Fruit modulation of the effects of fatigue on cognitive performance

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Abstract

Cognitive fatigue hinders performance in social, academic and physical environments and has a profound effect on the ability of a person to function and make decisions. Research into reducing or eliminating cognitive fatigue and its effects have been largely inconclusive but an emerging area of research is focussing on phytochemicals effects on optimising cognition. The present study examined the effects of blackcurrant supplementation on cognitive fatigue and physical markers of performance. This required 11mg of freeze-dried blackcurrant powder and water mixture to be consumed by participants before completing a psychometric and exercise test. Blackcurrants are a high source of phytochemicals but are under-researched compared to other berry fruit, such as blueberries. Sixty participants completed two sessions which composed of a familiarisation session and an intervention session. Participants were randomly assigned to the blackcurrant supplementation or the control group. Each session consisted of six blocks of the Stroop test, a Standard VO_2max test and followed by a post-exercise Stroop test. The purpose of the Standard VO_2max test to exhaustion was to induce physical and cognitive fatigue. At the intervention session, participants ingested either a blackcurrant or sugar-controlled juice one hour before testing. Analyses demonstrated that the blackcurrant supplement had no effect on cognitive performance or physical markers. However, it was questionable as to whether the study had enough statically power to test for the small effect sizes due to participants being unable to complete testing and some data sets unable to be used. Future research should focus on larger sample sizes and high doses of anthocyanin to observe if blackcurrant can have cognitive and physiological effects.

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Chapter 1 – Introduction

Cognitive and physical health are both vital to overall functioning and wellbeing. Exercise, fatigue and diet can positively or negatively affect health. Understanding and influencing how these factors interact is a significant research area and its influence on short- and long-term health can apply to all stages of human development. Cognition can be defined as “the mental action or process of acquiring knowledge and understanding through thought, experience and the senses” (Stevenson, 2010, p. 337). Cognition can be broken down into components such as memory, attention, information processing, problem-solving and decision making (Posner & Snyder, 2004). Many other components make up overall cognition, and it is a complex network of interlinking pathways that construct the human experience. Within these are executive functions which are a higher order cognitive processing that underpins selective and sustained attention, working memory, planning, mental flexibility, resistance to interference and volitional inhibition (Chan, Shum, Toulopoulou, & Chen, 2008). These functions are essential for human survival and depend on the frontal lobes, temporal and parietal cortices. Executive functions are well-researched due to their role in normal development, dysfunction in psychopathology and decline in ageing (Pennington & Ozonoff, 1996).

Cognitive fatigue is a fundamental experience in human life. Although normal, the costs of cognitive fatigue in relation to the prevalence of errors and impairment in physical performance can have devastating effects. Cognitive fatigue results in a decrease in the availability of mental resources and can be observed in various attentional and executive cognitive functions (Boksem & Tops, 2008). For example, difficulty suppressing irrelevant stimuli during selective attention, decrease cognitive control and increased time needed for information processing. Therefore, it is important to investigate the nature of mental fatigue and the cognitive processes that underlie its behavioural manifestations. Cognitive fatigue impairs executive functioning which may explain typical errors and sub-optimal performance seen when people are feeling fatigued (Van der Linden, Frese, & Meijman, 2003). Several studies suggest that a deterioration in executive control and behavioural disorganisation occurs in information processing at different levels of mental fatigue (Bartlett, 1943; Lorist et al., 2000). This highlights a theme called the "control view" where performance on simple or learned tasks can be executed automatically when the person is fatigued however complex tasks which require deliberate mental and behavioural control are difficult to perform when fatigued (Hockey & Hamilton, 1983). Research into cognitive fatigue has focused mostly on Chronic Fatigue Syndrome (CFS) rather than an overview for everyday fatigue experienced by otherwise healthy people. Studies investigating neurotransmitters and brains structures that are involved in CFS have identified a clear correlation between the level of serum acetylcarnitine, which has a role in inhibitory and excitatory functions in the brain, and fatigue rating score (Kuratsune et al., 1998). This finding cannot be generalised outside of CFS patients however future research may be able to investigate links between neurotransmitter functioning and cognitive fatigue.

Cognitive fatigue can be induced through continuously performing heavy loads of cognitive processing or exercise. Certain types and intensities of exercise can produce cognitive fatigue and affect an individual in a variety of ways. Some feel disorientated while others may feel more energised with increased clarity of thought (Brisswalter, Collardeau, & René, 2002). Studies have found that any exercise regime can have positive effects on long-term cognitive functioning, even though the mechanisms for this improvement are unclear (Colcombe & Kramer, 2003). Physiological effects of exercise on cognitive function have been well-documented however experimental research often has contradictory findings (Tomporowski, 2003).

Research has also investigated the link between human health, physical and cognitive performance with functional foods. Diet and nutrition provide the body with adequate energy, macro- and micronutrients to survive with particular food groups improving function beyond what is needed to survive. Adenosine triphosphate (ATP) is the body's energy source and is formed through consumption macronutrients and micronutrients. ATP is vital in almost every physiological function, and ATP depletion can threaten cell homeostasis, integrity and neuronal depolarisation (Owen & Sunram-Lea, 2011). Neuronal depolarisation causes a failure in membrane ion-transport systems which increases the quantity of calcium and glutamate released, producing oxidants and degenerative enzymes which further neuron damage (Vergun, Keelan, Khodorov, & Duchen, 1999).

Glucose, a derivative of carbohydrate, is the brain's main fuel source (Mergenthaler, Lindauer, Dienel, & Meisel, 2013). Ingestion of glucose or carbohydrate-rich food can improve cognitive and physical functioning (Brisswalter et al., 2002; J. M. Carter, Jeukendrup, & Jones, 2004; Hargreaves, Costill, Fink, King, & Fielding, 1987). This is a dose-response relationship where the glycaemic index of the food changes cognitive ability. The higher glycaemic index, the sharper the increase in blood glucose and insulin levels which imposes metabolic stress on the brain and can decrease cognitive functioning (Nabb & Benton, 2006). Stable metabolic conditions favour higher cognitive functioning and therefore consuming low glycaemic index carbohydrates result in a slower release of glucose into the system which results in more stable concentrations of metabolites (Bourre, 2006). Glucose's effect appears to particular pronounced in the hippocampal region which may explain its' positive effect on declarative verbal memory and tasks that require a relatively high cognitive load (Owen & Sunram-Lea, 2011; Scholey, Harper, & Kennedy, 2001). Glucose enhances performance on a number of cognitive measure such as choice reaction time tasks, the Stroop test and rapid information processing tasks (Benton, Owens, & Parker, 1994).

There are many dietary sources of carbohydrate, with a major source being fruit. Several studies have demonstrated that protective effects seen in berry fruits including blackberries, strawberries, blueberries, bilberry and blackcurrant have been attributed to antioxidants which have a growing body of evidence to suggest that their consumption may cause changes in physiological and cognitive processes (Aqualiano, Baldelli, Rotilio & Ciriolo, 2008; Joseph, Shukitt-Hale & Willis, 2009; Miller & Shutkitt-Hale, 2012; Spenser, 2010). Flavonoids are a group of antioxidants that contain commonly consumed compounds such as anthocyanins, tannins, flavones and others. These polyphenolic compounds that can be found in fruits and vegetables have potent anti-inflammatory and antioxidant abilities that interact with the cellular and molecular structures of the brain, protecting and enhancing neuro-cognitive function (Joseph, et al, 2009; Spenser, Vauzour & Rendiero, 2009; Spenser, 2010). Their function stems from the ability of polyphenols to cross the blood-brain barrier and scavenge free radicals, inhibit lipid peroxidation, encourage angiogenesis and decrease cognitive decline (Heim, Tagliaferro & Bobilya, 2002).

Metabolites in food have been found to affect brain cell structure and integrity through interacting with neurotransmission, metabolism and energy supply (Schmitt, et al, 2005). Research is currently focussed towards demystifying the interaction between food constituents and cognitive performance. Nutritional interventions are used to better both physical and mental wellbeing. Berry fruit have had widespread positive effects in animal studies, and human interventions have also shown positive results to overall cognitive health and preventing cognitive decline (Lamport, Dye, Wightman & Lawton, 2014; Miller & Shukitt-Hale, 2012). They have been shown to affect the availability of precursors and essential cofactors for enzymes which are vital for the synthesis of neurotransmitters (Isaacs & Oates, 2008).

Neuro-cognitive health and degeneration are becoming essential topics due to an increased lifespan and ageing population. Berry fruit metabolites have a role in mediating signal pathways and increasing blood flow which encourages neurogenesis, neuroplasticity and decreases inflammation (Miller & Shukitt-Hale, 2012). Since oxidation and inflammation have been connected to neurodegeneration and ageing, it has been theorised that increasing the consumption of antioxidants will prevent or ameliorate this damage.

There is contradicting evidence on whether the protective effects of berry fruit on cognitive changes can be attributed to flavonoids or the food matrix from which it is derived. Improving levels of bioactive compounds and identifying the mechanisms in humans as to how these works and why they are useful are crucial components of understanding how berry fruits affect cognition and how we can maximise its effectiveness.

