to be involved with cognition and fatigue, as well as describing two of the most common aspects of cognition affected by fatigue.

Neurocognitive Mechanisms of Cognitive Fatigue

Basal Ganglia

The basal ganglia has been identified as one of the primary neural structures associated with central fatigue (Leavitt & DeLuca, 2010), and is known to be involved in higher cognitive functioning and motor control (Chaudhuri & Behan, 2000). It consists of six interconnected nuclei: the caudate nucleus, putamen, globus

pallidus, substantia nigra, subthalamic nucleus, and amygdala (Chaudhuri & Behan, 2000). The amygdala links the basal ganglia to the limbic system and hypothalamus, which controls mood and hormone manufacture and release. It is also cortically associated with the prefrontal cortex and linked to striatothalamic pathways that project to the primary motor cortex and the entire frontal lobe (Chaudhuri & Behan, 2000). An interruption to this loop of striatocortical fibres is one of the most recently proposed explanations for the symptoms of central fatigue (Chaudhuri & Behan, 2000). For example, a number of disorders of central fatigue have a strong association with basal ganglia disease, such as Parkinson's disease, multiple sclerosis, chronic fatigue syndrome, and depression.

Anterior Cingulate Cortex

Research utilising neuroimaging and event-related potentials has found that the anterior cingulate cortex (ACC) is crucial for performance monitoring. It is also important for detecting the activation of conflicting responses and making adjustments to minimise errors. It has been found that neural activity in the ACC changes with time-on-task. This suggests that alterations in ACC functioning are related to fatigue. Lorist et al. (2005) found that 2 hours on a prolonged cognitive task negatively affected error monitoring controlled by the ACC. This was demonstrated by increased reaction time as participants attempted to compensate for the increasing occurrence of errors, presumably as greater demands were placed on the ACC. Brain imaging studies have also found the ACC to be involved with sustained attention (Helton & Russell, 2011).

Another way to examine the effects of cognitive fatigue is with error-related negativity (ERN or NE). The ERN is an event-related potential and is seen when an individual makes an error on a task or there are high levels of response conflict. This is called performance monitoring. The ACC detects conflicting responses when performing cognitive tasks and adjusts behaviour to mediate these problems. For example, when participants made an error on a cognitive task they slowed down. The association between ERN and the ACC in role monitoring means that ERN can be used to observe state variables, such as fatigue (Lorist et al., 2005). It has been shown that the ACC is activated on cognitive tests such as the Stroop. The ACC can also activate others areas, such as the dorsolateral prefrontal cortex, when increasing attention is required (Carter & Van Veen, 2007). Therefore, if the ACC is fatigued, it cannot delegate resources in the brain or control for conflicting responses. Thus, adaptive behaviour to maintain cognitive performance is not possible.

Cerebral Blood Flow

Most of the research pertaining to the mechanisms of cognitive fatigue (and cognition and exercise) refers to cerebral blood flow (CBF). Ogoh et al. (2014) point out that, although CBF does not have a prominent role in changing cognitive performance, it may play a part in increasing cerebral neural activity, which could indicate an increasing demand for oxygen in the brain. In addition, the resource depletion theory describes how CBF is implicated in cognitive fatigue. For example, declines in cognitive performance can be matched to declines in CBF on the right side of the brain with time-on-task activities. Blood flow to regions such as the ACC, thalamus, right prefrontal cortex, and parietal regions has been found to be indicative of cognitive resource supply (Helton & Russell, 2011). The resource depletion theory

describes the difficulty in maintaining a high level of attention resulting from a depletion of attentional resources. For example, the individual is only capable of focusing on one task at a time due to the difficulty and amount of attention required. Therefore, as time-on-task increases, the resource pool decreases, and a decline in performance is seen (Helton & Russell, 2011). CBF has been found to be fastest at 60% VO₂ max. This suggests that exercising at higher intensities detrimentally affects cognition due to changes in CBF.

Neurotransmitters

Serotonin

Newsholme and Blomstrand (1995) were some of the first researchers to propose that serotonin plays a role in the development of exercise-induced fatigue. They hypothesised that fatigue could be explained by disturbances in brain serotonin concentrations. The neurotransmitter, serotonin, is involved in changes in sleep–wakefulness, emotion, appetite, the hypothalamic–pituitary axis, and numerous physiological functions. Serotonin in the brain is suspected to play a major role in exercise-induced alterations in neurotransmitter function (Davis & Bailey, 1997), and has been found to affect cognitive performance (Kashihara et al., 2009). Serotonin was also the first neurotransmitter proposed to be a mediator of CNS fatigue. Increases in serotonin have been found to affect arousal, sleepiness, mood, and the perception of muscular fatigue. The central fatigue hypothesis posits that prolonged exercise produces an elevation in serotonin in some regions in the brain (Watson, 2008). Due to the role that serotonin plays in regulating sleepiness and lethargy, it has been suggested that this elevated production could produce a different sense of effort

and a loss of motivation to continue exercising (Watson, 2008). This explanation was suggested to provide a link between changes in physical exertion and the CNS. The shift in mobilisation occurs during exercise which in turn affects the synthesis of brain neurotransmitters (Watson, 2008). Thus, changes in the level of serotonin in the brain are affected by exercise resulting in feelings of fatigue and leading to changes in cognitive performance.

Dopamine

Dopamine (DA) has also been investigated as a neurotransmitter likely involved with CNS fatigue (Davis & Bailey, 1997). DA is involved in a number of neuro-behavioural disorders such as Parkinson's disease, attention deficit hyperactivity disorder, schizophrenia, and drug addiction, all of which demonstrate deficits in cognitive control. Its contribution to fatigue has yet to be confirmed, but it is thought to be at low concentrations when the individual is at, or near, the point of fatigue. The prefrontal cortex, involved with cognitive control, contains a large number of DA receptors, suggesting that this transmitter plays a major role in cognitive functioning (Cools & D'Esposito, 2011). DA has also been linked to working memory, planning, and cognitive flexibility in both human and animal studies (Cools & D'Esposito, 2011). However, recent research has revealed that the relationship between DA and cognition is highly complex due to the often contradictory results of improvements and impairments involving DA (Cools & D'Esposito, 2011). Nevertheless, there is mounting evidence that this neurotransmitter plays an important part in cognitive performance and fatigue.

Cognitive Fatigue and Cognition

Cognitive fatigue can be measured using cognitive tasks designed to assess specific aspects of cognition. It is important to understand how cognition can be affected and the underlying mechanisms responsible. Two key underlying factors are attention and reaction time.

Attention

One of the most commonly studied types of cognitive fatigue is attentional fatigue (Lim et al., 2010). Voluntary control of attention is particularly influenced by fatigue (Langner, Steinborn, Chatterjee, Sturm, & Willmes, 2009). Attention is studied frequently due to its importance in daily functioning. It allows individuals to process incoming information and actively ignore irrelevant stimuli, both of which are important for tasks, such as driving (Boksem, Meikman, & Lorist, 2005). A study conducted by Boksem et al. (2005) investigated the effects of cognitive fatigue on attention. The participants performed a visual attention task for 3 hours without rest. They used event-related potentials (ERP) and electroencephalographic (EEG) data to measure the physiological changes related to fatigue and attention. Increases in alpha, theta, and beta power across the 3-hour task were observed, indicating that a decrease in arousal and increase in effort occurred (Boksem et al., 2005). Performance on the task also deteriorated over time. Reaction time and the number of false alarms and missed targets also increased steadily across 3 hours. The ERP measures for selective attention found effects on early visual P1 and N1 components. The P1 amplitude was larger for stimuli presented at relevant locations, as opposed to irrelevant locations, and increased in amplitude over time. In contrast, the N1 amplitude decreased over

time and was larger for stimuli at irrelevant locations. Their study also demonstrated that participants showed increasing difficulty staying on task, staying alert, and performing at the required level.

Past studies investigating cognition and exercise have focused on the assumption that physical arousal associated with exercise will lead to a narrowing of attention (Brisswalter, Arcelin, Audiffren, & Delignieres, 1997). This assumption is in line with the U-hypothesis that moderate exercise would improve cognition and heavy exercise would lead to a decline. The U-hypothesis highlights how attention becomes narrowed as the intensity of the exercise increases. However, cognition affected by fatigue may also be a result of divided attention (McMorris & Keen, 1994). Perhaps participants may be focusing more on their physical discomfort from exercise, or attention may simply be split between exercise and cognitive performance. Divided attention should be considered when testing participants on cognitive tasks whilst they are exercising. Therefore, narrowing of attention is not the only possibility with exercise arousal. The changes observed may also be due to the allocation of attention, divided between exercise and cognition.

Fatigue and cognitive strain can be seen when performing a monotonous or simple task, leading to the idea that the ability to maintain a high level of attention decreases as the task goes on (Helton & Russell, 2011). The proposed resource depletion theory describes the difficulty in maintaining high levels of attention resulting from a depletion of attentional resources. As time-on-task increases the resource pool decreases and a decline in performance is seen (Helton & Russell, 2011). This effect is revealed as attentional lapses, increasing reaction times, and increasing subjective

fatigue. Brain imaging research has shown that there are several areas implicated in sustained attention. The ACC, right prefrontal cortex, right inferior and parietal regions, and the thalamus, are known to be associated with the maintenance of attention (Helton & Russell, 2011).

Reaction Time

Reaction time (RT) has been measured since the first half of the 19th century (Niemi & Naatanen, 1981). It continues to be one of the most important measures of many types of human performance, both in laboratory studies and in many aspects of everyday life, such as athletics. As previously described, cognition is a complex topic and the relationship between cognition and exercise highlights that clearly. However, a wide range of factors can affect RT but the results are rather confusing.

Investigations find that exercise can increase, decrease, or even have no effects on it (Tomprowski & Ganio, 2006).

It is important to note that the use of physical exercise to incur cognitive fatigue will result in physiological changes as well as cognitive changes. For example, exercise of a moderate nature will result in a rise of core body temperature, which can increase the speed of nerve transmission as well as producing a rise in arousal level.

Physiological changes such as these have the ability to influence RT. The varying nature of RT and the multitude of RT tasks available to measure cognitive performance are responsible for some discrepancies in the literature on the effects of physical fatigue on cognitive performance. For example, early research showed that RT was always negatively affected (increased) by exercise. However, later studies have found that during exercise RT may decrease (Brisswalter et al., 1997).

Complex RT is particularly affected by fatigue (Langner et al., 2010). Complex RT can be defined as any task that requires the participant to discriminate between multiple stimuli before reacting. Langner et al. (2010) theorise that when participants are fatigued they will often continue to function at high levels on simple, automated tasks. However, when the task is more complex, more effort is required to process the incoming information and the participant will now be more vulnerable to cognitive fatigue.

Although much of the literature on exercise and fatigue describes how complex RT changes with exercise, simple RT can also be affected by cognitive fatigue. McMorris and Keen (1994) conducted a study with recreational athletes to investigate the effect of incremental exercise to exhaustion on simple RT, establishing that exercise increased RT. They also found that when workload increased from 70% to 100%, simple RT further increased, demonstrating that it is detrimentally affected by high intensity exercise. A similar finding was obtained by Kashihara and Nakahara (2005) who investigated the effects of moderate exercise on choice RT (a type of complex reaction time). Six healthy participants were assigned to a cycle task for 10 mins. Exercise improved participants' performance on the choice RT task but only for 8 mins after they completed the exercise. They attributed this positive effect to the lactate threshold experienced while exercising, which begins to wear off over post-exercise time.

Likewise, Audiffren, Tomporowski, and Zagrodnik (2008) investigated the immediate and short term after effects of exercise on information processing. A choice RT task

was performed by 17 participants before, during, and after 40 mins of cycle exercise. The same tests were also conducted without cycling. They found that exercise improved RT. However, the facilitating effect of exercise was not seen until 19 mins into the exercise but remained until 39 mins of exercise had been completed. The authors theorise that mechanisms that drive the beneficial effects of exercise on RT are linked closely to motoric activity. In other words, the muscle fibres in the body are linked to arousal and activation in the brain due to exercise which influences RT. Studies such as this demonstrate that RT is affected negatively by exercise, but only immediately after exercise as the beneficial effects start to exert themselves 19 mins after the completion of exercise.

Summary

The mechanisms of cognitive fatigue are not well understood. The basal ganglia, ACC, and cerebral blood flow have all been implicated in in the relationship between exercise and fatigue. Similarly, the levels of neurotransmitters, serotonin and dopamine, are affected by exercise-induced fatigue. While cognition can be affected in numerous ways, RT and attention have been two of the variables most extensively studied. However, it appears that the assessment of cognitive performance after exercise is a critical issue; cognition may be enhanced straight after exercise, and then deteriorate a short while later.

Chapter 4

The Effect of Phytochemicals on Cognition

What are Phytochemicals?

Research began in the 1950's to examine the influence of phytochemicals on the human body (Casini et al., 2006). However, there is little research on the effects of phytochemicals on the brain and most of this research has been generated from animal trials (Talavera et al., 2006). Plant foods are thought to be beneficial for cognitive performance because they contain exogenous antioxidants and anti-inflammatory compounds (Miller & Shukitt-Hale, 2012). Berries contain phytochemicals, and are classed as a subtype of anthocyanins (Del Rio et al., 2010). Berries, such as blackcurrants, are an important component of an individual's diet specifically due to the anthocyanins they contain. Anthocyanins are a category of flavonoid that is responsible for the colourful pigments of the fruit (Miller & Shukitt-Hale, 2012). Flavonoids are a group of polyphenolic compounds that are found in plants, fruits, vegetables, tea, wine, and fruit juices. They exert effects on the brain that have been proposed to enhance, stimulate, and protect neurons (Vauzour et al., 2008). Vauzour et al. (2008) state that it is also possible that flavonoids promote memory, learning, and cognitive performance. Blackcurrants in particular have been found to contain the highest levels of anthocyanins, followed by blueberries, and then cranberries (Borges, Degeneve, Mullen, & Crozier, 2009).

Phytochemical Supplementation and Cognition

It is well known that a healthy lifestyle and good nutrition are important components for physical wellbeing and a long healthy life. Good diet and nutrition has been found to affect the brain in a multitude of ways, for example, by regulating neurotransmitter pathways, and by modulating synaptic transmission, membrane fluidity, and signal transduction pathways. The importance of diet is especially emphasized for growing children who need good nutrition for brain development and functioning (Meeusen, 2014). Aside from this, research on nutrition and cognition has for some time been dominated by age-related health disorders and how to delay their onset (Meeusen, 2014). Research with phytochemicals has previously focused on their ability to reverse age-related cognitive deficits (Andres-Lacueva et al., 2005). The neuroprotective effects of flavonoids have been given importance due to the growing cost of age-related diseases such as Alzheimer's, vascular dementia, and Parkinson's disease (Macready et al., 2009). This is most likely because anthocyanins are able to cross the blood-brain barrier, which may explain why they are able to directly influence areas of the brain responsible for memory and cognition. In particular, phytochemicals have consistently been reported to have beneficial effects on neurocognitive functioning (Casini et al., 2006; File, Hartley, Elsabagh, Duffy, & Wiseman, 2005). Their neuroprotective actions include defending neurons against neurotoxins, suppressing neuro-inflammation and promoting memory, learning, and overall good cognitive functioning (Meeusen, 2014).