The current study uses blackcurrant supplementation, and this is due to their previously demonstrated role in the prevention of various diseases associated with oxidative stress and their probable role in enhancing physical and cognitive abilities. (Scalbert & Williamson, 2000).

Chapter 2 – Exercise and Cognition

Relationship between exercise and cognition

The relationship between exercise and cognitive performance across a lifespan has been an interest to research and health professionals alike. The pioneering study of Spirduso and Clifford (1978) found that active men had faster simple, choice and movement reaction time compared to the sedentary group. Simple reaction time uses only one stimulus and one response, choice reaction time uses multiple stimuli, and each stimuli requires a different response while movement reaction time is the time in which an action is performed. Spirduso and Clifford (1978) argue that chronic physical activity may play an important role in maintaining central nervous system functioning and a healthy cardiovascular system. Further studies observed that older adults who exercised regularly had greater brain preservation and reduced risk of contracting neurological disorders such as Alzheimer's Disease (Beckett, Ardern, & Rotondi, 2015; Van Praag, 2009). The type and intensity of exercise may influence cognitive performance either positively or negatively. Aerobic exercise involves pumping oxygenated blood from the heart to working muscles while anaerobic exercise does not involve oxygen and is typically much shorter in duration compared to aerobic exercise (Weil & Stöppler, 2015). Due to an ageing and increasingly sedentary population, the effect of exercise on executive functioning and physical health is important to understand to reduce the risk of harmful diseases and improve life quality and longevity.

Aerobic exercise has benefits for both physical and mental health. Regular aerobic exercise can decrease the risk of developing several types of cancer, osteoporosis and cardiovascular disease (Thompson et al., 2003; Warburton, Nicol, & Bredin, 2006). Meta-analytic reviews demonstrate that acute exercise benefits cognitive performance with positive effects seen on neural systems, encouraging neuronal growth which improves learning and memory (Chang, Labban, Gapin, & Etnier, 2012; Vaynman & Gomez-Pinilla, 2006). The physiological responses to exercise which have an impact on cognitive function include changes in levels of brain-derived neurotrophic factor, heart rate and plasma catecholamines (Chang et al., 2012). Aerobic fitness training has also been found to increase activation in the superior parietal cortex and the middle frontal gyrus and decrease activation of the anterior cingulate cortex compared to non-aerobic groups (Colcombe et al., 2004). Animal models have demonstrated that aerobic exercise is associated with increased angiogenesis and neurogenesis in the learning and memory regions of the brain (Van Praag, Shubert, Zhao, & Gage, 2005). In human studies, Pereira et al. (2007) demonstrated that cerebral blood volume (CBV) in the dentate gyrus of the hippocampus increased during three-month fitness training. These increases were associated with improvements in memory and verbal learning. An increase of CBV in the dentate gyrus has been previously shown to increase neurogenesis (Brown et al., 2003).

Research with older adults using exercise interventions has demonstrated vast improvement in nearly all areas of cognitive functioning. Aerobic training in older adults shows benefits in executive function (using the flanker task, Hedges g=.68) with improvements in spatial (g=0.43) and reaction time (g=.27) tasks (Colcombe & Kramer, 2003). These results are further supported by Hillman, Erickson, and Kramer (2008) showing a strong relationship between physical activity and faster reaction time. Another study by Colcombe et al. (2006) randomly assigned young and older adults to a cardiovascular training or a stretching program for six months, using magnetic resonance imaging and maximal oxygen uptake (VO_2max) as measures of improvement in physical fitness. Cardiovascular training resulted in no changes among young adults, however, in older adults, significant increases of anterior white matter volume and in grey matter volume in the inferior frontal gyrus, superior temporal gyrus and anterior cingulate were observed. These findings support a strong biological basis for aerobic fitness and its role in maintaining and enhancing central nervous system health and cognitive function in older adults. In most studies, the significant improvements seen in the elderly adults after short-term exercise are not apparent in their younger counterparts, possibly due to optimal functioning in young participants. However, some studies have shown that exercise training may improve aspects of effect and visuospatial, but not verbal, memory in young adults (Stroth, Hille, Spitzer, & Reinhardt, 2009).

A Swedish cohort study (*N*= 1,221,727) found a relationship between cardiovascular fitness and intelligence with cardiovascular fitness at age 18 predicting educational achievement later in life. In this study cross-twin cross-trait analyses showed that individual specific, non-shared environmental influences explained more than 80% of covariation while heritability explained less than 15% (Åberg et al., 2009). In a recent meta-analysis, Chang et al. (2012) found positive and small effect sizes during exercise, immediately following exercise and after a delay. They also investigated potential moderators such as exercise duration and intensity, type of cognitive performance assessed and participant fitness. These changes in activation were associated with marked improvement in selective-attention tasks. Many studies have also indicated that aerobic exercise can ameliorate depressive and anxious symptoms, enhance self-esteem, improve mood and sleep and increase resilience to stress (Fox, 1999).

Anaerobic exercise is commonly used to simulate exhaustion and has frequently been used investigate effects of intense, acute exercise on cognition. Researchers have been unable to detect a clear relationship between anaerobic exercise and processes involved in discrimination, sensory integration or perception. Hancock and McNaughton (1986) evaluated the effect of anaerobic threshold exercise on trained orienteer’s ability to evaluate and interpret topographical maps. Global interpretations of information on the maps decreased but short-term memory and time estimations improved. A review conducted by Tomporowski (2003) showed that exercise affects low and high-level cognitive processes differently. Some studies found that anaerobic exercise facilitated cognitive performance in tests such as Visual Search and Short-Term memory (Allard, 1989; Hancock & McNaughton, 1986) whereas others found that anaerobic activity impaired cognitive performance in areas such as symbol interpretation and coincidence timing (Hancock & McNaughton, 1986; Isaacs, 1991). Other studies that incorporated both aerobic and anaerobic components supported a U-shaped facilitation in choice reaction time and impairment at highest load in simple reaction time (Levitt & Gutin, 1971; McMorris & Keen, 1994).

Participant’s fitness level may also modulate executive functioning. Highly fit individuals may experience an increase in executive functioning with shorter reaction times being shown on Stroop interference tasks in moderate exercise conditions while unfit individuals experience longer reaction times from exercise (Guiney & Machado, 2013). To the contrary, Lambourne and Tomporowski (2010) completed a meta-analysis and found that exercise has a small negative effect on Stroop performance, regardless of fitness level. However, their meta-analysis included children and older adult participants which may account for the difference seen compared to healthy adults due to the differences in functioning levels and improvements that can be seen within these populations (Lambourne and Tomporowski (2010).

Underlying mechanisms between exercise and cognition

Two main hypotheses have been used to describe the effect that exercise has on cognition. The Yerkes and Dodson (1908) U-hypothesis proposes that exercise has an inverted U-effect on physiological performance. As arousal increases, performance on a task progressively improve until an optimum point, beyond which increased arousal leads to decrements in performance. McMorris and Graydon (2000) further applied this to the area of exercise and cognitive domains and predicted that cognitive performance improves and peaks as physiological arousal increases and then deteriorates in the same curvature as physical performance. The inverted U-hypothesis is supported by findings in a review completed by Lambourne and Tomporowski (2010) which indicated that exercise improved cognitive performance after exercise (d= 0.20) but impaired cognitive performance during exercise (d= -0.14). Moderator variables, such as intensity and duration of exercise, timing and administration of cognitive task and study design were also investigated, but their study cannot be generalised due to the design.

Cue Utilisation Theory developed by Easterbrook's (1959) predicts that as physical arousal increases, attention becomes narrowly focused and eliminates irrelevant environmental cues. At high levels of arousal, relevant cues may also be eliminated. An over-aroused person will have a reduction in the availability of important information while an under-aroused person will have an influx of irrelevant information. In exercise physiology, a moderate exercise intensity corresponds to an intensity below the lactate threshold (<70% VO_2max), whereas heavy exercise is above the lactate threshold. Both moderate and heavy intensities are associated with changes in the arousal of the central nervous system and increases in 'brain arousal' have been observed through an increase in beta activity and a decrease in alpha activity (Kubitz & Mott, 1996). The concept of adrenaline threshold, defined by a sudden increase in adrenaline concentration with exercise intensity, has been put forward as an indirect measure of arousal. This concept was proposed after Chmura, Krysztofiak, Ziemba, Nazar, and Kaciuba-Uścilko (1997) found a significant increase in blood adrenaline concentrations at the same time as cognitive performance improved. High levels of blood adrenaline are associated with changes in the CNS which may improve attention and decision-making performance (Brisswalter et al., 2002).

Contemporary neurophysiological studies have also identified several mechanisms that may connect arousal, exercise and cognition. One hypothesis is that exercise over 20 minutes in duration leads to an increase in cerebral blood flow and neurotransmitters such as endorphin and catecholamines, which result in an improved cognitive performance (Grego et al., 2004).

Further studies have investigated the role of event-related brain potentials (ERPs), specifically the P300 component, and have provided evidence for the relationship between exercise-induced arousal and cognitive improvement (Polich & Kok, 1995; Stroth et al., 2009). P300 is related to neural activity which underlies basic cognition. Physical training has a positive effect on the P300 component through positively affecting ERP by improving cerebral blood flow (Brisswalter et al., 2002). After moderate or heavy exercise, an increase in P300 amplitude and a decrease in P300 latency can be observed indicating an increase in attentional resource allocation, proportional to the amount of attentional resources employed in any given task (Hillman, Snook, & Jerome, 2003). Grego et al. (2004) supported this hypothesis by attributing P300 as the manifestation of the CNS processing new information.