The beneficial effects of phytochemical supplementation on cognition have been previously demonstrated with studies on animals; flavonoids can benefit the animal brain. For example, studies with rodents have found that berry anthocyanins have the

ability to cross the blood-brain barrier (El Mohsen et al., 2006; Talavera et al., 2005), and similar studies with pigs have found anthocyanins in the brain and ocular tissue after the animals had been fed blueberries (Kalt et al., 2008). Talavera et al. (2005) investigated the distribution and metabolic fate of anthocyanins in rats, and demonstrated how quickly anthocyanins were metabolised. For 15 days the rats were fed a diet rich in anthocyanins. However, after only one dose, anthocyanins were present in the brain 30 mins after ingestion.

One of the first studies published on the beneficial effects of berries on cognition was conducted by Joseph et al. (1999). In this study aged 19-month old rats were fed blueberries or strawberries for 8 weeks. This study found that age-related declines in neuronal and cognitive function could be halted by high antioxidant feeding of fruit and vegetables. Both of these groups of rats showed improved performance on working memory tasks, as well as enhanced balance on a rod-walking task compared to a control group. A more recent study conducted by Andres-Lacueva et al. (2005) on aged rats found flavonoids present in the brain 10 mins after ingestion. They also found that the presence of flavonoids was associated with improved performance on memory and learning tasks. Similarly, in a study conducted by Milbury and Kalt (2010) pigs were fed blueberry anthocyanins to determine if they reached the brain and the quantities detectable. They found that anthocyanins were able to cross the blood-brain barrier being detectable in the brain tissue 18 hours after ingestion. The study could not determine whether the anthocyanins were synthesised in the brain or if they were synthesised in the kidneys and then transported to the brain after this process.

Studies with humans have found similar results. Human consumption of berry fruit juice has demonstrated some beneficial properties. For instance, anthocyanins can be found in numerous areas in the human brain after consumption of wild berry juice, such as in the hippocampus, neocortex, and other regions essential for cognitive functioning. Furthermore, the presence of anthocyanins has been detected in the human brain after only one dose and just 30 mins after ingestion (Krikorian et al., 2010).

However, how phytochemicals benefit cognition is not well understood, and investigations continue. It is believed that flavonoids exert their effects by modulating cellular signalling pathways, for example, the mitogen-activated protein kinase pathway (MAPK) and the phosphoinositide 3-kinase (PI3 kinase/Akt) signalling cascade (Macready et al., 2009). They have also been found to control the transcription of factors such as nuclear factor-kappa B (NF-kB). This helps to promote the expression of brain-derived neurotrophic factor, which is integral to neuronal and synaptic growth (Macready et al., 2009). Flavonoids have also been found to improve endothelial functioning by increasing the production of the signalling molecule, nitric oxide (NO). The latter leads to the relaxation of endothelial smooth muscles, which can help control blood pressure (Macready et al., 2009).

Studies on animals and humans have begun to establish the benefits of phytochemicals on cognition. The most recent research on phytochemicals has focused on their ability to decrease oxidative stress, and increase protective signalling as well as protecting neurons using hormetic effects (Meeusen, 2014).

In 2009, Spencer, Vazour, and Rendeiro suggested that the bioactivity of flavonoids seen in the brain after ingestion is debatable. The effect of flavonoids on the brain was initially attributed to their ability to help control antioxidant actions by scavenging reactive species. However, it has since been proposed that flavonoids can exert neuroprotective actions on the brain. This means that flavonoids have the ability to protect vulnerable neurons, enhance existing neuronal function, excite neuronal regeneration, as well as inducing neurogenesis (Spencer et al., 2009).

Macready et al. (2009) reviewed the neuroprotective effects of flavonoids from 15 human studies. They concluded that there is a positive association between the consumption of flavonoids and cognitive functioning. They note that only two of the 15 studies reported null findings, and just one study reported a decline in cognition. The rest of the studies found significant improvements in cognitive functioning, such as executive functioning, memory, and processing speed.

Recent research is beginning to demonstrate that particular cognitive functions benefit from phytochemicals. For example, Krishnaveni (2012) points out how blackcurrant has been found to improve short-term memory, which has been attributed to the activity of anthocyanins in the hippocampus. One of the major mechanisms thought to contribute to memory acquisition, consolidation, and storage in the brain is long-term potentiation (LTP). LTP is known to be regulated at the molecular level by the activation of neuronal signalling pathways. These pathways include the phosphatidylinositol-3 kinase/protein kinase B/Akt, protein kinase C, protein kinase A, calcium-calmodulin kinase, and mitogen-activated protein kinase, which are enzymes that initiate the transfer of phosphate groups to specific substances

(Meeusen, 2014). Research suggests that flavonoids, such as those contained in blackcurrant, exert effects on LTP by directly modulating the activity of kinases thereby improving memory.

Flavonoids have also been studied in long-term trials with good results. Letenneur, Proust-Lima, Le Gouge, Dartigues, and Barberger-Gateau (2007) examined flavonoid intake in relation to cognitive functioning. A total of 1,640 participants 65 years and over were examined four times, using three psychometric tests across a 10-year period. The results showed that flavonoid intake was associated with better cognitive functioning than baseline measures and also better evolution of cognitive performance over time. The participants who received the highest dose of flavonoids across this timeframe showed the greatest cognitive improvement.

Acute Versus Long-Term Phytochemical Intervention

Despite the evidence that suggests a positive association between phytochemicals and cognitive performance, the time needed before the benefits of phytochemicals are observed is largely unknown. It is important to understand the timeline of effects they can exert on the human body. For example, they may be able to offer immediate enhancement to cognitive performance similar to stimulants, such as coffee. A comparison of acute versus long-term studies is required to understand the possible range of effects that phytochemicals can elicit, and the cognitive mechanisms they affect.

Studies of acute supplementation are usually conducted across 1-6 hours. Watson et al. (2015) conducted a randomised, double blind, placebo controlled study with 36

young healthy participants to assess the effect of blackcurrant extract on cognition. The participants in the blackcurrant group were fed 525 mg of polyphenols per 60 kg of body weight, or 142 mL of cold-pressed blackcurrant fruit juice. Baseline cognitive measures included the Digit Vigilance task, the Stroop task, the Rapid Visual Information Processing task (RVIP), mood scales, and the Logical Reasoning task. The participants were then given their supplement and tested 60 mins after to allow for absorption. Participants then completed the cognitive battery again. The results showed that RVIP accuracy improved after supplementation from the blackcurrant extract and the blackcurrant fruit juice also lead to improvements in the digit vigilance task compared to placebo. However, the study did not account for practice effects. The cognitive tasks were used to cognitively fatigue the participants as well as assess for cognitive changes, leading to 14 repetitions of the three tasks.

Whyte and Williams (2015) also conducted a study using an acute dose of flavonoids to assess memory. A flavonoid-rich blueberry drink or placebo was administered to 14 children aged 8 to 10 years old. The intervention drink consisted of 200 g of blueberries, 100 mL of milk, and 8 g of sucrose, which is equivalent to 143 mg of anthocyanins. Two hours after consumption the children completed a cognitive test battery including the Go-NoGo, Stroop, Rey's Auditory Verbal Learning Task (RAVLT), Object Location Task, and a Visual N-Back task. The results showed that the RAVLT was the only task sensitive to the intervention. The RAVLT showed significantly improved scores in delayed auditory recall performance.

Longer interventions of phytochemical supplementation tend to find slightly different results compared to acute dose studies. For example, a 12-week study conducted by Krikorian et al. (2010) examined the ability of blueberry juice to improve memory in

older adults. Nine men and women with mild cognitive decline participated in the study. The participants were administered wild blueberry juice according to their body weight. Individuals weighing 54-64 kg were prescribed 444 mL/day, those weighing 65-76 kg consumed 532 mL/day, and those weighing 77-91 kg consumed 621 mL/day. The participants consumed their prescribed dose each day for 12 weeks. Cognitive tests were conducted before and after the intervention and included the Verbal Paired Associate Learning Test and the California Verbal Learning Test. Both cognitive tests showed significantly improved performance post-testing. Importantly, Kirkorian et al. accounted for practice effects by using analyses that controlled for the repeated cognitive testing.

Similar short term trials using rats have also found improvements in cognitive functioning and performance. Shukkit-Hale, Cheng, and Joseph (2013) fed rats a 2% blackberry supplemented diet for 8 weeks. At the end of 8 weeks the rats were assessed on three tasks that rely on balance and coordination. They improved on all three tasks, which include the accelerating rotarod, wire suspension, and the small plank walk. The rats also improved on the Morris water maze task to assess spatial learning and memory, which demonstrated improved working or short-term memory compared to the control rats.

As mentioned previously, long-term studies, such as Letenneur et al. (2007) demonstrate the effects of flavonoids over a 10-year period. The mean intake of flavonoids per day for 10 years was 14.33 mg. The psychological tests used to determine cognitive functioning and performance across this time included the Mini-Mental State Examination, Benton's Visual Retention Test, Isaac's Set Test for verbal fluency, Zazzo's cancellation test for visuospatial attention, and the Wechsler's Digit

Symbol Test. The baseline for each of these tests increased over time as flavonoid intake increased, demonstrating that flavonoids can exert beneficial effects on cognitive performance when consumed over an extended period of time. However, although Letenneur et al. conducted testing across 10 years they used the same cognitive tests at each assessment. This may have resulted in possible practice effects that were unaccounted for in this study.

The ability of phytochemicals to exert effects on cognitive performance is contingent upon digestion, absorption, and metabolism (Bell, Lamport, Butler, & Williams, 2015). However, phytochemical supplementation appears to be more effective when consumed over a longer period of time, such as 6-12 weeks. Studies such as those conducted by Letenneur et al. (2007) demonstrate that phytochemicals do have the ability to affect cognition. However, improvements are less likely to be observed from a single dose of flavonoid, opposed to studies across multiple weeks.

Physical and Cognitive Performance and the Influence of Mood

There is a strong association between mood and physical performance (Beedie, Terry, & Lane, 2000), and similarly mood can greatly affect cognitive functioning. The connection between mood, exercise, and cognition can be demonstrated with two examples. Firstly, mood can affect the ability of an individual to concentrate and process information. For example, depression is commonly associated with a number of deficits in episodic memory, learning, and cognitive performance (Austin, Mitchell, & Goodwin, 2001). Studies have consistently found that patients with depression are impaired in their ability to shift the focus of their attention, executive functioning, and their use of working memory (Austin et al., 2001). Secondly, mood

affects the ability and motivation of an individual to exercise. Exercise is associated with improvements in mood and cognitive functioning. Bartholomew, Morrison, and Ciccolo (2005) found that even a short walk was enough to improve mood in patients with Major Depressive Disorder.

Other mood states, such as anxiety, described as an aversive emotional and motivation state can also negatively influence cognitive performance (Eysenck, Derakshan, Santos, & Calvo, 2007). The effect of anxiety on cognitive performance has been explained by the processing efficiency theory. This theory proposes that efficiency and effectiveness of performance are integral to understanding the effect of anxiety. The negative effects of anxiety result in greater effort of processing efficiency rather than performing the cognitive task effectively. Furthermore, a central feature of anxiety is worry. When an individual is worrying, their cognitive processing is disrupted and their storage capacity for working memory is reduced (Eysenck et al., 2007). Anxiety also has detrimental effects on attentional control. Due to the increased allocation of attention to threat-related stimuli, attentional focus is reduced towards the current task. The idea that anxiety impairs attentional control is based on the proposition that there are two attentional systems, the goal directed attentional system and stimulus-driven attentional system (Eysenck et al., 2007). The goal directed attentional system involves top-down control of attention and the stimulus-driven attentional system involves bottom-up control of attention. Anxiety decreases the influence of the goal directed attentional system and increases the effect of the stimulus-driven attentional system. According to attentional control theory, anxiety impairs processing efficiency because it reduces attentional control. As a result the probability that processing resources will be diverted from task-relevant

stimuli to task irrelevant ones on tasks involving the inhibition or shifting functions is increased.

The importance of mood on the relationship between exercise and cognition has proven to be influential and should be considered when conducting research in this area. In order to account for mood, tests such as the Profile of Mood States (PoMS) can be used to assess psychological distress or to detect changes in healthy, physically ill, and psychiatric populations (Curran, Andrykowski, & Studts, 1995). The PoMS assesses global distress and presents six subscales that measure fatigue-inertia, vigour-activity, tension-anxiety, depression-dejection, anger-hostility, and confusion-bewilderment (Curran et al., 1995). It is one of the most commonly used instruments for the assessment of mood within sport and exercise, (LeUnes & Burger, 2000) and can be used to identify problematic psychological aspects of individuals.

Summary

Based on their findings, Spencer et al. (2009) and Spencer (2010) suggest that flavonoid-rich foods have the ability to limit cognitive decline, including memory deterioration, if they are consumed across an individual's lifetime. Although phytochemicals in general have been found to support cognitive functioning, and even reverse damage, the specific effects of flavonoid-rich foods still need to be investigated (Spencer et al., 2009). Furthermore, the limited research on acute and longer-term supplementation of phytochemicals has demonstrated that studies of extended duration have better effects on cognitive performance. Future studies should attempt to investigate causal links between flavonoid consumption and behavioural outcomes, as well as the most effective dosage of flavonoids. Letenneuer et al. (2007)

noted that there are few studies of flavonoids and cognition using humans and these studies have tended to focus predominantly on aging populations. To obtain a more complete picture, investigations employing young healthy participants are required. Mood has also been shown to have a strong influence on physical and cognitive performance. It can have a detrimental effect on physical and cognitive performance due to factors such as depression and anxiety. Additionally, mood should be considered in studies of exercise and cognition because exercise can influence mood and cognition positively and may alter results unexpectedly. Mood can easily be assessed with short form instruments, such as the PoMS. The latter is used in the present study to assess mood.

Chapter 5

The Current Study

The above chapters describe the effects of physical fatigue on cognition and the impairments that result. They also demonstrate the importance of nutrition and how specific foods have the ability to influence cognition. While the effects of nutrition on the brain have been studied extensively, there is little research that focuses on phytochemicals and cognition. Furthermore, the interaction between physical fatigue, cognitive performance and nutrition requires additional research. The present study adds to this relatively small body of research by investigating whether phytochemicals can ameliorate the effects of physical fatigue on cognitive performance.

An earlier Plant and Food study was conducted by Harold (2016). Her study investigated the impact of physical fatigue on cognitive performance and the ameliorating effects of a single dose of blackcurrant supplement. Harold's study included 72 healthy participants who completed 10 mins of high intensity cycling or 10 mins watching an emotionally neutral documentary (control group). Half of the participants received 250 mg of blackcurrant supplement one hour before the experiment began. Baseline cognitive tasks and mood questionnaires were administered before consumption of the blackcurrant extract, again before post-task assessments were completed, and also after the experimental session. Harold's study found that high intensity cycle sessions were successful in inducing physical fatigue. However, this did not carry over to affect cognitive performance; blackcurrant supplementation had minimal effects on cognitive performance. The present study

was a replication and extension of Harold's work. Both an acute dose of blackcurrant supplement as used by Harold and a longer term dose, given each day over 6 weeks, was administered to find out if the latter dosing regime has stronger effects than the acute dose.

Hypothesis One

In light of the recent research by Harold (2016), the present study hypothesised that *a single dose of blackcurrant would improve performance on cognitive tasks by ameliorating the effects of physical fatigue.*

Hypothesis Two

Based on previous research showing that phytochemicals administered over time are beneficial to cognition, it was hypothesised that *participants receiving an extended dose of blackcurrant across 6 weeks would perform better on all cognitive tests in comparison to a placebo group.*

Chapter 7

Method

Participants

Fifty healthy participants aged between 19 and 50 years ($M = 27$, $SD = 7.3$) volunteered to participate in this study. The participants had no known neurological or psychological conditions, and they were not physically impaired in any way. Of these 50 participants, 29 were female, mean age = 25, $SD = 6.4$, and 21 were male, mean age = 29, $SD = 7.9$. Participants were recruited through advertisements, word of mouth, and undergraduate classes at Massey University (see Appendix A). They were given a \$75 supermarket voucher to compensate for their time given to the research. All of the procedures and materials used in the present study were approved by the Health and Disability Ethics Committee (Protocol 15/STH/99).

Group Assignment and Statistical Power

The participants were randomly assigned to one of two conditions: blackcurrant or placebo. There were 25 participants assigned to each condition. All of the participants completed the exercise component of the study.

The power requirements of the study were examined using the G*Power program (Faul, Erdfelder, Lang, & Buchner, 2007). For a medium effect size, with a significance level of $p=.05$, and power = 0.8, a total of 128 participants were required, an impossible number to obtain given the time requirements of the study, the multiple

lab visits required, the donation of multiple blood samples, and the requirement to exercise to exhaustion. The actual number of participants obtained only met the power requirements for large effect sizes.

Apparatus and Cognitive Measures

Five cognitive tests were used in the present study (see Appendix B). All five tests were administered on the computer. The participants all partook in a familiarisation trial in which they completed each of the cognitive tests once.

Cognitive Tests

Participants completed a short test battery on the computer (20 min). These cognitive tests were chosen so that Harold's (2016) study could be replicated and extended.

However, one test from Harold's study (Digit Symbol Substitution) was replaced with the Serial Sevens task in the current study. This was replaced due to practice effects found by Harold. The following tests were included in the current study:

The Stroop Colour-Word Task: This test consisted of 73 colour names (red, green, and blue) each name shown individually. The ink colour was either congruent with the colour name, or incongruent with the colour name. For example the word 'blue' could be printed in red. Participants were instructed to press a key on the computer keyboard (R, G, or B) that corresponded to the colour of the ink of the word that was displayed on the computer screen. They were instructed to press the appropriate key as quickly as possible using only their left hand to replicate Harold's (2016) study and to keep the task consistent. Reaction Time (RT) and accuracy were the dependent

variables, and each was recorded for the congruent stimuli, the incongruent stimuli, and the combination of the two.

The Choice Reaction Time Task: This test consisted of 60 questions. A fixation point, represented by an X, appeared in the middle of the screen. After this, four yellow boxes appeared on the screen. One of the four boxes would then light up orange. The participant was required to press one of four keys, “Z” and “X” with their left hand and “N” and “M” with their right hand, that corresponded to the orange box as quickly as possible. RT and the number of correct responses and errors were recorded.

The Backwards Digit Span Task: This test generated a sequence of random numbers starting with 2 digits and progressing up to 9 digits. The participants were instructed to remember the sequence of digits and type them in the reverse order to which they initially appeared. The sequence of digits grew in length until the participant could not correctly recall the sequence. The participants were under no time restriction to recall each sequence. The greatest number of digits correctly recalled before an error occurred was recorded.

The Trail Making Task: In this computerised version of the Trail Making Test, 18 circles were haphazardly positioned on the screen. Half of the circles contained a letter and the other half contained a number. The participant was instructed to join the circles (using the computer mouse) in a sequence “A-1, B-2, C-3...” alternating between letter and number and continuing until all of the circles were joined. As each circle was approached a line connected it to the preceding circle. If the connection was correct, the line remained. If incorrect, a computer beep indicated the error and

the participant tried another connection. The time taken to complete the task to the nearest whole second was recorded.

The Serial Sevens Task: This test required participants to count down from either 100 or 104 in sevens. The participants were required to subtract seven until they got down to the last number closest to zero. If a wrong number was entered, the computer notified participants with a beep and they attempted another answer.

Physical Task

The physical fatiguing was completed on the Wattbike cycle ergometer (Gee, 2008). The participants were required to cycle for 30 s bouts of high intensity cycles with 1 min rest intervals. The target intensity of cycle bouts that the participants were given was normalised to their body weight and was typically between 3 to 6 watts/kg.

Immediately at the end of each bout the participant was asked to give a Rating of Perceived Fatigue (RPE) score. In addition, the rate of power decline for each bout and heart rate after each bout were immediately recorded. Participants repeated the cycle bouts until they were no longer able to maintain their target intensity for the majority of the bout. The fatiguing exercise was completed a number of times across the study. It was first completed at the familiarisation session to establish participants' fatiguing index and familiarise them with the type of exercise. At the main trial at Week 1, the fatiguing session was completed after the first set of cognitive tasks.

Participants were then given the supplement and instructed to rest for one hour. After this time, the fatiguing exercise was completed again and then the cognitive tasks were completed for the second time. Following this, participants completed one 30 s

all out cycle before the trial was finished, therefore completing three bouts of exercise overall. This process was again completed in the identical order at Week 6.

Heart Rate

Before the trial began the participants were fitted with a heart rate monitor strap around their chest, and a watch. Participants' heart rate was monitored throughout the trial and recorded at specific time points. Heart rate was recorded at the beginning of the trial, before the cycle test, at 30 s intervals during the cycle test, after the cognitive tests, after the 1-hour rest break, and again before, during, and after the second set of cycle tests. These heart rate data were not analysed for the present cognitive study.

Ratings of Perceived Exertion

Ratings of perceived exertion were also used to establish a fatigue index. Borg (1982) gathered information from peripheral and CNS fatigue to develop this scale. The scale does not begin at 1 because this suggests no physical exertion, for example resting. Therefore, the RPE scale begins at 6, equivalent to 'very, very light' and progresses to 'very, very hard', equal to 20 (Borg, 1982). Participants were asked to rate how difficult the cycle task was at each rest interval contingent on how much effort each bout required (refer to Appendix C).

Profile of Mood States (PoMS)

The PoMS (short form) questionnaire is a 30-item checklist used to measure current mood states. Examples of the items assessed included anger, hopelessness, fatigue, friendliness, and vigour. A computerised version of the questionnaire was administered once at Week 1, and once at Week 6, before any cognitive testing had

begun. For this questionnaire, the participants were required to rate the 30 mood states on a 5-point Likert scale from 0 to 4, where 0 represents 'not at all' and 4 represents 'extremely'. The total mood disturbance score was then calculated and used for analysis. The PoMS (short form) has been rated as an excellent alternative to the more time consuming longer version with good internal consistency for all subscales, and consistency with other measures of fatigue and mood (Curran et al., 1995). It has also been found to have good reliability (O'Connor, 2004) and validity (Terry, Lane, & Fogarty, 2003).

Baecke Questionnaire

The Baecke questionnaire was developed to evaluate physical activity in three dimensions; work activity, sports activity, and leisure activity (see Appendix D). Each question is scaled from 1 – indicating low activity, to 5 – indicating high activity. A total of 16 questions comprise the total score for habitual physical activity. The Baecke questionnaire was found to have adequate reliability (Baecke, Burema, & Frijers, 1982). It has also been found to relate well to similar exercise measures and have good validity in healthy populations (Pols et al., 1995). The data from the Baecke were collected for the research programme but were not used in the current study.

Phytochemical Manipulation

Supplement

Participants receiving the blackcurrant supplement were given a gelatine capsule that contained 240 mg of anthocyanin-rich blackcurrant (freeze dried powder) extracts.

The capsules were produced by an experienced and trained team (under the appropriate food safety regulation and conditions) at Plant and Food Research. The participants consumed the capsule 1 hour prior to the second exercise session at Week 1 and Week 6, and one capsule daily over the 6-week period.

Control

The participants in the control (placebo) condition were given a gelatine capsule containing fructose and glucose equivalent to the amount found in the blackcurrant extract.

Dietary Controls

Participants were instructed to avoid strenuous exercise and foods that contained similar properties to blackcurrant two days before they came in for the main trials (see Appendix F). They were instructed to continue with their normal diet and physical activity for the remainder of the time. They were also advised to make sure they had eaten breakfast before they came in for the trial so they did not run out of energy during the 3-hour period that the trial took. The participants were also asked to complete a weekly survey. The survey monitored the participants' diet and consumption of particular foods.

Design and Analysis

The study design was a 3-way repeated measures ANCOVA. The factors were Time (pre vs. post exercise), Feeding (blackcurrant vs. no blackcurrant), and Week (week 1 vs. week 6). The covariate was the total mood disturbance scores obtained from the PoMS at Week 1 and Week 6.

All statistical analyses were completed using the statistical package SPSS for Mac, version 24.0. Both the effect sizes and the significance levels are reported in the current study, calculated in accordance with the guidelines suggested by Cohen (1988). Effect size approximation was achieved using partial eta squared (η_p^2) and was calculated within SPSS. The family-wise significance level was set at $p = .05$.

Procedure

Familiarisation Session

The participants were given an information sheet about the study (see Appendix F) and any questions they had were answered. They completed a medical questionnaire (see Appendix G) and the Baecke Questionnaire for physical activity. If they decided to participate, they signed an informed consent form (see Appendix H). After this time, they were weighed in normal clothing and their height taken. A familiarisation session was then conducted for each individual participant, taking around 45 mins. During this session participants were fitted with a heart monitor and completed the cycle test on the Wattbike. This was to determine their personalised exercise

parameters necessary to induce physical fatigue. Participants were then familiarised with the cognitive tests. After this, they were given a list of the dietary requirements for the study.

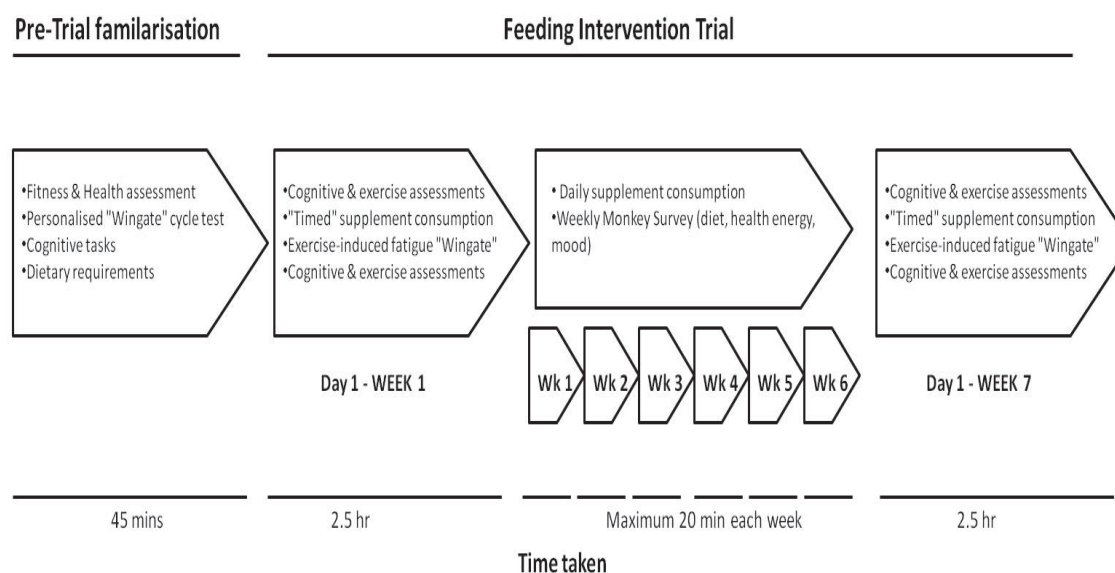


Figure 1. Outline of overall trial design showing cognitive and fatiguing assessments at Week 1 and Week 6, and consumption of the supplement over 6 weeks.

Main Trial (Week 1)

Figure 1 shows the trial design in the form of a time-line indicating the duration of each trial component. At the main trial, participants (individually) arrived having omitted the required foods from their diet 2 days beforehand. Each trial ran for 2 hours and 45 mins. The cognitive tests and Wattbike were situated in the same room. No other individual except for the participant and the trial coordinators were present in the room when the trial was being completed.

Cognitive Tests

When participants were ready they completed the PoMS test on the computer. Next, they completed the five cognitive tasks, which took no longer than 25 mins. The instructions were read aloud to the participant for each test and any questions were answered. Two of the cognitive tests had two equivalent versions, which were alternated at the two administration points during the trial to minimise practice effects. The Trail Making Task was administered first using version A. Similarly, the Serial Sevens Task, version 100, was used at this time point. The remaining three cognitive tasks used randomly computer generated stimuli that varied from trial to trial, and so did not require different versions to be used.

Fatiguing Session

Following the cognitive tests, participants were taken to the bicycle to complete the exercise task. Participants' exercise component was tailored to their level of fitness and Body Mass Index (BMI) that had been established at the familiarisation session. The exercise session took 10 mins or less, and was complete when the participant was fatigued. During the exercise session, participants were encouraged to cycle as hard and as fast as they could for each 30 s interval. The fatigue level was established by monitoring the heart rate, performance of the bicycle task (wattage output), and RPE scale.

Rest Period & Treatment

When participants were fatigued they stepped off the bicycle and gave blood (finger prick) from which lactate and glucose levels could be established. This took about 2-5

mins. After that, participants were taken to a separate room to relax. Following 20 mins of relaxation they were given their first dose of supplement (blackcurrant or placebo) and relaxed for a further 1 hour. During this time the participant was only allowed to drink water and was instructed to sit quietly. In this time, participants read, studied, or used their cell phones.

Resume Main Trial – Fatiguing Session

After 1 hour had elapsed participants were taken back to the exercise room. At this time point they again cycled to fatigue in less than 10 mins on the Wattbike. Heart rate and RPE was recorded during the exercise. After this, participants' lactate and glucose levels were again measured.

Cognitive Tests

Immediately after, the cognitive tests were complete for the second time with the alternate versions of the Trail Making Task and Serial Sevens Task being used.

Following the cognitive tests, participants gave blood once again.

Fatiguing Session

Next, participants were required to cycle on the Wattbike for one “all out” cycle session. They were encouraged to cycle as hard as they could for the duration of 30 s. After this, they gave one more blood sample to provide lactate and glucose readings, bringing the first stage of the trial to a close.

Treatment Across Six Weeks

At this point, the participants were given enough supplements to take one capsule a day, at the same time, for a week. They were informed that they could resume their normal diet for the next 6 weeks. One week after the first trial, participants individually came into the lab to give blood and their supplement compliance was checked. They were again given enough supplements for one week before returning to the lab. This cycle of activity continued over the 5-week period.

Main Trial (Week 6)

At Week 6, the participants returned to complete the trial. This second part of the trial replicated the exact same procedure used at Week 1. After the trial was over the participants were debriefed, thanked for their time, and given their shopping voucher.

Chapter 8

Results

Firstly, the physical data of the participants was reviewed to ensure that they had been physically fatigued as expected by the sessions on the Wattbike. Following this manipulation check, the data for mood differences between the blackcurrant and placebo groups were examined. Next, the analysis turned to investigating the cognitive effects of the blackcurrant supplement taken across 6 weeks. The placebo group and the blackcurrant supplement group scores were compared on the Trail Making Task, Backwards Digit Span, Choice Reaction Time, Serial Sevens, and the Stroop. Three separate analyses were conducted on the Stroop: the overall Stroop scores, the congruent scores, and the incongruent scores. The reason for this is that extant studies use all three types of Stroop analysis. Thus, for comparison purposes all three types of analysis are reported.

ANOVA Assumptions

A 2 (Condition) x 2 (Mood) analysis of variance (ANOVA) was conducted to compare the mood differences between the blackcurrant ($n = 25$) and the placebo ($n = 25$) groups. Prior to beginning the analysis the general assumptions underlying the ANOVA were tested. The Shapiro-Wilk, F_{max} and Levenes test statistics showed the assumptions of normality and homogeneity of variance, respectively, were not violated.