A more recent proposal, the transient hypofrontality hypothesis, adds to the main hypotheses by suggesting that the complete motor patterns, autonomic regulation and sensory inputs during exercise need extensive neural activation (Dietrich, 2003). This results in a transient decrease of neural activity in brain structures that are not essential for performing the exercise, including decreased neural activity in the prefrontal cortex which is responsible for executive functioning (Dietrich, 2006). This hypothesis can account for the decreased cognitive performance seen with increasing exercise duration and intensity. Del Giorno, Hall, O'Leary, Bixby, and Miller (2010) conducted a study to test this hypothesis and how executive control can be altered as a function of exercise. This study demonstrated that changes in executive control tasks occurred immediately and following exercise, providing support for the transient hypofrontality theory. Specifically, the Wisconsin Card Sorting Tasks had a significant time and condition effect on errors made of F (3, 27) = 3.17, $p < .05$, partial $\eta^2 = 0.26$ indicating that post-exercise, participants had more difficulty performing this test due to possible decreased activity in the prefrontal cortex and subsequently decrease in executive functioning.

Research has shown that physical activity can have positive effects on brain structure and cognitive performance in humans. In animal research, brain-derived neurotrophic factor (BDNF) is a protein is found in the brain and periphery that contributes to neuronal transmission, modulation and plasticity (Cotman & Berchtold, 2002). BDNF can be found in the hippocampus, cortex and basal forebrain which are areas vital in memory, executive functioning and learning. This protein can also contribute to an increase in glutamatergic activity, an excitatory neurotransmitter while also preventing the signalling of the inhibitory neurotransmitter, GABA (Henneberger, Jüttner, Rothe, & Grantyn, 2002). In combination with its effects on the NMDA receptor, this increase in glutamate can influence the activity of receptors involved in processes of memory, neurogenesis, learning and environmental responses (Cotman & Berchtold, 2002).

During exercise, peripheral BDNF levels are significantly elevated, and the magnitude of its increase is dependent on exercise intensity (Huang, Larsen, Ried-Larsen, Møller, & Andersen, 2014). As BDNF can cross the blood-brain barrier, the intensity of the exercise can modify the effect seen on cognitive performance. Animal studies conducted by Vaynman and Gomez-Pinilla (2006) showed that animals exercising for a short duration improved their learning and recall compared to sedentary counterparts. Animals that were faster learners and had better recall also had higher expression of BDNF and CREB mRNA levels. In human trials, Ferris, Williams, and Shen (2007) found a significant difference in serum BDNF levels after exercise, relative to baseline levels in the higher-intensity exercise group. A review conducted by Huang et al. (2014) also demonstrated that serum BDNF concentrations did not change significantly in warm-up exercises but increased significantly during ramp test to exhaustion. The authors proposed that BDNF is stored in platelets and in response to exercise, leads to an increased response of the platelets that contribute to serum BDNF concentrations. Additionally, BDNF can be synthesised by contracting skeletal muscle cells, but not released into circulation (Matthews et al., 2009).

Adequate BDNF levels are essential for cognitive health. Altered levels can lead to mental stress and major depressive disorder. In rodent studies, physical activity upregulated the expression of BDNF for several days and increased proliferator-activated receptors (PGC-1α and FNDC5) that regulate BDNF expression (Berchtold, Chinn, Chou, Kesslak, & Cotman, 2005). These proteins can interact with neuroinflammatory and neuroplasticity pathways and improve cognitive performance and affect. Significant associations have been found between acute exercise-induced changes in BDNF and cognitive performance in memory tasks and non-significant improvements revealed in non-memory cognitive domains (Hillman et al., 2008).

Motivation plays a role in mediating cognitive and physical effort. Motivation can be influenced by factors such as personality type, self-efficacy, perceived competence or monetary compensation (Roberts, 2001). The ventral striatum represents a common motivational role between cognition and physical output. Activity in the ventral striatum can predict variations in behavioural performance that cannot be accounted for by task difficulty (Schmidt, Lebreton, Cléry-Melin, Daunizeau, & Pessiglione, 2012). The ventral striatum also modulates cognitive regions during mental effort and motor regions during physical activity. A neuroimaging study conducted by Schmidt et al. (2012) showed that the ventral striatum could boost behavioural performance through ventral striatum activity being correlated with activity in cognitive regions such as the caudate when placed under high cognitive demand. Under high physical demand, the motor regions, predominantly the putamen illustrated high activation. This is consistent with the central striatum having a generic motivation role. Ventral striatum activity was also significantly linked to monetary incentive and task performance; however this relationship was dependent on task effort and difficulty with the relationship becoming dissociable over time (Schmidt et al., 2012).

Heart rate variability has been studied as a vital marker of autonomic nervous system (ANS) modulation. The ANS compromises the sympathetic nervous system which is associated with the fight-or-flight response and the parasympathetic nervous system which is associated with digestive activity and rest (Lane et al., 2009). This interplay between systems requires adequate prefrontal cortex functioning, as neural mechanisms have an important role in mediating the response to exercise which often involves rapid changes in heart rate and blood pressure (Aubert, Seps, & Beckers, 2003). Hansen, Johnsen, and Thayer (2003) reported that participants with higher prefrontal cortex activity and higher heart rate variability performed better on executive tasks but did not differ in simple reaction time tasks compared to participants with low heart rate variability. Recent research by Aubert et al. (2003) shown that physical activity level can affect heart rate variability through increased vagal tone which may contribute to a lower resting heart rate, providing high rates of heart rate variability. Regular exercise is also shown to beneficially change the brain structures and subsequently, cognitive performance (Thomas, Dennis, Bandettini, & Johansen-Berg, 2012). Consequently, untrained people who have higher heart rates provoked by exercise of long duration can experience diminished cognitive performance especially on tasks that require high amounts of information processing. Isaacs (1991) found that cognitive performance was affected by exercise when heart rate was 150 to 175 bpm.

Heart rate variability is also associated with maximal oxygen consumption (VO_2 max) and physical fitness (Kaikkonen, Nummela, & Rusko, 2007). Oxygen use during exercise is a key factor in a person's ability to maintain exercise intensity and duration. Efficient muscular oxygen consumption increases the body's ability to use carbohydrates and fat as fuel, which can lead to an increase in exercise duration or physical effort (Weil & Stöppler, 2015). The average sedentary adult will have an oxygen consumption around 35ml/kg/min in a VO_2max test (Weil & Stöppler, 2015). This means that the person can consume 35 millilitres of oxygen for every kilogram of body weight per minute. Elite athletes can reach 90 ml/kg/min. VO_2max is influenced by muscle mitochondrial oxidative capacity, but in endurance exercise, oxygen delivery from the cardiovascular system exerts a greater effect on VO_2max (Bassett Jr & Howley, 2000). Cognitive performance appears to be significantly improved using incremental protocols from 40 to 60% VO_2max, most likely due to the activation of the CNS and increased levels of catecholamines (Brisswalter et al., 2002). The concept of how adrenalin release at different levels of exercise intensity can affect cognitive performance has been explored by

Chmura et al. (1997) who suggested that high adrenalin concentrations in the brain improve memory and information processing ability.

Cognition can also be affected by factors outside of physiological changes in response to fatigue or exercise. Excessive exercise can lead to signs of fatigue, which can be seen through increases in metabolic load associated with increased heat stress and the appearance of central (reduced neural drive) and peripheral (reduced muscle excitability) fatigue (Shei & Mickleborough, 2013). This can lead to dehydration which in turn may compromise both information processing and memory function (Lambourne & Tomporowski, 2010).

Physical activity may also act through vascular mechanisms to benefit cognitive functions. In rat studies, regular exercise has been associated with an increase vascular perfusion and the formation of new capillaries as a direct result of exercise (A. D. Brown et al., 2010). Angiogenesis is stimulated through exercise and as the brains vascularity can be altered throughout the lifespan, increasing the brains vascularisation can increase cognitive performance and reduce age-related cognitive impairment. Cerebral blood flow is also increased during exercise and can provide resources for the brain to use, such as glucose and oxygen, which allow for continued effort either during physical or cognitive tasks (Helton & Russell, 2011).

Although many hypotheses and individual physiological factors have been proposed, the actual mechanism behind the relationship between exercise, arousal, fatigue, neurotransmitters and cognition are still unknown and are certainly complex.

Exercise-induced fatigue and cognitive performance

Mental capacity during and after exercise varies between individuals. Some individuals find that exercise leads to an increased ability in mental tasks and clarity of thought while others may feel disorientated or unable to concentrate (Lambourne & Tomporowski, 2010). Cognitive performance can vary depending on task demands and energy allocation to these demands (Brisswalter et al., 2002). This reinforces the contradictory relationship between exercise and cognition currently in research. The current study assumes that exercise-induced fatigue induces cognitive impairment due to the use of a submaximal exercise test which has been previously demonstrated to affect physical and cognitive fatigue.