Profile of Mood States

The Profile of Mood States Test is a self-report assessment tool used to quickly gather information about transient and fluctuating feelings as well as affective mood states.

Table 1

Means and Standard Deviations (SD) for the Profile of Mood States Test administered at Week 1 (PoMs1) and Week 6 (PoMS2)

Condition	PoMS1		PoMS2	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
BC	46.00	6.95	45.12	5.81
PC	51.20	9.12	52.12	8.24

Note: Values shown for the PoMS 1 and 2 are Total Mood Disturbance scores. Higher scores reflect more negative emotions. BC = Blackcurrant; PC = Placebo.

From Table 1 it can be seen that there was an unexpected difference in mood between the two conditions at both Week 1 and Week 6. However, there was no main effect for Mood over 6 weeks, $F < 1$, and no interaction between the PoMS and Condition, $F < 1$. Surprisingly, there was a strong main effect for Condition, $F(1, 48) = 10.49$, $p = .002$, $\eta_p^2 = .18$.

Due to the mood differences between these two groups it was thought best to treat the PoMS 1 and 2 as covariates to control for any effects these mood differences might be having on the main variables. Consequently, for all of the dependent measures, analysis of covariance (ANCOVA) was employed.

Manipulation Check: Fatiguing Effect of Physical Exercise

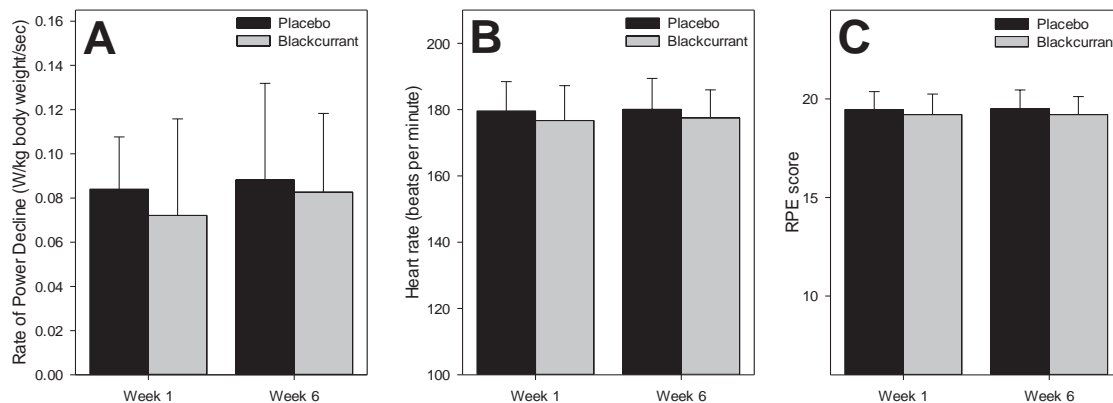


Figure 2. Physiological and subjective measures of physical exertion during the final high intensity interval cycle where participants were no longer able to maintain their target cycling intensity.

Figure 2 shows small differences between the placebo and black currant groups. However, paired *t*-tests revealed no statistically significant changes in the rate of power decline (A) and heart rate (B) between Week 1 and Week 6 within treatment groups. This was also true for RPE scores (C). ANOVA revealed no significant differences in rate of power decline, heart rate and RPE between treatments groups at Week 1 and Week 6 ($p > 0.05$). These results confirm that all participants, regardless of trial week or treatment group, were equally fatigued after their final high intensity interval cycle prior to their performing the cognitive tests.

ANCOVA Assumptions

A repeated measures mixed ANCOVA with Week (1, 6) and Exercise (pre, post) as the within-group factors, and Condition (blackcurrant, placebo) as the between-groups factor was used to investigate the effects of blackcurrant extract on various measures of cognitive performance across 6 weeks with participants exercised to the point of exhaustion. The independent variable was the type of supplement the participants received; blackcurrant or placebo. The dependent variables consisted of scores on five cognitive tests described in the Method section: the Stroop (congruent, incongruent, and mixed), Choice Reaction Time, Trail Making, Backwards Digit Span, and Serial Sevens.

Data from one participant was excluded as an extreme outlier only for the Serial Sevens Task. All remaining data from the other four cognitive tests for this participant were used.

Before beginning the main analysis, the assumptions underlying ANCOVA were tested for each of the cognitive tests. Examinations of the Shapiro-Wilk statistics and histograms for each group indicated that the ANCOVA assumption of normality was supported. Scatterplots indicated that the relationship between the covariate (PoMS) and the dependent variables were approximately linear. Finally, the assumptions of homogeneity of regression slopes and homogeneity of variances were supported by the absence of significant independent variable-by-covariate interactions and non-significant Levenes tests, respectively.

Cognitive Measures

ANCOVAs were individually conducted for each of the five cognitive tasks. It was expected that blackcurrant supplement would ameliorate some of the negative effects of exhaustion on cognitive performance, resulting in relatively better performance by the blackcurrant group compared to placebo. That is, there would be an interaction between Week and Condition.

Trail Making

The Trail Making task involved linking letters and numbers in a predetermined order.

The dependent measure was the time taken in seconds to complete the task.

Table 2

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing

Time for the Trail Making Task

		Condition			
Week	Exercise	Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	56.05	15.83	55.09	10.24
	Post	48.05	9.87	48.71	11.63
6	Pre	57.39	11.67	57.38	12.80
	Post	52.20	11.70	53.51	11.15

Note: Values for Trail Making task are given in seconds.

From Table 2, it can be seen that, overall, there is slight increase in time taken to complete the Trail Making Task from Week 1 to Week 6. The main effect of Condition (blackcurrant, placebo) was not significant in the Trail Making Task, $F < 1$. Neither was the predicted interaction between Exercise and Condition, $F < 1$. All other main effects and interactions also failed to reach significance with no effects apparent.

Backwards Digit Span

The Backwards Digit Span Task entailed recalling increasing numbers of digits in backwards order from the order the digits were originally presented in. The dependent variable was the maximum number of digits correctly recalled.

Table 3

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing Time for the Backwards Digit Span Task

Week	Exercise	Condition			
		Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	6.08	1.94	6.56	1.79
	Post	6.64	1.87	6.48	1.56
6	Pre	6.36	1.73	7.08	1.68
	Post	6.60	1.87	7.24	1.67

Note: Values for Backwards Digit Span Task are the mean number of digits recalled.

Table 3 shows that there was a small increase in the number of digits recalled in the Backwards Digit Span task between Week 1 and Week 6, but this occurred for both the blackcurrant and placebo groups. Consequently, there was no significant difference between blackcurrant and placebo, $F < 1$. The interaction between Exercise and Condition was not statistically significant $F(1, 46) = 1.70, p = .20, \eta_p^2 = .04$, nor was the interaction between Week and Exercise, $F(1, 46) = 1.14, p = .28, \eta_p^2 = .03$. Both effect sizes are very small. However, there was a significant interaction between Week and Condition, $F(1, 46) = 5.31, p = .03, \eta_p^2 = .10$, accounting for approximately 10% of the experimental variance. Figure 3 shows that prior to exercise the blackcurrant group improved from $M = 6.08$ to $M = 6.36$ across 6 weeks. However, the placebo condition also improved from $M = 6.56$ to $M = 7.08$ across 6 weeks. As can be seen in Figure 4, post-exercise, the participants' scores in the blackcurrant group ($M = 6.64$) at Week 1 were *higher* than the participants' scores in the blackcurrant group ($M = 6.60$) at Week 6. Contrary to expectations, the scores for the placebo group ($M = 6.48$) at Week 1 *improved* across 6 weeks ($M = 7.24$). The improvement in the placebo groups' score suggests that the changes observed over the 6-week interval were due to practice, or increasing familiarity with the task and the procedure. Taken at face value, this interaction appears to show that the blackcurrant supplement taken over the 6 weeks had a slight negative effect on performance.

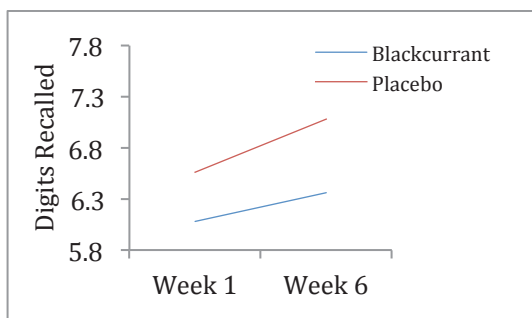


Figure 3. Pre-exercise mean scores for Backwards Digit Span across Week 1 and Week 6.

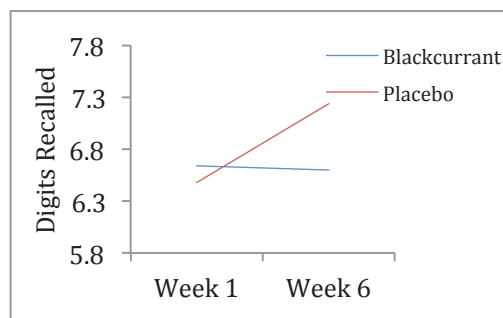


Figure 4. Post-exercise mean scores for Backwards Digit Span across Week 1 and Week 6.

Serial Sevens

The Serial Sevens Task involved counting down from 100 to zero by steps of seven. To minimise the effects of practice, the repeat of this task post-exercise started at 104. The dependent variable was the number of seconds taken to count down to zero.

Table 4 shows that testing time decreased across Week 1 to Week 6 post-exercise for both the blackcurrant and placebo groups. However, pre-exercise the blackcurrant group increased from 59.55 s to 61.52 s as opposed to the placebo group who decreased in time from 69.21 s to 59.44 s. It was expected that the blackcurrant group would outperform the placebo group across 6 weeks and this would be demonstrated by an interaction between Week and Condition, but the interaction did not occur, $F < 1$.

Table 4

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing

Time for the Serial Sevens Task

		Condition			
Week	Exercise	Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	59.55	27.85	69.21	29.01
	Post	60.36	52.37	51.29	23.84
6	Pre	61.52	33.96	59.44	35.49
	Post	51.39	20.51	45.29	15.39

Note: Values for Serial Sevens Task are shown in seconds. A decrease in time

represents an improved score.

There was a near significant interaction between Exercise and Condition, $F(1, 45) = 3.62, p = .06, \eta_p^2 = .08$, with a moderate effect size. Pre-exercise, only the placebo group showed decreases in time. However, post-exercise the blackcurrant group decreased from 60.36 s to 51.39 s, similar to the placebo group, which decreased post-exercise from 51.29 s to 45.29 s. Therefore, the interaction suggests that the effect was likely due to practice and not improvements from blackcurrant. There were no significant interactions between Week and Condition, $F < 1$, Week and Exercise, $F(1, 45) = 1.37, p = .25, \eta_p^2 = .03$, or Week, Exercise, and Condition, $F(1, 45) = 2.48, p = .12, \eta_p^2 = .05$. Finally, there were no main effects for Condition, Week, or Exercise, all $F_s < 1$.

Choice Reaction Time

The Choice Reaction Time Task required the participants to press one of four keys that corresponded to the box on the screen that was highlighted. The dependent variable was the time taken in ms to respond.

Table 5

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing Time for the Choice Reaction Time Task

Week	Exercise	Condition			
		Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	451	52	439	53
	Post	431	47	433	49
6	Pre	413	38	412	42
	Post	413	42	407	49

Note: Values for Choice Reaction Time are shown to the nearest ms.

From Table 5, it can be seen that performance improved from Week 1 to Week 6 but did so for both the blackcurrant and placebo groups. Pre-exercise the blackcurrant group (38 ms) improved more than the placebo group (26 ms). But post-exercise the placebo group (25 ms) improved more than the blackcurrant group (17ms). However, these are very small changes for both groups. So, contrary to expectations, there were no main effects for Condition, Week, or Exercise, all $F_s < 1$. Similarly, there were no interactions between Week and Condition, Exercise and Condition, Week and

Exercise, or Week, Exercise, and Condition, all $F_s < 1$. The results of the Choice Reaction Time Task fail to support the hypothesis that the blackcurrant supplement would improve reaction time after 6 weeks of supplementation.

Stroop Congruent Condition

In the Stroop Congruent Task the stimulus word matched the stimulus colour. The dependent variable was the time taken to respond by pressing the colour-coded key that matched the colour of the font of the word. The means and SDs for the congruent Stroop are given in Table 6.

Table 6

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing Time for the Stroop Congruent Task

Week	Exercise	Condition			
		Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	547	68	557	66
	Post	532	58	520	53
6	Pre	517	58	502	49
	Post	499	54	491	43

Note: Values for Stroop Congruent condition are shown to the nearest ms.

There were improvements in RT from Week 1 to Week 6 for both the blackcurrant and placebo groups, and at both pre- and post-exercise points. Relative to the size of

the SDs, these improvements are quite small. Consequently, there were no main effects for Week or for Condition, both $F_s < 1$. Neither did the expected interaction between Week and Condition occur, $F < 1$. Further, there were no interactions between Exercise and Condition, or Week and Exercise, both $F_s < 1$. However, there was a near significant 3-way interaction for Week, Exercise, and Condition, $F(1, 46) = 3.46, p = .07, \eta_p^2 = .07$. The Week x Exercise x Condition interaction explained 7% of the variance demonstrating a medium effect size. Figures 5 and 6 pinpoint most of this 3-way interaction as being due to the interaction between Week and Condition for the pre-exercise data. The improvement of scores for both the blackcurrant group and the placebo groups suggests that practice effects remained at the pre-exercise time point after 6 weeks. Once again, the placebo group showed slightly faster RTs than for the blackcurrant group at Week 6 for both pre- and post-exercise conditions, suggesting that these changes are indeed due to practice.

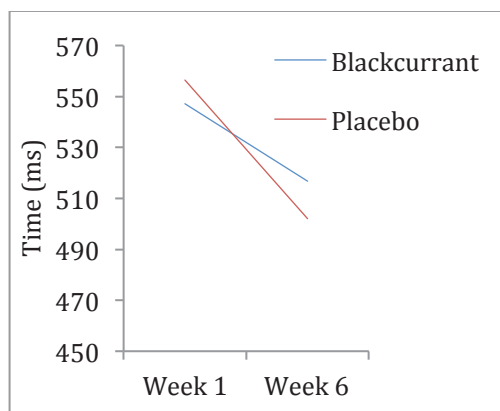


Figure 5. Pre-exercise at Week 1 and Week 6 for the Stroop congruent task.

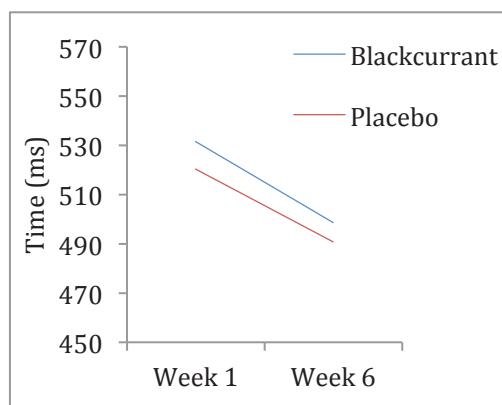


Figure 6. Post-exercise at Week 1 and Week 6 for the Stroop congruent task.

Stroop Incongruent Condition

The Stroop Incongruent Task involved stimulus words incongruent with the stimulus colour. The dependent variable was the time taken to respond to the written word by pressing the corresponding coloured key. Table 7 displays the results obtained from this cognitive task.