Fatigue can be either central or peripheral. Both types of fatigue can occur at rest or during vigorous exercise. Central fatigue results from decreased signalling in the central nervous system to neuromuscular junctions (Davis, Alderson, & Welsh, 2000). This is thought to occur due to a change in the synaptic concentrations of neurotransmitters, notably serotonin, noradrenaline and dopamine, which affects muscle functioning and exercise performance.

Peripheral fatigue is caused by impairments between neuromuscular transmission to the actin-myosin cross bridges. Peripheral factors of fatigue involve changes in peripheral organs such as muscle substrate depletion, metabolite accumulation, metabolic inhibition and excitation-contraction coupling failure (Gibson et al., 2003). Accumulation of intramuscular metabolites and depletion of muscle glycogen in high-intensity exercise may also be implicated in the development of muscle fatigue as well as reduced excitability of neuromuscular transmission (Evans & Lambert, 2007). These changes result in afferent feedback to the brain, inducing the sensation of fatigue. The gradual loss of body fluids and increased metabolic, cardiovascular and thermoregulatory strains can increase peripheral fatigue (Meeusen, Watson, Hasegawa, Roelands, & Piacentini, 2006). As evidenced, peripheral fatigue arises from events that occur independently of the central nervous system.

Exercise-induced fatigue has been researched due to its practical implications throughout sports and workforce operations. Identifying the effects of fatigue on cognitive performance could help to inform guidelines which promote the facilitation of cognitive functioning. Fatigue has various definitions depending on the field. In exercise physiology, fatigue is defined as an acute impairment due to metabolite accumulation and substrate depletion of exercise performance which leads to the inability to produce maximal force (Hagberg, 1981). Within neurophysiology, fatigue is described as a reduction in efferent motor commands to active muscles, resulting in a decline of force or tension as a part of a centrally controlled process (Gandevia, 2001). Gibson et al. (2003) introduces the concept that fatigue is a conscious sensation of changes in body functions such as increased breathlessness, heart rate, perspiration and reduced muscle power generation. These sensations then combine with cognitive functions such as motivation, the memory of the previous exercise, and decision-making components to determine a level of fatigue.

High levels of fatigue do not result in a sudden decrease in physical and cognitive performance. More frequently, attention and task engagement decreases progressively and leads to decrements in performance (Hillman et al., 2008). Physical fatigue is complex, and the perception of fatigue is predominantly experienced through muscle feedback channels. Prolonged effort to activate muscle fibres and muscle neurons increase an individual’s awareness of perceived effort and fatigue (Lorist, Kernell, Meijman, & Zijdewind, 2002). These submaximal contractions place increased demand on the central mechanism responsible for the fatiguing muscles and drive the awareness of fatigue and effort. Lorist et al. (2002) demonstrated that cognitive performance is severely affected by motor fatigue and that participants had slower reactions times and made more errors due to the high levels of submaximal exercise experienced. The increased amount of errors may be attributed to participant’s use of heuristics to lessen the investment of attentional resources on the cognitive tasks and use mental shortcuts that lessens effort. Due to the effect of exercise-induced fatigue on cognitive performance, experimental procedures often induce physical and cognitive fatigue through an incremental-load exercise to voluntary exhaustion or prolonged, physically demanding steady-state exercise protocols. It has been hypothesised that cognitive performance would be impaired during and immediately after exercise. A review conducted by Tomporowski (2003) concluded that acute exercise had selective facilitative effects on cognition often in areas such as information processing, problem-solving, inhibition and goal orientated actions.

Several methods exist to provoke exercise-induced fatigue. HIIT programmes are the most efficient way to achieve cognitive fatigue and both physical and cognitive fatigue extensively affect reaction time and attention (Brisswalter et al., 2002; Tomporowski, 2003).

Cognitive effort is known to increase with time during prolonged exercise. During submaximal exercise, the perception of effort is increased as mental fatigue increases. Marcora, Staiano, and Manning (2009) showed that exercise tolerance is limited through mental fatigue and perception of effort rather than through cardiorespiratory or musculoenergetic fatigue.

Incremental exercise is exercise that increases in intensity over time. Variables such as initial workload and workload increases, increments and duration can be modified to suit the purpose of research (Bentley, Newell, & Bishop, 2007). Two variables can be obtained through incremental exercise: the maximal rate of oxygen consumption (VO_2max) and ventilation threshold (VT). During incremental exercise, there is an increase in the body's ventilatory system, specifically the first VT which is the maximum for elderly and the second VT used for elite athletes (Richards, Lonac, Johnson, Schweder, & Bell, 2010). A VT is a transition from predominantly aerobic exercise to anaerobic exercise. In untrained populations, the overall duration for incremental exercise can be between 8-17 minutes for optimal determination of VO_2max (Bentley & McNaughton, 2003). Literature has demonstrated that VO_2max peak was similar in both short and long stage incremental tests.

Saanijoki et al. (2015) found that HIIT exercise-induced considerably more stress to participants compared to the endurance exercise, resulting in higher ratings of fatigue. Tomporowski and Hatfield (2005) also demonstrated this through participants cycling for two hours, causing physical fatigue, produced a faciliatory effect on the Stroop task. Studies using shorter, more high-intensity exercises found that complex cognitive skills were negatively affected (Hancock & McNaughton, 1986; Isaacs, 1991). This could signify that endurance exercise facilitates cognition due to less stress placed on the body and therefore more resources can be allocated to cognitive tasks.

Physical fatigue becomes more apparent during exercise through afferent feedback from the muscles, heart and lungs (Amann & Secher, 2010). However, this appears to occur independently from perceived exertion suggesting that the ACC, insular cortex, thalamus, dopamine and other endogenous opioids are more important in the person's perception of exertion and cost-benefit analysis of continuing the exercise, compared to physiological responses (Marcora, 2009).

Rating of perceived exertion (RPE) is frequently used during exercise as a measure of self-assessed fatigue. It is accepted that the RPE score given is a combination of cerebral activity and afferent signals which represent peripheral physiological changes (Gibson et al., 2003). The centrally regulated effort model shows that a linear increase in RPE scores during constant workload exercises indicate peripheral metabolic compound changes during exercise (Lambert, Gibson, & Noakes, 2005). Several peripheral and cardiopulmonary signals are integrated by the central nervous system and effect RPE during exercise. Heart rate and oxygen consumption (VO_2) are cardiopulmonary signals which can unconsciously influence the increase in RPE during exercise (Hampson, Gibson, Lambert, & Noakes, 2001). RPE can predict time to exhaustion due to the accumulation of these cardiopulmonary and metabolic systems fatiguing such as muscle glycogen depletion or breathlessness (Pires et al., 2011). However, as cognition plays a vital role in physical performance, this affects RPE scores and volitional exhaustion. The brain can regulate RPE and physical performance based on the awareness of metabolic reserves. Internal negotiation takes place throughout exercise where the person compares the current effort to previous experiences (Eston, 2012).

From this observation, muscle fatigue reveals four themes: task dependency, force-fatigability relationship, muscle wisdom and sense of effort that reflect a group of acute effects that impair motor performance (Enoka & Stuart, 1992). Task dependency illustrates that the demand of the task can alter the underlying mechanisms and site of muscle fatigue. Participant motivation and the intensity and duration of activity influence CNS fatigue mechanisms. The force-fatigability relationship suggests that a hyperbolic relationship exists between force elicited and endurance time. The greater the force exerted, the more rapidly the muscle fatigues. Muscle wisdom uses the CNS to optimise the force produced and allow economical activation of the fatiguing muscle, minimising fatigue. Perceived effort is associated with the participant's judgments of effort required to generate and maintain the force. These factors interact to allow the person to make a judgement on their power output and motivation to complete the physical task.

The human structure has developed several protective mechanisms to regulate exercise and damage to the body through interaction of central and peripheral fatigue. Inhibitory efferent processes control force output, muscle wisdom down-regulates power output and cardiac protection occurs during maximal incremental exercise (St Clair Gibson, Lambert, & Noakes, 2001). These changes have evolved to stop exercise activity and maintain muscle reserve to ensure that no body system is damaged beyond its capacity. This suggests that fatigue is a sensory process of a balance between neural regulation and peripheral metabolic changes. This coincides with earlier research by Kent-Braun (1999) who indicated that central factors contribute to approximately 20% of muscle fatigue with the remainder being intramuscular metabolic factors.

Perceived effort and physical effort

Actual and perceived effort have a variety of influences in physical, cognitive and self-restraint domains. Effort is experienced when making decisions, inhibiting responses and vigilantly attending to a task (Posner & Snyder, 2004). Mental effort is often accompanied by physiological responses such as an increase in cortisol, heart rate and corrugator superciili activity (Preston & Wegner, 2009). Cognitive effort is determined through presenting information about the difficulty of a task and the energy required for completion. Physical effort involves the excitation-contraction coupling of muscles that exert force (Enoka & Duchateau, 2008). Perceived effort is the conscious sensation of how strenuous a physical task is (Marcora, 2010).