Table 7

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing Time for the Stroop Incongruent Task

Week	Exercise	Condition			
		Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	574	85	584	97
	Post	545	62	554	60
6	Pre	537	63	535	53
	Post	518	60	523	55

Note: Values for Stroop Incongruent Condition are shown in ms.

For both groups, RT decreased from Week 1 to Week 6. At Week 1 and at Week 6 both the blackcurrant and placebo groups produced slightly faster RTs. However, the expected interaction between Week and Condition did not occur, $F < 1$. Similarly, there were no interactions between Exercise and Condition, Week and Exercise, or Week, Exercise, and Condition, all $F_s < 1$.

In addition, there were no main effects for the three factors of Condition, Week, or Exercise, all $F_s < 1$. The results from the Stroop Incongruent task clearly show no support for the hypothesis that a blackcurrant supplement taken over 6 weeks would improve RT.

Stroop Mixed Condition

The Stroop Mixed Task involved stimulus colours that were either congruent or incongruent to the written words. The participant was required to distinguish and swap between these variations by responding to the correct stimulus colour. The dependent variable was the time taken to respond by pressing the correct colour corresponding key.

Table 8

Means (M) and Standard Deviations (SD) as a Function of Condition and Testing Time for the Stroop Mixed Task

Week	Exercise	Condition			
		Blackcurrant		Placebo	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
1	Pre	565	78	568	67
	Post	541	59	543	56
6	Pre	530	59	524	50
	Post	511	56	512	48

Note: Values for the Stroop Mixed Condition are shown to the nearest ms.

Table 8 shows that in both the blackcurrant and placebo conditions RT decreased from Week 1 to Week 6. Contrary to expectations, there was no main effect for Condition, Week, or Exercise, all $F_s < 1$. Similarly, there were no interactions between Week and Condition, $F < 1$, Exercise and Condition, $F < 1$, or Week, Exercise, and Condition, $F(1, 46) = 1.89, p = .18, \eta_p^2 = .04$. However, a significant interaction was found between Week and Exercise, $F(1, 46) = 4.3, p = .04, \eta_p^2 = .09$. Figure 7 shows the interaction between Week and pre-exercise. Conversely, Figure 8 shows no interaction between Week and post-exercise. The interaction between Week, Exercise, and Condition displayed a small effect size and the interaction between Week and Exercise was the main component of this interaction.

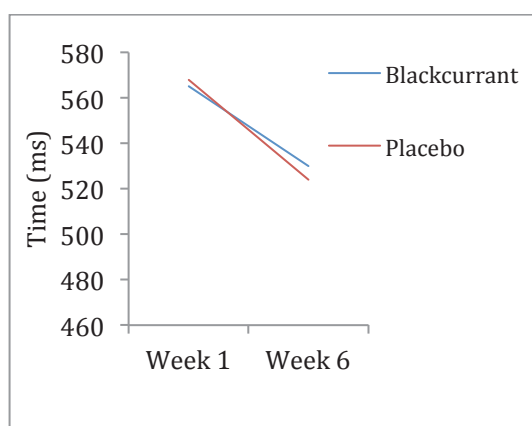


Figure 7. Pre-exercise scores in milliseconds for the Stroop mixed task.

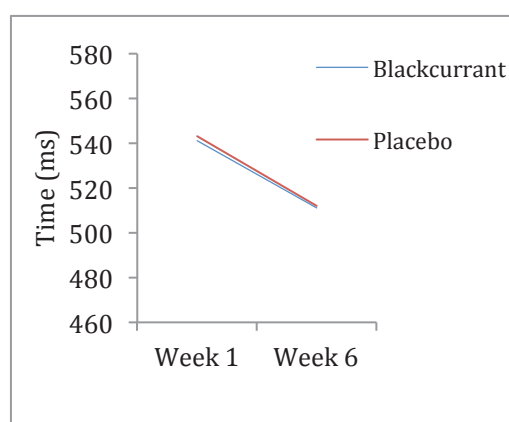


Figure 8. Post-exercise scores in milliseconds for the Stroop mixed task.

Chapter Nine

Discussion

The present study had two aims. First, to confirm Harold's (2016) finding that an acute dose of phytochemical supplementation (blackcurrant) does not ameliorate the effects of physical exhaustion on cognitive performance. Second, to find out if the blackcurrant supplement, taken daily over a 6-week period, has different effects on the recovery rate from physical exhaustion and on cognitive performance compared to the acute dose. The present results provided support for Harold's results, but no evidence for any effect of the supplement taken over the longer term.

In order to investigate the aims of the current study physical fatigue was used to induce cognitive fatigue. Therefore, it is important to discuss the intensity, type, and duration of exercise that the current study used to induce fatigue.

The U-hypothesis is well known in the exercise and cognition literature. This hypothesis states that intense exercise has an inverted U-effect on cognitive performance. Therefore, cognitive performance is negatively affected only when completing high intensity exercise (Brisswalter et al., 2002). By using sprint interval training, such as HIIT, many of the physiological and biochemical systems that are used in aerobic exercise become stressed. HIIT also affects skeletal muscles and increases blood lactate, body carbohydrate, and fat oxidation (Bayati et al., 2011). The high level of intensity of the exercise in turn affects the CNS, which influences

cognition. When arousal is increased due to increased exercise intensity, attention is narrowed, and an optimal level of performance can only be attained when relevant cues are processed with increased effort (Brisswalter et al., 2002). Thus, the present study expected that physical fatigue from HIIT cycle exercise would result in declines in cognitive performance.

High intensity exercise is characterised as 77-95% of maximal heart rate (Gillen & Gibala, 2014). Participants in the current study exercised at a wattage that was tailored to their height and body weight. Heart rate, work output, and RPE were all monitored to establish the fatigue threshold for each participant. Research has consistently shown that HIIT is an effective and time-efficient way to induce fatigue in a number of populations, and it has been found to simulate similar results to endurance exercise.

Ensuring that the participants were fatigued was integral to the current study. However, the relationship between physical fatigue and cognitive performance is complex. The current study based its hypotheses on the findings that the physiological mechanisms of fatigue would influence the biological mechanisms that control cognition. This relationship can be briefly summarised by reference to two types of fatigue: peripheral fatigue and CNS fatigue (Ament & Verkerke, 2009). Similarly, it is well known that fatigue is experienced both physiologically and psychologically because both of these functions shape how individuals experience fatigue and exhaustion, and their subsequent physical and cognitive performance (Paul et al., 1998).

Many previous studies have used exercise to induce cognitive fatigue. For instance, Moore et al. (2012) examined the influence of exercise-induced fatigue on cognitive functioning. Thirty participants completed 60 mins of cycling at 90% of their ventilatory threshold, while a control group rested. After the exercise, all the participants completed a simple and complex visual perceptual discrimination test, a 40 min memory test, and a repetition of the visual perceptual discrimination test. They found that the participants in the exercise group showed declines in performance on complex perceptual-discrimination tasks compared to the participants who rested. The response time of the exercise group during the memory task was also found to be slower than for the rest group. Interestingly, the participants did not show decrements in target detection across the vigil. Similar to the study conducted by Moore et al. (2012), the current study used self-report measures of RPE, as well as heart rate, and wattage output. However, the present study did not include a control rest group. So, although analysis of heart rate, RPE, and power output demonstrated that the participants were physically fatigued, it was not possible to confirm that they experienced cognitive fatigue as a result of the exercise. This is because changes following the fatiguing exercise could have been confounded by practice effects.

A comparable study by Hogervorst et al. (1996) investigated cognitive performance after strenuous exercise. Psychomotor and cognitive tests were conducted on 15 athletes before and after exercise. The participants cycled on a stationary bike at 75% of their maximal work capacity and equivalent to 1 hour of exercising. The results showed that simple RT was significantly lower after exercise than at baseline. However, complex RT improved after exercise and no extra errors were made. This study suggests that, although the participants were fatigued, their performance on

different cognitive tasks varied. Different aspects of cognition may be affected differently by fatigue and may help to explain some of the discrepancies in cognitive performance in the literature. Therefore, it was important to closely monitor the participants fatigue due to the ability of moderate exercise to improve cognition. The results from heart rate and wattage power performance demonstrated that all participants reached their fatigue threshold and were sufficiently exhausted.

In summary, the current study has shown that HIIT induces physical fatigue in the participants. However, it is unclear if the participants' cognitive performance was affected as a result of the physical exercise due to possible confounding with practice effects. Therefore, although participants may have reached their fatigue index, this may not have been sufficient to carry over to impair cognitive performance in a manner that could be tested to determine the efficacy of the blackcurrant supplement. If the participants did not experience sufficient cognitive fatigue, then slight changes from the blackcurrant supplement may not have been noticeable.

Hypothesis 1: An acute dose of phytochemical supplementation will ameliorate the effects of physical exhaustion on cognitive performance.

The first hypothesis was tested by looking at the effects of a single dose of blackcurrant supplement on cognitive performance in physically fatigued participants. Earlier research by Harold (2016) found that a very similar-sized dose of blackcurrant did not ameliorate the effects of fatigue to improve cognitive performance. The present study was a partial replication of Harold's work. A second set of results

showing no effects of an acute dose of a BC supplement on cognitive performance would strengthen Harold's conclusion.

Previous research has shown that phytochemicals positively influence the brain in numerous ways. Phytochemicals have been found to enhance, stimulate, and protect neurons, as well as promote learning and cognitive performance. Phytochemicals have been extensively researched in animals, but the majority of the research in human studies has focused on preventing neurocognitive decline in the aging population. Recent animal studies have found that anthocyanins are able to cross the blood-brain barrier and have been found in the brain and brain tissue 18 hours after ingestion (Milbury & Kalt, 2010). The ability of anthocyanins to cross the blood-brain barrier probably contributes to their direct ability to affect cognition. Reviews of the current research on phytochemicals in humans have found that the majority of studies show a positive association between phytochemical consumption and cognitive performance (Macready et al., 2009). These findings have also been seen in long-term studies. For instance, over a period of 10 years a high intake of flavonoids has been linked with better cognitive functioning than at baseline (Letenneur et al., 2007).

The present study examined the influence of phytochemicals on five cognitive tasks: Choice Reaction Time, Backwards Digit Span, Serial Sevens, Trial Making, and Stroop. It was expected that improvements would be seen on all five cognitive tests across six weeks. However, no statistically significant interactions were found between Week, Exercise, and Condition. Similarly, no statistically significant interactions were seen for Week and Condition, with the exception of the Backwards Digit Span task. However, this interaction was mainly due to the placebo group

outperforming the blackcurrant group post exercise at Week 6 (see Figure 4). That is, the interaction is in the opposite direction to that predicted. This interaction suggests that the blackcurrant supplement decreased performance on the backwards digit span task. However, in light of the failure to see any significant changes on the other cognitive tasks and taking into account previous research findings, it seems likely that this outcome was a chance result.

Furthermore, no statistically significant improvements were found for the interaction between Week and Exercise except for the Stroop (mixed), suggesting a change at the pre- and post-exercise time points. Contrary to expectations, the current study found a significant Exercise x Week interaction for performance on the Stroop mixed condition. This finding suggests that the participants improved from pre-exercise to post-exercise testing within the 1-hour testing period at week 1. Figure 7 shows that the blackcurrant condition improved by 24 ms, which is the same as the 24 ms improvement in the placebo group. This unexpected (but very small) interaction between Exercise and Week did not involve Condition, and demonstrates that both groups improved at the same rate from pre- and post-exercise. Therefore, the ability of the participants improved or they became more practiced at switching between the two stimuli on this task. It is interesting that statistically significant results were found on the Stroop mixed condition and not the Stroop incongruent or Stroop congruent tasks. It may be that the complexity of this particular task (congruent and incongruent tasks intertwined) was enough to utilise higher cognitive processing skills and made it more vulnerable to fatigue and subsequent improvement.

The null findings of the current study and those of Harold (2016) may be partially due to the dose of the blackcurrant. The dose used in the current study was 240 mg, while Harold used a dose of 250 mg. Recent research by Dodd (2012) has shown that acute supplementation of blueberries can lead to improvements in working memory in younger adults and immediate word recognition in the older group when assessed 5 hours after ingestion. Besides the longer interval between ingestion and cognitive testing, Dodd's study used 575 mg of flavonoids, a much higher dosage than the current study. Furthermore, Dodd found that when cognition was tested 2 hours after ingestion (blueberry supplement) there were no effects seen for executive functioning, working memory, or mood. This research applies to the current study in two ways. Firstly, it raises the question of whether assessment of cognitive performance 1 hour after ingestion of the blackcurrant supplement was long enough. Secondly, the dose in the current study compared to Dodd's study was distinctly smaller. When feeding an acute dose of supplement the requirements of the dosage may need to be bigger for any effects to be seen. In any event, studies are required that vary the quantity of the supplement to establish whether the size of the dose needs to be taken into account.

In summary, previous research has investigated the effects of phytochemicals on cognition. However, there is little research that specifically examines the phytochemicals extracted from blackcurrant on cognitive performance. It is possible that the dose was not high enough to have an effect on cognitive performance. As well, more time may be needed before administering cognitive tests so that the blackcurrant can be metabolised and absorbed by the body. The results failed to support the hypothesis that a single dose of 240 mg blackcurrant supplement would

ameliorate the effects of physical fatigue on cognitive performance, lending support to Harold's (2016) same finding.

Hypothesis 2: Blackcurrant supplement will ameliorate the effects of physical fatigue on cognitive performance when taken daily over 6 weeks.

The main research question for the current study was to investigate whether blackcurrant supplement would ameliorate the effects of physical fatigue on cognitive performance when taken every day over a 6-week period.

The present study follows from one of the first studies (Harold, 2016) to investigate the ameliorating effects of blackcurrant supplement on cognition in participants that are physically fatigued. It was expected that the blackcurrant extract would ameliorate the effects of physical fatigue, perhaps by speeding recovery rate, thereby reducing cognitive fatigue. In other words it was expected that blackcurrant extract would improve cognitive performance compared to the placebo group. However, with the exception of an interaction between Condition and Exercise on the Backwards Digit Span, and an interaction between Week and Exercise on the Stroop (mixed), the results for the other cognitive tests showed that there were no significant interactions between Condition and Week, Condition and Exercise, or Condition, Week, and Exercise. Participants in the blackcurrant group did not experience any mediating effects on their cognitive performance compared to placebo. Both groups performed similarly on the cognitive tasks and demonstrated the same changes, which suggests various explanations.

Similar to the first hypothesis, it may be that the size of the dose of blackcurrant was a contributing factor to the null findings for the second hypothesis. Some previous research has used doses similar to the 240 mg dose used in the present study, whereas others have used doses in excess of 500 mg. For instance, Watson et al. (2015) feed 525 ± 5 mg of polyphenols per 60 kg of bodyweight from an anthocyanin-enriched blackcurrant extract and found improvements in rapid visual information processing and reaction time tasks. On the other hand, more recent studies such as Whyte and Williams (2015) administered a single dose of 143 mg of blueberries and observed a significant improvement in delayed word recall - a dose size little more than half that used in the current investigation. Whyte, Schafer, and Williams (2015) used 253 mg of blueberries and found improvements in immediate word recall, delayed word recognition, and improved accuracy compared to a 127 mg dose. The above results, and several other similar studies, suggest that cognitive effects can be observed over a range of dose sizes. Despite the evidence for the beneficial effects of phytochemicals it is difficult to define the dosage and period of consumption that will improve cognition or prevent cognitive fatigue. It has been suggested that the preparation of the blackcurrant extract and the source of the blackcurrants may contribute to the discrepancies in the research (Bell et al., 2015).

Analysis of the Backwards Digit span task found no interaction between Week, Exercise, and Condition, or Week and Exercise. However, statistically significant results were found for the interaction between Week and Condition. This interaction suggests that blackcurrant had an effect across 6 weeks. Therefore, the participants' short-term working memory improved from the assessment at week 1. However, on closer inspection of the results Figure 4 shows that the placebo group improved and

the blackcurrant group declined in performance. This can only be explained by a chance effect. Due to the number of tests conducted, the current study would have used a total of over 100 significance tests at $p = .05$. Therefore, it can be expected that chance statistically significant results would arise when conducting a large number of tests (at $p = .05$, one in five tests, on average). The backwards Digit Span task also shows improvements from week 1 to week 6 pre-exercise, as seen in Figure 3. This suggests that the participants in both the placebo and blackcurrant groups were experiencing practice effects due to the repetitive nature of the task and increasing confidence in their ability to complete it.

This probable practice effect was in evidence for all of the cognitive tasks used. Although the five cognitive tests administered in the present study are frequently used to assess performance after fatigue, there is no physiological research supporting these choices. Evidence that demonstrated that these cognitive domains are affected by physical fatigue would help to clarify that ambiguity surrounding physical fatigue and cognitive performance. Additionally, physiological research supporting the connection between physical fatigue and cognitive performance would provide insight into the cognitive mechanisms that are affected by fatigue and subsequently what cognitive domains may be affected by a nutritional supplement. The cognitive tests utilised in this study may not have been sensitive enough to detect slight changes in cognitive performance, though they have produced significant effects in many previous studies (Cho et al., 2003; Duffy, Wiseman, & File, 2003; File et al., 2001).

A review of 39 studies covering a total of 121 cognitive tests, all investigating the effects of phytochemicals or micronutrients, found that none of the studies had used

cognitive tests designed to assess specific nutritional interventions (Macready et al., 2009). However, the literature shows that the Trail Making task is a popular choice for cognition and nutrient studies, as well as the Stroop Colour-Word task. Despite its popularity the Trail Making task has been found to have limited sensitivity and demonstrated negative effects for the treatment of flavonoids in 13 of the included studies. Similarly, the Backwards Digit Span task is frequently used in flavonoid studies, yielding significant differences, even with small samples. The Choice Reaction Time task is also a popular choice and has been found to be sensitive to vitamin B treatments and multivitamin interventions (Macready et al., 2009). Nonetheless, despite the popularity of the chosen cognitive tests only 31% of the 121 tests included in the review conducted by Macready et al. were found to be sensitive to chronic supplementation.

The cognitive tests being used in nutritional studies, such as the current blackcurrant trial, may not be finding effects because of the limited understanding of how physical exercise influences cognitive performance. Macready et al. (2009) note that despite strong findings from animal studies that phytochemicals have a positive effect on cognition, human trials have failed to build on this research. For example, researchers may be administering cognitive tests that are assessing cognitive domains not being influenced by the nutritional intervention. Macready et al. points out that in flavonoid studies conducted with animals, spatial memory was found to be one of the most sensitive measures to flavonoid interventions. However, spatial memory is not frequently tested in human trials. This indicates that current research investigating phytochemicals could be administering and assessing domains of cognition that are not consistently affected by the supplementary interventions. Future studies should

carefully select cognitive tests based on previous research that has shown they are consistently sensitive to small nutritional changes in cognitive domains that are known to be influenced by nutritional interventions. It is also recommended that at least two measures of a cognitive domain are included in a test battery when possible. For example, in the current study, the Choice Reaction Time Task and the Trail Making task both measure information processing. The former measures rapidly made responses whereas the latter measures processing speed over a longer period of time.

It is essential that the cognitive tests selected are suitable to the population being studied. The literature shows that studies generally based their choice of cognitive tests on earlier research. However, reviews of the literature have shown that there is little correspondence between studies regarding the selection of cognitive tests. For instance, the same cognitive tests are frequently used with varying populations (Macready et al., 2009). This may mean that the tests being used are not normalised to the age of the participants they are being administered to. Furthermore, a majority of the previous research on phytochemicals has focused on an aging population. Researchers should select cognitive measures that are suitable for normal and younger populations who do not have dementia or cognitive impairment. The previous focus on the aging population and the reported improvements from phytochemicals may also be attributed to age-related changes. For example, the hippocampus may be more receptive during childhood development and decline in old age. Similarly, the frontal lobes may be sensitive during adolescence. The use of phytochemicals in a younger population may not be as apparent and the natural changes in the brain that occur with aging may influence the effect and uptake of the phytochemicals differently than in a younger population (Bell et al., 2015).

The ability of flavonoids to cross the blood-brain barrier (BBB) is often assumed to mean that the supplement can influence the brain. However, this may not be the case. Crossing the BBB does not mean entry into neurons in which flavonoids are thought to exert neuroprotective effects (Youdim, Shukitt-Hale, & Joseph, 2004).

Phytochemicals, which include anthocyanins, make up a large proportion of the existing literature. However, phytochemicals in different foods are comprised of different chemical components that are absorbed differently by the body. So although the literature shows promising and significant findings regarding the ability of phytochemicals to influence cognitive performance, it is becoming apparent that the research needs to be narrowed to specific chemical components and specific fruits. For example, Balentine et al. (2015) recommend that terms, such as polyphenols, antioxidants, and flavonoids should be avoided due to the broad family they are comprised of. This is very important if the potential of phytochemicals on the human body are to be researched efficiently. So far, research on the absorption, metabolism, and excretion of flavonoids in humans has produced rather inconsistent results (Nijveldt et al., 2001).

Another explanation for the null results in the current study may be to do with the length of the intervention over which the supplement is being taken. The previously discussed review conducted by Macready et al. (2009) included 39 studies that investigated nutrition and cognition. Of these 39 studies, 16 reported that their interventions, which ranged from 4 weeks to 2 years, were too short in duration. Other phytochemical studies have varied in the duration of feeding. For example, Talavera et al. (2005) fed rats a blackberry-enriched diet for 15 days and found anthocyanins

present in the brain after this time. Similarly, Shukkit-Hale et al. (2013) fed rats a 2% blackberry supplemented diet for 8 weeks. The results showed that the rats' motor performance, working memory, and short-term memory improved. Long term studies have also been conducted over ten years, such as the study by Letenneur et al. (2007) in which they found improvements in cognition in the aging population. Acute studies have found that blackcurrant is available in the brain within 18 hours, suggesting that it could be affecting cognitive performance in this short time frame. Therefore, it is interesting that studies that feed phytochemicals for less than 1 week often do not find significant changes in cognitive test scores. Among the explanations given for this finding is that the cognitive tests are not sensitive enough to detect the slight changes that may arise from nutritional interventions. It is also suggested that the testing that is taking place is not assessing the type of cognition and regions of the brain that are potentially being affected by phytochemicals.

In summary, the present study failed to support the hypothesis that blackcurrant supplement fed across 6 weeks would ameliorate the effects of physical fatigue on cognitive performance. The blackcurrant supplement was found to have no influence on cognitive performance on four of the five cognitive tests, and the effect observed for the Backwards Digit Span task was likely a chance effect.

Limitations of the Present Study and Suggested Further Research

At completion of the present study it became apparent that it suffered from a number of limitations.

Firstly, the small sample size ($n = 50$) may have contributed to the null results. Previous studies have used varying sample sizes, with File et al. (2001) basing their findings on 27 young adults, while Elsabagh, Hartely, Ali, Williamson, and File (2005) recruited 40 young men and women. However, in the present study the analyses clearly show that almost all statistical tests lacked sufficient statistical power. Even for large effect sizes the recommended total number of participants is 52 (G*Power: Faul et al., 2007), just over the number of participants used in the current study. The handful of small effect sizes observed were not large enough to yield a statistically significant outcome at the $p = .05$ level. Future studies will produce clearer results with larger sample sizes. However, many effect sizes are likely to be small to medium making an appropriate sample size beyond the means of many studies. Once a few more studies have been run, it should be possible to use meta-analysis to get a clearer picture of the ability of blackcurrant extract to speed up recovery from physical exhaustion.

A further limitation may have been the inclusion of four participants who did not speak English as their first language. This may have affected their ability to complete the cognitive tests and/or benefited their ability to complete them. For example, the Stroop task relies on participants' ability to process language faster than their ability to differentiate the stimuli. Thus, if participants are able to ignore the written word they will easily be able to focus on the colours in the test instead of being distracted. On the other hand, participants who do not speak English as their first language may take more time to process the words, lengthening the time taken to complete a task. Future studies should consider this when including cognitive tests, such as the Stroop, which require automatic discrimination between colours and words.

The 6-week timeframe of the present study required a great deal of compliance from the participants. The participants were responsible for taking their supplements at the same time each day. Whilst they were required to collect their new supplements from the study coordinators each week, it is possible that they may not have closely adhered to the weekly schedule. In addition, the participants were required to avoid foods that may have interfered with the study 2 days before each main trial. The conformity of both of these requirements could only be regulated by self-report from the participants and may not have been accurately reported. It is advised that future research attempts to adhere to a stricter protocol that more frequently checks in with the participants, or reminds them to take their supplement each day, and at the same time each day.

Unlike the preceding study by Harold (2016), the present study did not include a control rest group. Due to the difficulty in recruiting participants for a study requiring more than one visit to the lab, the nature of the tasks undertaken, and the requirement to donate blood, recruiting 50 participants was difficult and a control group would have required a total of around 75. However, this means that the physically fatigued participants could not be compared to a control. Therefore, it was impossible to know how participants performed on the cognitive tests without exercising to exhaustion. Such a control group would have made it clearer how practice was affecting performance on the cognitive tasks, for example. This is important because research shows that exercise has the ability to positively influence cognition (Brisswalter et al., 2002), which could be misinterpreted and attributed to the effect of the phytochemical intervention.

The sensitivity of the cognitive tests may also have been a limitation of the current study. The current study was a replication of Harold's (2016) study and therefore required the use of the same cognitive test battery. However, previous studies have argued that using basic cognitive tests such as the Choice Reaction Time task and the Visual Recognition task are not sensitive enough to evaluate changes in higher cognitive abilities that may be affected by exercise (Dietrich & Sparling, 2004). Earlier reviews have concluded that demanding tasks with delayed memory performance are the most sensitive to macronutrient interventions (Hoyland, Lawton, & Dye, 2008; Macready et al., 2009). Macready et al. (2009) describes frontal functioning (Reversal Task), inhibition (Stroop Task), planning (Stockings of Cambridge Task), and sustained attention tasks (Paced Auditory Serial Addition Test) to be the most sensitive to flavonoid interventions. In addition to using these types of cognitive tasks, this limitation could further be overcome in the future by developing a better understanding of the mechanisms that are influenced by phytochemicals, as well as how physical fatigue influences cognitive performance.

An additional limitation of the current study may be practice effects on the cognitive tasks. The participants were required to complete the cognitive tests numerous times across the main trials and at the familiarisation trial. It is possible that the tests became predictive in some aspects. It was anticipated that practice effects may become apparent and therefore, the cognitive tests that did not generate test information randomly, such as the Stroop and the Trail Making task, had alternating tests. However, due to the nature of the current study, the participants were required to repeat each cognitive test more than five times. For almost all the cognitive tests

used, performance increased across 6 weeks in both the blackcurrant and placebo groups. This increase in performance was most likely due to practice.

The time of day that the participants completed the main trials may have also been a limitation of the current study. Research has shown that time spent awake and homeostatic sleep pressure can influence cognitive functioning and neurobehaviour, which can vary across the day (Schmidt, Collette, Cajochen, & Peigneux, 2007). Due to the busy schedules of participants, and the demanding protocol of the study, the participants were assessed for the main trail at any time of the day they could afford. Although many participants in the current study were able to meet at the same time at week 1 and at week 6 it is still possible that this affected the results. Future studies should consider that time of testing may influence the results and forewarn potential participants of strict time requirements. The different times of the day that the cognitive tests were administered may have affected the present results.

Summary and Conclusions

The present study investigated the ameliorating effects of phytochemicals on cognitive performance in physically fatigued individuals. The cognitive tasks used to assess performance were Choice Reaction Time, Serial Sevens, Backwards Digit Span, Stroop, and Trail Making. Past research has found that phytochemicals have the ability to improve cognitive performance. The present study followed from a previous study conducted by Harold (2016) who investigated the effect of a single dose of blackcurrant supplement on cognitive performance in physically fatigued participants. The present study built on this research to determine if feeding blackcurrant over a period of 6 weeks would improve cognitive performance after physically induced

fatigue. However, similar to Harold, the current results found that blackcurrant did not ameliorate fatigue or improve cognitive performance after a single dose of blackcurrant or across 6 weeks of daily supplementation. Overall, there was little or no evidence for any beneficial effects of the blackcurrant supplement, either when taken in the short term or the long term. The limitations of the present study include the use of a small sample, difficulty determining the effect of physical fatigue on cognition, the sensitivity of the cognitive tests, the first spoken language of some of the participants, and adherence to the study protocol. In conclusion, the presence of ameliorating effects of blackcurrant on cognitive performance has not been confirmed by this study. These findings are consistent with the current literature that has found mixed results in regard to the effects of phytochemicals on cognitive performance. Further research should focus on the possible interactions between blackcurrant and the type of cognitive task, and the size and duration of the phytochemical supplement. Additional research is also needed to understand the relationship between physical fatigue and cognition and the biological mechanisms influenced by phytochemical supplementation.

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Appendices

Appendix A

Recruitment Advertisement

VOLUNTEERS WANTED

Plant and Food Research are conducting a study to evaluate the effect of fruit supplement on exercise and cognitive performance

This study is across a 6 week period

Participants will be required to take part in:

- Computerised “brain game” tasks
- Short (30 sec) cycle tests
- Consumption of a fruit supplement for 6 weeks



Participants will be reimbursed for their time

**For more information please contact Olivia McKenzie at Massey University on 02102891770 or Dominic Lomiwes at Plant and Food Research on 06 353 6224 or txt/call 021 0357844
dominic.lomiwes@plantandfood.co.nz**

Appendix B

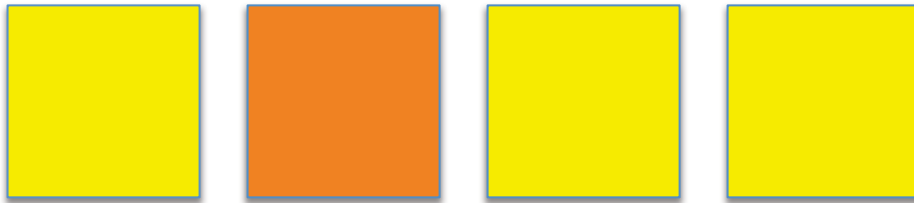
Examples of cognitive tasks: Choice Reaction Time, Trail Making B, Serial Sevens, the Stroop, and the Backwards Digit Span

Choice Reaction Time

'X' indicated that participants are required to wait for the stimulus.

X

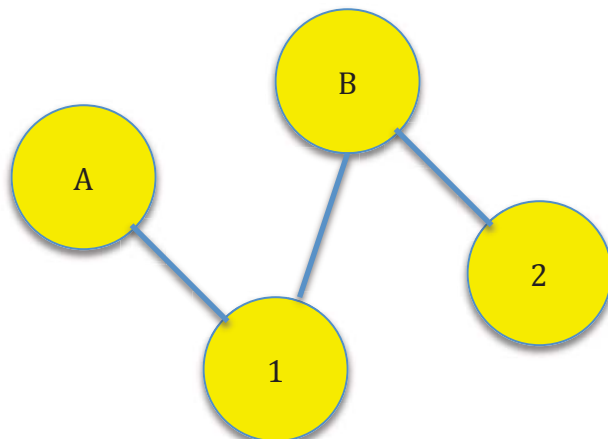
Example of the four boxes that appeared on the screen. The participants were required to respond to the orange box by pressing the corresponding key.