Facial electromyography (fEMG) can be used to assess cognitive and physical effort through monitoring facial muscle activity (Larsen, Berntson, Poehlmann, Ito, & Cacioppo, 2008). It is a reliable measure of emotional valence and is sensitive to weak muscular responses that have previously been unobservable. Facial displays of emotion are dynamic and when related to exercise, facial activity in the corrugator superciili can correlate with effort (de Morree & Marcora, 2010). The activation of corrugator muscle is thought to link to the immobilisation of mental resources and task engagement (Silvestrini & Gendolla, 2009). The corrugator superciili muscle is located above the eyebrow and is partially controlled by the anterior midcingulate cortex which is sensitive to pain, negative affect and exertion of effort (Boxtel & Jessurun, 1993; Cacioppo, Petty, Losch, & Kim, 1986).

Traditionally, the effort of physical tasks has been measured by Rating of Perceived Exertion scales (RPE) introduced by Gunnar Borg (1971). This scale ranged from 6-20 and relies on the participant self-rating the level of effort they are exerting. The scale illustrates effort increasing relative to workload (Borg, 1998). This is supported by research showing that perception of effort grows exponentially to force exerted (Hampson et al., 2001). This perception of effort can be influenced based on the major muscle group involved in the task. However, self-rating may not be representative of the participant's true ability as there appears to be a perceptual barrier where maximal effort does not relate to the true range of the participant's maximal performance ability (Banister, 1979). RPE scores also can be altered by gender of the researcher or the presence of co-participants, highlighting the need for an objective measure of effort (Boutcher, Fleischer-Curtian, & Gines, 1988).

Other physiological measures can be used alongside RPE scales to assess effort. Heart rate and fEMG activity are positively correlated with RPE and increased power output during incremental workload cycling (Huang, Chou, Chen, & Chiou, 2014). Facial EMG measures can complement RPE scales as fEMG accurately reflects the perception of effort in a dynamic mode, offering more insight into exact moments of effort during exercise or cognitive tasks (Blanchfield, Hardy, & Marcora, 2014; de Morree & Marcora, 2012). Facial activity has been shown to positively correlate with RPE and muscle EMG in physical tasks while a similar effect was shown in cognitive tasks (de Morree & Marcora, 2010). Activity of the corrugator superciili is sensitive to task difficulty and capacity to complete the task.

A combination of RPE and fEMG can be vital to understanding effort. Social psychological theory shows that RPE is influenced by external cues such as the perceived duration of the task and the participant's perception of self. Although Borg (1971) found a correlation between heart rate, a physiological marker of physical effort, and RPE, a self-rated marker of perceived effort, he acknowledged that motivation could affect this relationship. Physiological variables account for 60% of the variance in RPE, leaving 40% to be mediated through psychosocial variables (Morgan, 1973). Past experience, distraction strategies, personality type, emotional status and motivational cues can all influence perception of exertion (Buckworth, Dishman, & Tomporowski, 2013). Being able to assess effort is a vital skill as information regarding task difficulty can give another person an estimate of the individual's ability to complete a physical task. This proposition is supported indirectly through studies demonstrating that physical work capacity can be estimated through RPE scores at submaximal workloads (Coquart et al., 2009). The "face of effort" may have enabled our ancestors to understand if other hunter-gathers were coping with physical activities (de Morree & Marcora, 2010). Currently, facial expression can provide a source of non-verbal communication for athletes and other individuals. For example, athletes in team sports may use facial expressions to assess effort and improve cooperation or adapt their game strategy.

Facial displays of emotion have a social communication role. Facial EMG provides information about a person's emotional experience more accurately than self-report methods (Lajante, Droulers, & Amarantini, 2017). High levels of activation of the corrugator superciili muscle communicates the level of effort to others (Huang et al., 2014). The corrugator superciili muscle is correlated with negative emotions and appears to have a punitive role in effort exertion where mental effort itself can be aversive (Kool, McGuire, Rosen, & Botvinick, 2010). Studies have found that corrugator superciili activity is related to self-reported effort during mental and physical tasks (de Morree & Marcora, 2010; Waterink & Van Boxtel, 1994). Using fEMG can be beneficial in complementing RPE methods of measuring effort as it eliminates social desirability concerns and other social factors in self-rated scales, as mentioned above (Hutchinson & Tenenbaum, 2007).

Several theories exist that emphasise the importance on CNS information processing and the perception of effort. Conflict monitoring theory stipulates that monitoring occurs moment-to-moment to determine potential conflicts so that inhibitory responses can be implemented and to provide a more effective response (Botvinick, Braver, Barch, Carter, & Cohen, 2001). This process occurs in the ACC. For example, reading is an overlearned response in literate adults, and when presented with a word 'red' in blue ink, the initial urge to read the word will conflict with the Stroop test goal of naming the colour of the ink. When this conflict is detected, a secondary regulatory system, the dorsal anterior cingulate cortex and dorsolateral prefrontal cortex, biases behaviour towards goal-relevant responses and inhibits incompatible responses (Botvinick et al., 2001). However, the literature shows that accuracy is not usually increased after post-error slowing (Notebaert et al., 2009). An extension of this theory is that the ACC also encodes information regarding effort and conflict may serve as a guide for the mental effort required (Walton, Bannerman, Alterescu, & Rushworth, 2003). Consistent with this, the ACC becomes active during cognitively difficult tasks, and the induction of conflict can be considered as a feature of difficult tasks (Paus, Koski, Caramanos, & Westbury, 1998). This evidence may suggest that ACC monitors the conflict and mental effort demanded and enter into a cost-benefit analysis to determine the allocation of mental resources.

The psychobiological model of endurance performance postulates that the central motor command sends an efferent copy of fatigue from the motor to the sensory areas of the brain, creating a sense of effort (Poulet & Hedwig, 2007). The increased central motor command increases muscle activation and muscle spindle firing to lift a heavier or the same weight with weaker muscles, compensating for muscle fatigue. This sensory input from the muscles feeds back to the sensory areas of the brain and generates an increase in perceived effort (Noakes, 2000). The more intense the exercise, the greater frequency of corollary discharges. This sensory information combined with motivation, the perception of effort and knowledge of the exercise protocol influences exercise engagement (de Morree & Marcora, 2012). It is important to highlight the neurocognitive link between endurance performance and perception of effort. Pageaux, Lepers, Dietz, and Marcora (2014) found a strong link between perception of effort and inhibition of a response. These authors completed a study using congruent and incongruent Stroop tasks followed by time trial running. The incongruent group reported higher levels of perceived effort during the exercise component. The ACC is known to be associated with response inhibition, the perception of effort and decision making. Therefore, the response inhibition task may have required more mental effort which transferred over to perceived effort of the physical task.

Motor overflow may also explain the higher facial muscle activity accompanied with increased effort. Motor overflow is an involuntary movement that occurs with voluntary movement (Hoy, Fitzgerald, Bradshaw, Armatas, & Georgiou-Karistianis, 2004). The primary motor cortex contains functional areas of the face, arms and legs that control muscle force. As these areas are overlapping and interconnected, intense effort produces a motor overflow that activates not only the active muscles but irrelevant muscles such as the facial muscle (Hoy et al., 2004). Motor overflow is mostly seen in tasks that require considerable effort and is often an automatic process. Dai, Liu, Sahgal, Brown, and Yue (2001) conducted a brain imaging study that found a significant correlation between the activation of the motor areas of the brain and EMG of active muscles during forceful muscle contractions. de Morree and Marcora (2010) subsequently made the connection between EMG activity in active muscles and fEMG which resulted in a significant correlation and suggests that frowning is caused by muscle overflow and is an indirect measure of motor-related activity in the brain. In addition, a positive correlation found between active muscle EMG and RPE scores supports the hypothesis that perception of effort in physical activity is proportion to motor commands in the CNS (Marcora et al., 2009).

Methodological differences in previous research

Methodological differences in exercise and cognition research can account for the many differences in findings. Studies often had varied methods for assessing the physical fitness of participants; the intensity and duration of the exercise administered; the nature of the psychological task and the time when the psychological task was administered which makes it difficult to compare literature and arrive at a well-supported conclusion regarding cognition and exercise (Grego et al., 2004).

Methodological protocols across studies are varied. Some studies have conducted a dual task design where cognitive tasks and exercise are tested simultaneously (Brisswalter et al., 2002; Brisswalter, Durand, Delignieres, & Legros, 1995). Literature reviews of dual-task designs have shown that cognitive performance is influenced by the timing of the cognitive measurement or complexity of the exercise administered and not by the exercise intensity (Brisswalter et al., 2002; Tomporowski, 2003). Other studies have administered cognitive tests following an exercise task. Previous research demonstrated that these produce significant effects but are moderated by exercise intensity (Ciani, Guidi, Bartesaghi, & Contestabile, 2002; Hogervorst, Riedel, Jeukendrup, & Jolles, 1996).

Studies investigating the relationship between BDNF, exercise and cognition also have contradictory findings that might be attributed to methodological differences. Methodological differences such as the assessment used for types of memory (e.g. visual, spatial, long-term) was often varied; different measures of BDNF and its isoforms; and no statistical analysis that has looked at the mediating role of BDNF in the exercise and cognitive performance relationship which may be used to make causal inferences (Hillman et al., 2008).

Psychometric tests have also varied from simple tasks that involve speed, such as reaction time tests, to complex tasks that involve engagement of multiple domains and executive functioning, such as the Stroop test. It is possible that exercise may influence different cognitive domains and therefore have different effects on tasks that vary in complexity. As exercise and cognition literature is varied, it is difficult to draw conclusions for the relationship between acute exercise and cognitive performance.

Summary

The current literature regarding exercise and cognition highlights a complex relationship. Cognitive performance is likely to be moderated by a wide range of individual factor and methodological differences. Reproducibility of studies is also difficult due to large batteries of neuropsychological tests used. Individual factors such as fitness should also be controlled for, possibly by using individualised exercise tasks relative to an individual's level of fitness of physical functioning. These controls may be able to help provide a definitive answer of the underlying mechanisms involved in the exercise and cognition relationship. Further research is required to ascertain which circumstances will result in cognitive deterioration or improvement.

Chapter 3 - Cognition and Fatigue

Role of cognition and psychometric tests

Cognition is a complex phenomenon, one which decades of research have attempted to define and measure. Psychometric tests have been developed to help identify certain areas of cognition and a person's functioning that can be used in assessment, diagnosis and research. Researchers must rely on the dose, duration and expected change of the intervention along with choosing psychometric tests that are sensitive enough to test significant cognitive outcomes from nutritional interventions (Macready, et al, 2009). These tests must be repeatable, simple and specific to the cognitive domains being assessed. The choice of cognitive test can be informed by previous studies or a combination of measures to target global cognitive functioning.

Testing of several different areas of functioning are often reported in fatigue and cognition literature. Executive functioning involves top-down processing that requires effort. The three primary domains of executive functioning include inhibition and inference control, working memory and cognitive flexibility (Miyake et al., 2000). From these domains, higher order functioning such as reasoning and problem-solving are built. Executive functions are essential for physical and mental health along with success and achievement in life (Diamond, 2013). Psychometric tests have been developed to assess these different domains to allow a comprehensive analysis of a person's executive functioning skills. Examples of neuropsychometric tests are the Stroop test, verbal and design fluency test, and the Tower of

London which focus on verbal production, inhibition, switching and planning components of executive functioning respectively (Chan et al., 2008).

Inhibition controls behaviour, attention and thoughts which can override basic impulses or external reward to complete what is appropriate or functional. Inhibitory control of attention allows us to selectively attend to stimuli whilst ignoring irrelevant information. The subthalamic nucleus appears to play a critical role in inhibition by presenting premature responding or impulsivity (Frank, 2006). Examples of neuropsychological tests that can test inhibition include the incongruent condition in the Stroop task, stop-signal tasks, and go/no-go task (Cragg & Nation, 2008; MacLeod, 1991; Verbruggen & Logan, 2008). The Stroop task can assess inhibition; from childhood we are trained to read rather than identify superficial characteristics such as font colour or style. Incongruent Stroop trials require inhibition of the automatic response to read the word and instead attend to the colour of the ink. This cognitive process results in people making more errors and becoming slower as a result of the time it takes to inhibit and correct the learned response in favour of the demand required (MacLeod, 1991).

Working memory involves holding information and manipulating it within the mind (Diamond, 2013). It has also been correlated to executive attention through its role in controlling sustained attention in the presence of distracting and interfering stimuli. Building upon traditional models of dual-store memory, Unsworth and Engle (2007) proposed that working memory is a combination of active maintenance, otherwise known as primary memory, and controlled retrieval from secondary memory. Secondary memory is essential in cognitive performance when primary memory reaches its capacity; secondary memory can help retrieve information to solve complex problems. Secondary memory requires activating the dorsolateral prefrontal cortex and supports inhibitory control by holding relevant information in the mind as to what is appropriate to inhibit. This process increases the likelihood that the instructional goal will guide behaviour and inhibitory errors will decrease. The most frequently used psychometric test to assess working memory are the Working Memory Rating Scale and the forward and backward digit span tests (Stone & Towse, 2015).

Cognitive flexibility allows switching between mental processes that generate appropriate behavioural responses (Dajani & Uddin, 2015). This can include changing spatial perspectives such as the theory of mind or changing how we think about something. As cognitive flexibility requires the interruptions of automatic cognitive processes, the incongruent Stroop condition can also assess this area of cognitive functioning. Conflict adaptation in Stroop tasks refers to the decrease in interference seen after incongruent trials compared to congruent trials (Egner & Hirsch, 2005). Conflict adaptation is theorised to be caused by flexible cognitive control, supported by behavioural and neuroimaging studies. Conflict-adaptation network of brain activity shows an increase in focal activity in the dorsal and anterior cingulate gyrus, lateral prefrontal cortex and parietal areas (Egner & Hirsch, 2005). A functional integration analysis proposes that cognitive control relies on modulation of the temporal gyrus and frontal regions which are involved in response inhibition; the right anterior cerebellum that is involved in sensory discrimination processes; and right supramarginal gyrus, involved in visuospatial attention (Egner & Hirsch, 2005).

The Stroop test has gained such strong recognition within literature due to its high reliability with individual differences and its ability to assess multiple domains of functioning.

Neuropsychological studies suggest that the incongruent condition elicits Stroop interferences through an interference in verbal processing (Golden & Freshwater, 1978). The stimuli during the Stroop test appear to activate automatic verbal processing responses that interfere with the conscious naming of the colour. Increased attention is needed to name the actual colour rather than simply reading the text – this is known as the Selective Attention Theory (Golden & Freshwater, 1978; Stroop, 1935). Reaction time in this condition is affected by the individual either sequentially reading the word and then naming the colour or by using volitional control to suppress the automatic word-reading response – this is otherwise known as the Speed of Processing Theory (Golden & Freshwater, 1978; Stroop, 1935). The task of making an appropriate response can be attributed to activity in the anterior cingulate cortex which engages in a wide variety of cognitive processes. Directed attention can also be assessed in the Stroop test through its role in managing the focus of our thoughts. Directed attention can inhibit certain irrelevant social or environmental stimuli as well as internal distractions to allow concentration on relevant stimuli (Naatanen & Näätänen, 1992).

Mechanisms of cognitive fatigue

Fatigue is a common experience for all and is exacerbated by physical and cognitive exertion or illness. Fatigue is associated with the conscious perception of body function changing with increasing physical activity duration and intensity. Observed changes could be breathlessness and increased ventilation, increased cardiac output, increase in body temperature and sweating, and the sensation of increased muscle activity (Hampson et al., 2001). Fatigue is also associated with cognitive functions such as level of motivation, a memory of prior exercise and decision-making based on the personal relationship between memory of previous events and current sensorimotor input (Ulmer, 1996). As there are no mechanisms known to be directly responsible for the onset of fatigue, it is suggested that a number of different afferent and non-sensory inputs, such as motivation and psychological, are integrated into brain structures, with fatigue subsequently arising from stimulation of these brain structures (Gibson et al., 2003). Most of the research involving the sensation of fatigue is based on individuals with Chronic Fatigue Syndrome (CFS) (Kuratsune et al., 1998; Lewis & Wessely, 1992; Togo, Lange, Natelson, & Quigley, 2015). CFS is a debilitating condition with sensations of excessive fatigue. Excessive fatigue has been affiliated with brain structures responsible for the conscious perception of fatigue rather than one physiological or metabolic alteration in the causation of fatigue sensations (Gibson et al., 2003).

Several interactions between neurocognitive mechanism and neurotransmitters influence central fatigue which is a common symptom in many neurologic disorders. Central fatigue can also occur transiently in healthy populations due to stress, infection or lack of sleep (Chaudhuri & Behan, 2000).

One of the primary neural structures associated with central fatigue is the basal ganglia, which is involved in motor movements, procedural learning, cognition and emotion (Leavitt & DeLuca, 2010). The basal ganglia are a group of subcortical nuclei made up of the dorsal striatum (the caudate nuclear and putamen), the ventral striatum (the nucleus accumbens and olfactory tubercle), globus pallidus, ventral pallidum, substantia nigra and subthalamic nucleus

(Fix, 2008). The striatum receives input from other brain areas and communicates with the rest of the basal ganglia. The pallidum receives information from the striatum and sends inhibitory signals to the motor-related area while the substantia nigra regulates dopamine (Haber & Knutson, 2010). The basal ganglia are cortically associated with the prefrontal cortex with links to the striatothalamic pathways, these pathways project to the entire frontal lobe and primary motor cortex (Chaudhuri & Behan, 2000). Central fatigue may be caused by an interruption in the loop of striatocortical fibres or suppression of cortical activation through the striato-thalamo-cortical loop (Chaudhuri & Behan, 2000). Dysfunction in the basal ganglia highlights its importance for normal brain function and behaviour. Tourette’s syndrome, obsessive-compulsive disorder and movement disorders can result from dysfunction in the basal ganglia and the neurotransmitter dopamine.

The ACC has also been related to fatigue through its role in performance and conflict monitoring and subsequent correctional procedures. Neural activity in the ACC changes depending on time engaged with a task, suggesting that its function is related to fatigue (Lorist, Boksem, & Ridderinkhof, 2005). Brain imaging studies have found that ACC activation is involved with sustained attention, working memory and the acquisition of new information (Gevins, Smith, McEvoy, & Yu, 1997). The interaction between ACC and fatigue was demonstrated by Lorist et al. (2005) who found that two hours completing a cognitive task resulted in a decrease in error monitoring and increased reaction time, indicating that greater demands are being placed on the ACC. Individuals who were fatigued also had a reduction in the error-related negativity (ERN) and error negativity (Ne) amplitude (Boksem & Tops, 2008).