First box = 'Z' key, second box = 'X' key, third box = 'N' key, fourth box = 'M' key.

Trail Making B

Example: A - 1, 1 - B, B - 2...



Serial Sevens

From 100: 100, 93, 86, 79, 72, 65, 58, 51, 44, 37, 30, 23, 16, 9, 2

From 104: 104, 97, 90, 83, 76, 69, 62, 55, 48, 41, 34, 27, 20, 13, 6

The Stroop

Congruent example: Green, Blue, Red.

Incongruent example: Green, Blue, Red.

The participants are required to respond to the colour of the ink the word is presented in and to ignore the text.

Backwards Digit Span

Participants were required to recall the digits presented to them in backwards order. For example:

Presented	Recalled
1. 6, 3, 2	1. 2, 3, 6
2. 7, 2, 5, 9	2. 9, 5, 2, 7
3. 8, 1, 0, 6, 2	3. 2, 6, 0, 1, 8
4. 1, 5, 6, 2, 2, 7	4. 7, 2, 2, 6, 5, 1

Appendix C

Rating of Perceived Exertion Scale

Rating of perceived exertion (RPE)

Subject # _____

Date _____

Time _____

15 point scale

6 -	20% effort
7 -	30% effort - Very, very light (Rest)
8 -	40% effort
9 -	50% effort - Very light - gentle walking
10 -	55% effort
11 -	60% effort - Fairly light
12 -	65% effort
13 -	70% effort - Somewhat hard - steady pace
14 -	75% effort
15 -	80% effort - Hard
16 -	85% effort
17 -	90% effort - Very hard
18 -	95% effort
19 -	100% effort - Very, very hard
20 -	Exhaustion

I certify that these data are complete and correct

Researcher's name: _____ **Date:** _____

Researcher's signature: _____

Appendix D

Baecke Questionnaire

The Questionnaire for Measurement of a Person's Habitual Physical Activity

Overview:

*Baeke *et al.* (1982) developed a questionnaire for evaluating a person's physical activity and separating it into three distinct dimensions. The authors were from the Netherlands.

*Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 1982; 36:936-42.

Indices for physical activity:

- (1) work activity
- (2) sports activity
- (3) leisure activity

Work Index

Question	Response	Points
What is your main occupation?	Low activity	1
	Moderate activity	3
	High activity	5
At work I sit	Never	1
	Seldom	2
	Sometimes	3
	Often	4
	Always	5
At work I stand	Never	1
	Seldom	2
	Sometimes	3
	Often	4

	Always	5
At work I walk	Never	1
	Seldom	2
	Sometimes	3
	Often	4
	Always	5

Question	Response	Points
At work I lift heavy loads	Never	1
	Seldom	2
	Sometimes	3
	Often	4
	Always	5
After work I am tired	Very often	5
	Often	4
	Sometimes	3
	Seldom	2
	Never	1
At work I sweat	Very often	5
	Often	4
	Sometimes	3
	Seldom	2
	Never	1
In comparison with others of my own age I think my work is physically	Much heavier	5
	Heavier	4
	As heavy	3
	Lighter	2
	Much lighter	1

Where: • The work activity is according to the Netherlands Nutrition Council with (1) low activity including clerical work, driving, shopkeeping, teaching, studying, housework, medical practice and occupations requiring a university education; (2) middle activity including factory work, plumbing, carpentry and farming; (3) high activity includes dock work, construction work and professional sport.

Work index = $((6 - (\text{points for sitting})) + \text{SUM}(\text{points for the other 7 parameters})) / 8$

Sport Index

Question	Response	Points
Do you play sports?	Yes then calculate sport score	(see below)
	• sport score ≥ 12	5
	• sport score 8 to <12	4
	• sport score 4 to <8	3
	• sport score 0.01 to <4	2
	• sport score = 0	1
	No	1
In comparison with others of my own age I think my physical activity during leisure time is	Much more	5
	More	4
	The same	3
	Less	2
	Much less	1
During leisure time I sweat	Very often	5
	Often	4
	Sometimes	3
	Seldom	2
	Never	1
During leisure time I play sport	Never	1
	Seldom	2
	Sometimes	3
	Often	4
	Very often	5

Data on Most Frequently Played Sport	Finding	Value
What sport do you play most frequently	Low intensity	0.76
	Medium intensity	1.26
	High intensity	1.76
How many hours do you play a week?	< 1 hour	0.5
	1-2 hours	1.5
	3-4 hours	3.5
	> 4 hours	4.5
How many months do you play in a year?	< 1 month	0.04
	1-3 months	0.17
	4-6 months	0.42
	7-9 months	0.67
	> 9 months	0.92

Where: • The sport intensity is divided into 3 levels: (1) low level (billiards, sailing, bowling, golf etc) with an average energy expenditure of 0.76 MK/h; (2) middle level (badminton, cycling, dancing, swimming, tennis) with an average energy expenditure of 1.26 MJ/h; (3) high level (boxing, basketball, football, rugby, rowing) with an average energy expenditure of 1.76 MJ/h.

Data on Second Most Frequently Played Sport	Finding	Value
What sport do you play most frequently	Low intensity	0.76
	Medium intensity	1.26
	High intensity	1.76
How many hours do you play a week?	< 1 hour	0.5
	1-2 hours	1.5
	3-4 hours	3.5
	> 4 hours	4.5
How many months do you play in a year?	< 1 month	0.04
	1-3 months	0.17
	4-6 months	0.42
	7-9 months	0.67
	> 9 months	0.92

Simple sports score = ((value for intensity of most frequent sport) * (value for weekly time of most frequent sport) * (value for yearly proportion of most frequent sport)) * ((value for intensity of second sport) * (value for weekly time of second sport) * (value for yearly proportion of second sport))

Sport index = (SUM(points for all 4 parameters)) / 4

Appendix E

Dietary Controls

List of foods to avoid before the main trial

The trial coordinator will ask you to avoid eating these foods two days before during the main part of the trial.

Fruit and Fruit Juice: Blackcurrants, Blueberries, Kiwifruit, Raspberry, Strawberry, Apples, (green and red), Plums, Blackberries, Cherries, Cranberries, Citrus fruit (Oranges, Grapefruit, Lemons etc) Grapes, (black and red), and Figs.

Vegetables and Vegetable Juice: Aubergine, Beans (red and kidney), Potato/Sweet Potato (red and purple), Onion (red), Cabbage (red), Broccoli (purple), Beetroot, Corn (purple), Olives (black), and Avocado.

Miscellaneous: Wine (red), Coffee, Chocolate (dark), and Tea (black and green).

Dietary Supplements: (e.g. all supplements containing berries, 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 the trial coordinator.

Appendix F

Participant Information Sheet

Note: The long and detailed information sheet was a requirement of the principal Investigator of the overall research programme.

Information Sheet (*Version2, 17/07/2015*)

Title of Project: Effects of daily consumption of blackcurrants on cognitive performance after physical fatigue.

Locality: Plant & Food Research,
Food Industry Science Centre,
Fitzherbert Science Centre,
Batchelar Road
Palmerston North 4474

Phone: 06 953 7700

Principal Investigator	Dr Suzanne Hurst
Trial co-ordinator	Dr Dominic Lomiwes
Co-investigators	Assoc. Prof. John Podd, Olivia McKenzie (MA student), Prof. Roger Hurst

Introduction

You are invited to take part in a health research study that looks at how daily consumption of a blackcurrant extract for 6 weeks influences the effect of strenuous exercise on mental processing.

This participation information sheet will help you decide if you would like to take part. It tells you why we are doing the study, what you need to do, and what the benefits and possible risks to you might be.

We will go through the form (there are 9 pages) with you and answer any questions you may have; this will take about 10-15 mins. You may also like to take away the form to talk through the study with family, whānau, friends, or your general medical practitioner.

Please make sure you have read all the pages. If you require an interpreter, this can be arranged.

You have a period of 2 weeks to make a decision about whether you wish to take part (participate).

Taking part in this study is entirely your choice. If you agree to take part, you may withdraw from the study at any stage, without giving a reason. If you agree to take part in this study, you will be asked to sign the consent form at the back of this document. You will also be given a copy of both the participant information sheet and the consent form to keep. Free parking is available at the facility.

Background and purpose of this research

Moderate exercise, such as a 30 min walk, has been shown to maintain mental health (i.e. cognitive performance). However, strenuous exercise that causes physical fatigue appears to have the opposite effect and actually impairs cognitive ability. Daily consumption of fruit, especially berryfruit, has been shown to help recover from strenuous or unaccustomed exercise. Since eating blackcurrants supports mental health, it is feasible that eating blackcurrants or a blackcurrant product regularly or prior to strenuous exercise will help maintain cognitive performance. In this study, we look at the relationship between exercise-induced physical fatigue and the daily consumption of a blackcurrant supplement for 6 weeks on the ability to carry out mental tasks.

How will participants be recruited?

As a participant (individuals who agree to take part), you will be recruited through hearing about it at seminars located at Plant & Food Research or Massey University or from flyers posted at specific sites throughout Palmerston North: local gyms, AgResearch and Fonterra.

As an interested individual, trial investigators (Olivia McKenzie and/or Dominic Lomiwes) will contact you to make sure you meet study criteria.

If you are a healthy individual, (any gender) aged between 16 and 50 years old, you may take part in the study.

You will not be considered for this study if you have blood-borne diseases (such as hepatitis), clinically diagnosed high/low blood pressure, are pregnant or planning to get pregnant, have had a recent bacterial or viral illness, or take medication for blood disorders, e.g. anti-clotting medication.

You will not be able to take part if you have a health conditions that affect your ability to perform the exercise task required for this study (this includes problems with your heart or breathing during exercise, joint or back pain, a hernia or injury/recovery from injury).

Furthermore, since this study involves doing computer “brain games” you will not be able to take part if you have any of the following health concerns: colour blindness, migraines or epilepsy brought on by rapid moving computer images.

In addition, if you have intolerance, sensitivity or allergy to berryfruits or berry fruit products you will be unable to take part in this study.

You will be asked to complete a health questionnaire. The completed questionnaire is assessed for your suitability to take part in this study. Only the principal investigators of this study will see your health questionnaire and will be either returned to you (upon your request) or destroyed at the end of the study.

You may also like to seek advice from your general medical practitioner or primary care individual if you have any concerns about taking part in this study; however, this will be at your own expense.

What happens in this research?

This study will look at the relationship between exercise-induced physical fatigue and consumption of a blackcurrant extract on cognitive health. You will initially be asked to attend a familiarization session, where you will have your fitness assessed, do a series of bicycle exercises that determines how you experience physical fatigue and be shown how to complete a computer “brain game” task. This should take about 45 mins. During this session you will be asked to wear a heart monitor and donate a small amount of blood (ear prick) at specific times.

In the main trial, you will be asked to fill out a questionnaire that asks you about how you feel, followed by a computer “brain games” task and a cycle exercise. You will then be asked to take a gelatin capsule containing a fruit extract or a fruit sugar equivalent capsule and be asked to relax for about 1h, after which you may be asked to do a series of cycle exercises until you are fatigued (i.e. can’t do any more). Then you will be asked to fill in some more forms and questionnaires, repeat the computer “brain games” task and perform another cycle exercise. This main trial should take about 2½ hrs. In addition, you will be asked to donate a small amount of blood (from an ear prick) at specific times throughout the main trial.

Over the following 6 weeks you will be asked to eat an edible capsule (containing the blackcurrant extract or fruit sugar equivalent [placebo]) every day at the same time. Also, at a convenient time each week you will be asked to meet with the trial coordinator at Plant & Food Research who will ask you to complete an on-line survey and donate a small amount of blood (ear prick). At the end of the 6 weeks you will be asked to repeat the main trial involving the exercise-induced fatigue session you did in week 1.

Specific details of the study, and what is expected of you.

Familiarisation session (~ 45 mins).

On the morning of this familiarization session, you will be asked to come to Plant & Food Research in your gym wear. The trial investigators (Olivia McKenzie, Dominic Lomiwes) will meet you, answer any of your concerns about the trial, and take you to the exercise gym. You will then be guided through a series of computerised brain games and allowed time to familiarise yourself with this computer task.

The trial co-ordinators will then ask you to fill out forms that will assess whether you meet the study requirements (health and fitness forms), and then you will then be weighed. They will then show you how to use the Wattbike stationary bike and ask you to try it. If you feel comfortable, you will then be asked to do a short cycle. This will allow you and the trial co-ordinators to assess whether you are capable of taking part in this study. If you still would like to proceed, the trial coordinator (Dominic Lomiwes) will put a heart monitor around your chest and ask you to do a series of short cycle exercises, which will involve you cycling as fast as you can for 30 s followed by a 1 min rest. After each cycle exercise, you will be asked how you feel and whether you would like to continue. You will also be asked to donate a small amount of blood (taken from an ear prick) before and after each 30s cycle exercise (maximum of 4 times). At the end of this exercise session the heart monitor will be removed, and since you will probably be feeling very tired, you will be given something to drink and eat and allowed time to recover.

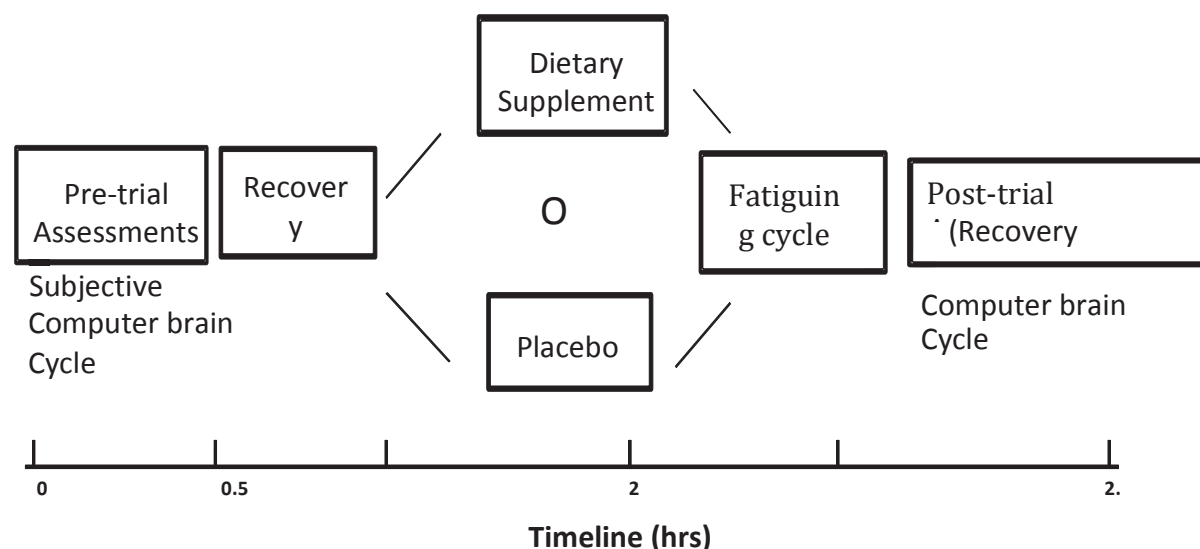
After you have recovered and before leaving, the trial investigators will ask you how you feel and answer any questions you may have. They will also provide you with a list of dietary supplements and foods to avoid eating two days before the start of the main trial and discuss any concerns you may have about that. You will also be advised to discuss with your health practitioner about not taking any dietary supplements you regularly take. In a couple of weeks the trial co-ordinator will inform you whether you meet the requirements of the study and ask whether you would like to take part.