These processes result from a decrease in mesencephalic dopaminergic neurons following reward prediction errors. The ERN/Ne processes are thought to be related to motivational processing and are seen during performance monitoring, roles known to be a part of the ACC system. The association between ERN and ACC in performance monitoring signifies that ERN may be used to observe central fatigue (Lorist et al., 2000). The ACC is activated on psychometric tests such as the Stroop test and activates other areas, such as the dorsolateral prefrontal cortex, when increased attention is required (Carter & Van Veen, 2007).

Dopamine is a neurotransmitter that can regulate energy expenditure and may also be involved in producing the ERN/Ne, suggesting a role in central fatigue (Boksem & Tops, 2008). Central fatigue is thought to originate in the striato-thalamo-cortical fibres from hampered dopaminergic functioning (Chaudhuri & Behan, 2000). The prefrontal cortex plays a role in cognitive control, decision making, personality and planning complex cognitive behaviour (Yang & Raine, 2009). The prefrontal cortex has a large number of dopamine receptors, suggesting that it could play a role in cognitive functioning and effective decision making. Dopamine is also involved in working memory, cognitive flexibility and planning brain functions (Cools & D'Esposito, 2011). Furthermore, dopamine is also thought to be involved in effort exertion behaviour, with a depletion in dopamine in the striatum or an increase of dopamine antagonist showing a cessation in effortful behaviour (Dobryakova, Genova, DeLuca, & Wylie, 2015). The effect of dopamine on cognition follows an inverted "U' shaped function, similar to the effect of exercise on cognition. Too much or too little dopamine can lead to impaired cognitive performance (Dobryakova et al., 2015). Dopamine imbalance can cause central fatigue through reduced connectivity in frontal control networks that receive dopaminergic projections or reduced mesocorticolimbic connectivity.

The importance of dopamine is highlighted by its role in a number of disorders such as attention-deficit hyperactivity disorder, schizophrenia, Parkinson's disease and drug addiction which all demonstrate a loss in cognitive functioning (Noble, 2003). However, the relationship between dopamine and cognition is complex and often has had contradictory results between null effects and improvements with increased dopamine (Robbins, 2003).

The Central Fatigue hypothesis suggests that central factors of fatigue may be mediated by serotonin (5-HT) where an increased amount of brain 5-HT can lead to central fatigue during prolonged exercise (Newsholme, 1987). This relationship is thought to be due to an increase in the delivery of tryptophan, a precursor to 5-HT, which can cross the blood-brain barrier. This pathway can also be affected by other activities that link to altered behaviours such as high carbohydrate meals, depression, eating disorders, and liver and renal disease (Curzon, 1996). Brain 5-HT and dopamine have an inverse relationship in areas of the brain. A low ratio of 5-HT to dopamine results in improved performance (i.e. increased arousal, neuromuscular coordination and motivation) while a high ratio of 5-HT to dopamine decreases performance (i.e. decrease motor coordination, motivation and tiredness) which would constitute central fatigue (Davis & Bailey, 1997). Due to the role that serotonin has in emotion, sleep-wake cycles, the hypothalamic-pituitary axis and many other physiological functions, it has been suggested that increased levels of serotonin cause a loss of motivation and increased sensation of perceived effort (Davis et al., 2000).

Central fatigue may also result from a depletion of energy for attentional processing. Cognitive processing requires considerable workload and stress. In a meta-analysis by See, Howe, Warm, and Dember (1995) of 42 studies, it was shown that sustained attention decreased with task difficulty, possibly due to a depletion of resources. Helton and Russell (2011) also supported the resource depletion theory by demonstrating a decline in performance as time on task increases, indicating a depletion of the resources pool. Therefore, in theory, if there were more resources available, attention and other cognitive processes might be sustained for longer periods of time. Matthews et al. (2010) found that cerebral blood flow volume may influence cognitive performance during vigilance tasks. This increase in cerebral blood flow appears to provide a general energisation process which aligns with theories that emphasise the mobilisation of resources dependent on task demands (Young & Stanton, 2002). Imagining studies have shown increased blood flow to regions such as the thalamus, right prefrontal cortex, ACC and parietal regions which is indicative of cognitive resources supply (Helton & Russell, 2011).

Fatigue and cognitive performance

Understanding how fatigue can affect executive control functions is important when identifying factors that can increase cognitive function and ameliorate effects of fatigue. Cognitive control of behaviour changes under fatigue through reorganisation of actions that make decisions or guide goal-directed behaviour (Van der Linden et al., 2003). Complex cognitive tasks which require deliberate concentration or application of a particular behaviour are most affected by fatigue due to the deterioration of executive control (Lorist et al., 2000).

Performance monitoring occurs in everyday life and effective control within multiple domains, allowing for the adjustment of behaviours to avoid adverse consequences (Elkins-Brown, Saunders, & Inzlicht, 2016). Research has demonstrated that motivation, emotion and peripheral nervous system play a role in performance monitoring. This type of control is present within psychometric testing such as the Stroop test and in everyday behaviours that require self-regulation (Braver, 2012; Nederkoorn, Houben, Hofmann, Roefs, & Jansen, 2010). Improvements in performance monitoring and control result in higher academic performance, financial stability, relationship satisfaction, health and well-being (Elkins-Brown et al., 2016).

Performance monitoring can be observed using the corrugator superciili whose activity increases within 100-300 milliseconds following an erroneous response (Elkins-Brown et al., 2016). During these erroneous response, ERN and error positivity responses (Pe) are measured by facial electromyography. The ERN shows a sharp negative deflection approximately 50-100 milliseconds after an error while Pe is represented by broad positive deflection, resulting in error awareness, that occurs 200-400 milliseconds after an error (Gehring, Goss, Coles, Meyer, & Donchin, 1993).

The autonomic nervous system is also engaged throughout error-related responses resulting in pupil dilation and increased heart rate deceleration which arouses and orients towards motivationally relevant stimuli (Wessel, Danielmeier, & Ullsperger, 2011). These factors complement error-related activity in the peripheral nervous system through corrugators association with negative affect such as angry, pain or fear (Burrows, Waller, & Parr, 2009). A study conducted by Elkins-Brown et al. (2016) found that error processing activates the corrugator superciili and that increases in corrugator activity are correlated with slowing of responses after errors, indicating that caution is taken after mistakes. This error-processing interaction in the study was measured using Stroop tasks where more error-related potential over the corrugator muscle was seen when the stimuli were answered incorrectly. This finding suggests that error-related corrugator activity is associated with performance monitoring where behaviour becomes more careful in an attempt to reduce the probability of another error occurring. However, as central fatigue increases, performance monitoring decreases and the cognitive system fails to detect erroneous behaviour, resulting in a decline in correct erroneous responses and slowing down after committing an error (Boksem, Meijman, & Lorist, 2006). These results suggest that when fatigued, individuals no longer strategically adjust their behaviour after incorrect responses. Lorist et al. (2000) found that fatigued subjects also did not prepare themselves for upcoming trials during testing which resulted in decreased reaction time and accuracy. These findings suggest that central fatigue negatively affects effort and cognitive performance.

Certain areas of information processing require effort and attention. Wickens (1991) proposed a model that created a hierarchy of information processing and the resources dedicated to the different task dimensions. These dimensions were: stages of information processing (perceptual, cognitive and response), codes of perceptual and cognitive processing (verbal versus spatial), modalities of input (visual versus auditory), and modalities of output (manual versus vocal). This model is similar to others proposed in the literature. Sanders (1983) emphasised the role that effort places on information processing during intense or sustained task performances. As the intensity of effort increases, body systems are activated to mobilise resources. Several facial muscles, such as the frontalis, corrugator superciili and orbicularis oris inferior, showed a proportional increase in EMG activity when task load was increased. These facial responses were revealed to be stronger and more reliable than other physiological responses, such as heart rate, eyeblink reflexes and calf muscle EMG responses (Waterink & Van Boxtel, 1994). A more recent study by Boksem, Meijman, and Lorist (2005) also confirmed that high levels of effort required for task demands increased fatigue ratings, theta and lower alpha EEG band power. Erroneous responses and reaction times also increased, and subjects were unable to inhibit automatic attentional shifting to irrelevant stimuli.

Research indicates that directed attention is a finite mental resource. Directed attention is effected when individuals become fatigued, a finding demonstrated by Boksem et al. (2005). When fatigued, subjects no longer selected relevant stimuli, instead selecting irrelevant stimuli and becoming easily distracted. These behaviours are due to lack of directed goal-oriented attention which, under normal circumstances, filters out irrelevant information to allow for focus on a task. This level of distraction implied that individuals had difficulty extracting necessary information by applying a top-down modulation of early sensory processing which resulted in a reduction of overall cognitive performance.

Methodological differences in previous research

Reviewing the scientific literature on fatigue is challenging due to the diverse definitions of central and peripheral fatigue. There are also a lack of good techniques or measures to differentiate between central and peripheral fatigue during exercise which has severely limited research into these mechanisms.