Main trial (~ 2½ hours).

Pre-trial. The trial co-ordinator will be in contact with you two days before the start of the main trial to ask you if you would like to take part in the study and to stop eating certain foods on the list provided to you in the familiarization session. You will also be asked not to do any strenuous exercise. If you have lost the food list, the trial coordinators will provide another one for you, and deal with any concerns you might have. They will also arrange with you a time to do the main trial and ask you to eat breakfast on the day of the trial at least 1h before.

Day of the trial. You will be asked to come to Plant & Food Research in your gym wear and the trial coordinators will meet you, take you to the exercise gym and ask if you would like to carry on with the trial and ask whether you have had breakfast and at what time. They will also ask you if you have eaten any foods or dietary supplements on the list provided, or taken part in any strenuous exercise. If you are happy to do the trial, then the trial coordinators and co-investigators will guide you through a series of tasks.

The diagram below shows what you will be expected to do and the time it will take at each stage of the trial.



The trial investigators (Dominic Lomiwes & Olivia McKenzie) will be present throughout the whole trial and will answer any concerns you may have. Before you start you will be fitted with a heart monitor, which you will be asked to wear throughout the duration of the trial. You will also be asked to donate a small amount of blood from ear pricks a maximum of 6 times throughout the course of the trial.

At the start of the trial you will then be asked by a co-investigator (Olivia McKenzie) to fill in some forms that ask how you are feeling. She will then ask you to complete a computer “brain games” task similar to the one you did in the familiarisation session. After this, the trial coordinator (Dominic Lomiwes) will ask you to donate a blood sample (ear prick) and then ask you to perform a 30 s “all out” fatiguing cycle test on the Wattbike (similar to the one you did in the familiarization session) as fast as you can. You will be coached by the trial co-ordinator to complete this test. Immediately after this you will be asked to donate another blood sample (ear prick) and then asked to relax for about 30 mins. During this period you will be taken to a separate area where you can watch a video or just relax (if you are a Plant & Food Research employee, you may return to work but must not do any strenuous exercise). During this rest period you will only be allowed to drink water.

After this time, you will be asked to donate another blood sample (ear prick) and then given a small gelatine capsule to take with water. You will then be asked to rest for a further 1 hr; during this period you will be able to leave but be advised not to do any exercise and only drink water. However, if you feel you need something to eat, the trial coordinators will provide you with food.

After 1 h you will be asked to donate another blood sample (ear prick) and asked to go to the gym where the trial coordinators will ask you to do a series of 30 s cycles with 1 min rest intervals. The trial co-ordinators will coach you through this and you will be asked how you feel after you finish each 30 s cycle. You will be asked to continue until you can no longer do it or the trial coordinators tell you to stop. Since this exercise is designed to cause physical fatigue, the trial coordinator will monitor you throughout the exercise and offer you a drink and/or glucose sweet at the end of the exercise.

After this (should only be about 10 mins) you will be asked to donate a blood sample again (ear prick) and fill in forms asking about how you feel. You will then be asked to immediately do a computer “brain games” task similar to the one you did at the beginning of the trial.

After 20 mins, you will be asked to repeat the 30 s “all out” fatiguing cycle test you did at the beginning of the trial. Upon completion, the heart monitor will be taken off and you will be offered something to drink and eat and asked to relax for about 10 mins, depending upon how you feel. Once you have recovered, you will be free to leave.

Daily supplements for 6 week. During this 6-week period you will be allowed to eat your normal diet but will be asked to eat an edible gelatine capsule at the same time every day. In addition, once a week you will meet with the trial co-ordinator (at Plant & Food Research) who will ask you (i) to complete an on-line survey about how you feel and (ii) donate a blood sample (ear prick). He will also give you enough capsules for the following week. At the end of week 6, the trial coordinator will arrange a time for you to come into Plant & Food Research in your gym wear to repeat the exercise trial you did in week 1. You will be

contacted 2 days before and advised not to eat certain foods or take part in strenuous exercise, and also not to take your capsule on the day of the repeat trial.

Reassurances.

On first reading, this participation information sheet may appear complicated and too much to remember. To reassure you, the trial investigators will promise to do the following:

Provide you with the necessary information for you to decide whether you would like to take part in this study.

Keep you informed about what is expected from you at each step of the study.

Monitor your wellbeing at each step of the study.

Coach you through each of the cycle exercise session

Provide drink and food and an area for you to relax when required.

What are the benefits of this study?

Personal benefit. This study will provide you with an insight into how exercise and appropriate nutrition influences your mental health and your ability to do mental tasks that are important for everyday activities such as recalling items on a list.

Scientific benefit. This study will advance the scientific knowledge underlying the relationship between physical fatigue and nutritional intervention on mental health. Findings from this study will fulfil the requirements for an MA thesis submission (Olivia McKenzie, Massey University) and be published in scientific journals, and presented at seminars at Massey University, Plant & Food Research, and national/international scientific conferences.

What are the discomforts and risks of this study?

Changes in Diet. You may feel unsure about what foods, drinks and supplements you are able to eat before doing this study. Moreover, you may feel uneasy about stopping taking dietary supplements or taking a fruit supplement. There is also small chance that you may experience digestive problems or a skin rash after taking the supplement.

Exercise requirements. You may feel uneasy about performing a series of cycle exercises designed to induce fatigue.

Blood sampling. You may feel uneasy about giving blood. There may be minor physical discomfort and you may experience some ear stinging after donating blood. You may also feel light-headed and dizzy after donating blood, especially after exercise to fatigue. This is not caused by blood loss from the amount of blood you will be asked to give in this study, but due to the possibility that you may feel queasy from the appearance of blood. You may also have cultural concerns about donating blood.

How will these discomforts and risks be alleviated?

Changes in Diet. As a participant, you will be reassured and advised throughout the entire study and closely monitored by the trial coordinator who will assist you on what foods will be OK to eat / drink during the trial period.

The blackcurrant supplements you will be asked to eat will be within a gelatine capsule. The amount of fruit we are asking to take within this trial lies within the recommended daily amount advised by New Zealand dieticians. It should have no adverse effect on your health. However, if you do experience digestive and/or skin problems relating to taking the blackcurrant supplement then you are asked to let the trial coordinator know and consult with your general medical practitioner (GP).

Consultation with your GP or other health practitioner will be at your own expense.

Exercise requirements. Before you agree to take part in this study you will be asked to fill out a form that assesses how often you exercise; as well, you will be shown how to use the Wattbike stationary bike. The trial coordinators will be present to coach you through the cycling exercises throughout the familiarisation session and the main trial. If at any time during these exercises you feel uncomfortable then you do not have to continue. First Aiders will be on hand in the unlikely case of injury.

Blood sampling. To ease any concerns you may have about giving blood, the trial coordinator, who holds a NZ MedLab certificate, and First Aiders will be the only persons taking your blood. The blood sample we are asking you to donate is small and only involves an ear prick. Nevertheless, if you feel light headed after having blood taken you will be asked to lie down with your feet slightly elevated, which will minimize the feeling of any light headedness/dizziness after blood giving. This only happens with a small percentage of the population. You will also be given something to eat and drink. If you have cultural concerns about giving blood, you should talk to your whānau support before taking part in this study. We can assure you that the blood donated in this study will not be given to anyone else and although it cannot be returned to you for health and safety reasons, we will destroy it by incineration.

What treatment is immediately available should an injury occur?

The Trial coordinator is a First Aider and will be on hand in the gym if you hurt yourself during the exercise sessions. There are also a number of trained First Aiders based at Plant & Food Research who will be on hand to provide immediate medical treatment, if required. In the unlikelyhood that you require medical attention, we will arrange transport to the nearest hospital, medical centre or health practitioner of your request.

What if something goes wrong?

If you were injured as a result of taking part in this study, which is unlikely, you will be eligible for compensation from ACC.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

What are the costs involved in this research?

There is no cost to you taking part in this study, except your time.

How will my privacy be protected?

No material, which could personally identify you, will be used in any reports or on the labelling of samples. All personal records, physiological data, biological samples and cognitive performance information will be kept in a secure area at Plant & Food Research or in the School of Psychology, Massey University, with access limited to

the principal investigator and the co-investigators named on the first page of this document.

The information gathered is confidential and will only be used for this project. Data will be stored for a maximum of 7 years and will be the responsibility of the principal investigator, after which it will be destroyed.

The blood collected in the study will not be passed on to anybody else and will be destroyed by incineration.

The health questionnaire will be assessed against the study's requirements and only the principal investigator will have access to your personal information. At the end of the trial, the health forms will be destroyed by incineration or, if requested, returned to you.

Will I receive feedback on the results of this research?

At the end of the study, you will be invited to a seminar at Plant & Food Research, Palmerston North in which the main findings of the study will be presented.

Unfortunately, since your blood will be used in bioassay analysis, we will be unable to provide you with specific information about your personal samples. However, the principal investigator will gladly give you an overview of the main findings of the study and answer any questions you may have about this research.

Please note that a significant delay may occur between data collection and the publication of any results, which is normal.

What support can I access to better understand this research?

You may have a friend, family or whānau support to help you understand the risks and/or benefits of this study and any other explanation you may require.

Since this study requires you to take part in exercise where you are asked to donate blood, we strongly advise that you discuss this with your whānau. Your blood will ONLY be used in this study and afterwards will be destroyed by incineration.

Blood or its products will not be stored and will not be passed on to any other research groups.

If requested, an interpreter will be provided.

What do I do if I have concerns about this research?

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact an independent Health and Disability Advocate at 0800 555 050 or at advocacy@hdc.org.nz.

For general information we advise participants read the ERMA document, which outlines the development of the ethics framework in New Zealand. This can be found on the ERMA Webpages. <http://www.ermanz.govt.nz/about/eap.html>

For Maori issues we advise participants consult the ERMA Webpages.

<http://www.ermanz.govt.nz/resources/publications/policy/no/consultmaorihuman.html>

Employees of Plant & Food Research can also contact the General Manager for Human Resources, Craig Jensen (09 815 4200 or cjensen@hortresearch.co.nz).

Who do I contact for further information?

Dr Suzanne Hurst (Principal Investigator)
Plant & Food Research Ltd.,
Palmerston North, Private Bag 11600
Palmerston North 4442
Tel. 06 355 6231
Email. Suzanne.Hurst@plantandfood.co.nz

Dr Dominic Lomiwes (Trial Coordinator)
Plant and Food Research Ltd.,
Palmerston North, Private Bag 11600
Palmerston North 4442
Tel. 06 953 6224
Email. Dominic.Lomiwes@plantandfood.co.nz

This study has been approved by Dr Jocelyn Eason (General Manager, Science) and the Health and Disability Ethics Committees (15/5TH/99).

Appendix G

Health Screening Form



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HEALTH SCREENING FORM (Version 1, 12/06/2015)

Personal details

Name: _____

Age: _____

Contact details _____ : _____

Emergency contact

Name: _____

Contact details _____ : _____

Family doctor

Name: _____

Contact details _____ : _____

Health history

Have you or anyone in your family ever experienced any of the following (tick for yes)?

- High blood pressure
- Low blood pressure
- Heart problems
- Stroke
- Breathing problems
- Cancer or tumours
- Asthma

- 🍏 Diabetes
- 🍏 Epilepsy
- 🍏 Arthritis
- 🍏 Kidney/bladder disorders
- 🍏 Stomach disorders
- 🍏 Hernia
- 🍏 Allergies/asthma
- 🍏 Blood disorder/diseases e.g. hepatitis
- 🍏 Chronic conditions e.g. lupus, arthritis
- 🍏 Other (please identify) _____

If yes to any of the above, please explain:

Are you currently pregnant (or planning)?

Are you currently taking any medication and/or dietary supplements (what/why)?

Do you any have food sensitivities/allergies (if yes, please give details)?

Do you smoke (how many/day)?

Do you drink (how much, frequency)?

Exercise history

Do you currently exercise or participate in any sport? _____

Details (frequency, duration, type, intensity):

Have you ever experienced any injuries or on-going pain (where, when, how bad)?

Working computers.

Do you suffer with headaches, migraines? If so when and how bad?

Do you suffer with any visual disturbances (i.e. flashing lights) when using working with computers? If so when and how bad?

Do have problems distinguishing between different colours (i.e. colour blindness)? If so which colours do you particularly have problems with?

Is there any other information, not discussed, that you feel is relevant?

I (print name) _____ have given true and complete information to the best of my knowledge.

Signature: _____ Date: _____

Researcher: _____ Date: _____

Appendix H

Consent Form

Consent Form (version 1, 12/06/2015)

Title of Project: Effects of daily consumption of blackcurrant on cognitive performance after physical fatigue

Principal Investigator: Dr Suzanne Hurst

English	I wish to have an interpreter.	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.	Ae	Kao
Cook Island	Ka inangaro au i tetahi tangata uri reo.	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au.	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu.	E	Nakai
Samoan	Ou te mana'o ia i ai se fa'amatala upu.	Io	Leai
Tokelaun	Ko au e fofou ki he tino ke fakaliliu te gagana Peletania ki na gagana o na motu o te Pahefika.	Io	Leai
Tongan	Oku ou fiema'u ha fakatonulea.	Io	Ikai

(circle one)

I have read and I understand the information sheet dated _____ for volunteers taking part in the study designed to assess the effects of blackcurrant consumption and physical fatigue on cognitive performance. Yes / No

I have had the opportunity to discuss this study and I am satisfied with the answers I have been given. Yes / No

I have had the opportunity to use family, whānau support or a friend to help me ask questions and understand the study. Yes / No

I have had the opportunity to discuss with my family, whānau about the use of my blood in this study and agree that it should only be used in this study and all samples be destroyed afterwards. Yes / No

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without giving a reason. Yes / No

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study. Yes / No

I understand that data will be stored until a maximum of 7 years and will be the responsibility of the principal investigator, after which it will be destroyed.	Yes / No
I understand that at the end of the study all blood samples will be destroyed, and health, fitness and subjective forms and questionnaires will either be destroyed or returned.	Yes / No
I understand that my health questionnaire will only be assessed against the inclusion and exclusion criteria of the study. Only the principal investigator will see or have access to this information. No other co-investigator will see your questionnaire.	Yes / No
I understand that the investigation will be stopped if it should appear harmful to me.	Yes / No
I understand the compensation provisions for this study.	Yes / No
I have had time to consider whether to take part.	Yes / No
I know who to contact if I have any side effects during the study.	Yes / No
I know who to contact if I have any questions about the study.	Yes / No
I would like the researcher to discuss the outcomes of the study with me.	Yes / No

I _____ (full name) hereby consent to take part in this study.

Date: _____

Signature: _____

Researchers contact details:

Dr Suzanne Hurst (Principal Investigator) Tel. 06 355 6231, Email. Suzanne.Hurst@plantandfood.co.nz

Dr Dominic Lomiwes (Trial coordinator) Tel. 06 355 6224, Email. Dominic.Lomiwes@plantandfood.co.nz

Olivia McKenzie (Co-investigator) Tel. 02102891770. Email. oliviamckenzie@hotmail.co.nz

Assoc.Prof. John Podd (Co-investigator) Tel. 06 356 9099, Email. J.V.Podd@massey.co.nz

Prof. Roger Hurst (Co-investigator) Tel. 06 953 7677 E mail. Roger.Hurst@plantandfood.co.nz

Project details explained by: (print name) --- _____

Project role: _____

Date: _____

Signature: _____