An abundance of literature exists regarding the effects that physical exercise has on fatigue. What is less researched though, is central fatigue in a neurotypical population and how it can affect cognitive performance. A significant amount of the research in central fatigue focuses on individuals with impairments, disorders, lesions or injuries meaning that this data cannot be used to provide recommendations to healthy populations. Methodological problems also include the inadequate familiarisation or practice of a task, resulting in practice effects being analysed as "real" effects from an intervention. Any effect in fatigue rating can also be attributed to alterations in psychological factors such as motivation, attention or perception of effort and pain. For example, force generation and electromyographic (EMG) activity during repeated maximal voluntary contractions can be enhanced through encouragement, whilst fatigue is more evident in participants that concentrate on performance and is decreased in those who are distracted (Marchant, 2011).

Summary

Fatigue is a complex phenomenon currently not well-defined within literature, making it difficult to compare research and develop knowledge around this area. Central fatigue appears to be influenced by brain structures such as the ACC and the neurotransmitters and pathways connecting different brain sites. Fatigue can increase reaction time and decrease performance monitoring, sustained attention and motivation due to a depletion of available resources. However, although it is known that cognitive tasks are affected by both peripheral and central fatigue, few psychometric tests are available to specifically test the effect of fatigue on cognition.

Chapter 4 – Phytochemicals, Exercise and Cognition

What are phytochemicals?

Fruit and vegetable consumption has been long-known to benefit human health. Plants produce phytochemicals through primary or secondary metabolism that plays a role in plant growth or defence mechanisms (Liu, 2003). Phytochemicals can be classified into categories such as carotenoids and polyphenols. Polyphenols include stilbenes, phenolic acids and flavonoids (Dillard & German, 2000). Flavonoids can be further classified into anthocyanins, flavanols, flavones and isoflavones. Flavanols can then be divided into catechins, proanthocyanidins and epicatechins.

Phytochemical-rich foods have become a focus for researchers searching to influence human health and functioning positively. Evidence currently supports the hypothesis that phytochemicals can alter age-related decline through neuromodulatory and neuroprotective effects (Shukitt-Hale, Carey, Jenkins, Rabin, & Joseph, 2007). Both animal and human studies have indicated that flavonoid benefits on cognition are wide-spread and occur within healthy and ageing populations (Rendeiro, Guerreiro, Williams, & Spencer, 2012). These phytochemical compounds, found in fruits and vegetables, have potent anti-inflammatory and antioxidant abilities that interact with the cellular and molecular structures of the brain, protecting and enhancing neuro-cognitive function (Joseph, Shukitt-Hale, & Willis, 2009; Spencer, 2010; Spencer, Vauzour, & Rendeiro, 2009). Their function stems from their ability to scavenge free radicals, inhibit lipid peroxidation, encourage angiogenesis and decrease cognitive decline (Heim, Tagliaferro, & Bobilya, 2002).

Anthocyanins produce a bright red, blue or purples pigment and are a class of flavonoids that are most frequently consumed by humans (Miller & Shukitt-Hale, 2012). Anthocyanins have potent anti-inflammatory and antioxidants properties. Once consumed, there is an increase in serum antioxidant level which is neuroavailable and resides in tissue longer than in plasma (Miller & Shukitt-Hale, 2012). Theoretically, this would lead to positive effects on brain structures and inflammation within the brain, however, there is a paucity of research on the extent of neuroavailablity and neuroprotection in humans.

There have been multiple proposed pathways in which berry fruits influence cognition. Berry fruits appear to not only scavenge free radicals but increase activation of protein pathways that involve cognition and activation of protection signals (Goyarzu et al., 2004). Specifically, the protective effects of berry fruits including blackberries, strawberries, blueberries, bilberry and blackcurrant have been demonstrated through many studies due to their ability as polyphenols to cross the blood-brain barrier and scavenge reactive oxygen and nitrogen species (Aquilano, Baldelli, Rotilio, & Ciriolo, 2008; Miller & Shukitt-Hale, 2012; Spencer, 2010). By encouraging healthy brain function and neurogenesis, neural morphology and cerebral atrophy are less likely to occur and affect cognitive functioning.

Metabolic mechanisms of phytochemicals

Berry fruits have been suggested to increase the number and strength of connections through their interaction with ERK and Akt pathways which increase the amount of brain-derived neurotrophic factors (BDNF) (Spencer et al., 2009). Stimulation of these pathways encourages growth and differentiation of new synapses and neurons while supporting cell survival. BDNF is found active in the hippocampus, cortex and basal forebrain which are areas vital to memory, learning and executive functioning (Huang & Reichardt, 2001). The ERK signalling pathway is activated by flavonoids found in berry fruit possibly due to the structure of the flavone backbone closely resembling that of the modulators of ERK signalling (Spencer et al., 2009). These pathways then continue to influence angiogenesis and synaptic plasticity which encourages neuronal survival and increases memory and cognitive performance.

Long-term potentiation (LTP) is widely acknowledged as the mechanism through which learning and memory formation is developed in the brain (Lamprecht & LeDoux, 2004). LTP is a constant increase of chemical synaptic strength which encourages synaptic plasticity which is vital for both implicit and explicit memory. This process is triggered by CREB activating genes associated with synaptic plasticity, and increases in neuronal spine density and morphology which are vital for learning and memory (Spencer, 2009).

Angiogenesis is the formation of new blood vessels from existing vascular networks (Wang & Stoner, 2008). Angiogenesis encourages neurogenesis due to increase delivery of oxygenated blood to specific regions of the brain. Flavanols can also cause vasodilation by inducing nitric oxide production and activating eNOS and AKT pathways, leading to an increase in the bioavailability of endothelial nitric oxide in the hippocampus, causing angiogenesis and neurogenesis (Zhao, Deng, & Gage, 2008). Flavonoids have been proven to affect peripheral blood flow and endothelial function. Increased endothelial health and access to resources provided through blood flow are significant in increasing neurogenesis, especially within the hippocampal cells which proliferate around the blood vessels and influence memory retention (Jagla & Pechanova, 2015). Blackberry strengthens blood circulation which allows more oxygen to reach the brain and increases neural activity, oxygen availability and clearing of toxins within the brain enabling augmented cognition (Raichle et al., 2001). However, it is important to note that flavonoids are found at low concentrations in the brain, and therefore their positive effects cannot be solely based on scavenging free radicals. Instead, these polyphenols appear to protect and enhance neuronal functioning and stimulate neurogenesis through critical signalling pathways that control LTP, memory and differentiation (Spencer, 2008). A study using fMRI and berry fruit juice was conducted by Krikorian et al. (2012) using anthocyanin nutritional intervention in grape juice. Greater activation was found in posterior and anterior regions of the brain, resulting from increased neural activity and blood flow.

Metabolic biochemical studies have shown that anthocyanins and their respective metabolites peak at one to two hours and also six hours after consumption of blueberry supplementation which corresponded with the two distinct peaks of cognitive benefits that are observed with blueberry (Rodriguez-Mateos et al., 2013). Peak vasodilation through increased peripheral blood flow is also seen at precisely the same times as above. This coordination of processes is thought to be due to enhanced activity and expression of eNOS, increasing levels of nitric oxide which is also implicated in the regulation of transcription factor CREB which is essential for synaptic plasticity and neuron survival (Ciani et al., 2002). eNOS itself can assist with the upregulation of BDNF expression, an increase of which can be observed after blueberry supplementation (Dodd, 2012). However, it is important to note that trends involving

BDNF are often only seen in chronic supplementation studies. These effects on the endothelial cells also extend to the cerebral spinal fluid, which increases one hour after acute flavonoid supplementation.

Phytochemicals effect on exercise

Phytochemicals have been associated with positive effects on the cardiovascular system and endothelial health. As research continues to explore ways that phytochemical supplementation can ameliorate or prevent disease states, parallel research investigating how phytochemical supplementation might benefit exercise performance and recovery.

Flavanols, such as quercetin, myricetin and kaempferol, are found in blackcurrants (Gopalan et al., 2012). Quercetin has antioxidant and anti-inflammatory activity that enhance endurance capacity through its role as an adenosine-A receptor antagonist which blocks receptors in the brain (Ferré, 2008). This results in an effect similar to the psychostimulant effects of caffeine. In untrained people, 500mg quercetin supplementation twice a week results in an increase in VO_2max and endurance capacity (Davis et al., 2010). In rat studies, quercetin also has the ability to increase mitochondrial biogenesis in muscle and brain tissue (Davis, Carlstedt, Chen, Carmichael, & Murphy, 2010). Quercetin supplementation also increases fat oxidation relative to carbohydrate, resistance to fatigue and lactate thresholds (Calvo et al., 2008). Anthocyanins are also found in blackcurrants, and clinical trials have found that blackcurrant ingestion improves blood flow to muscles during exercise which may improve muscle stiffness and fatigue (Matsumoto et al., 2005). Blackcurrant supplementation studies have shown that blackcurrant intake can restore oxyhaemoglobin levels and improve peripheral circulation during and after a typewriting task, leading to a reduction of muscle fatigue (Matsumoto et al., 2005).

Although the production of ROS during exercise is normal, an excess can cause fatigue by reducing calcium sensitivity and reuptake (Reid, 2008). Exercise-induced oxidative stress results from the accumulation of ROS that damage muscles and increases fatigue while slowing recovery times (Powers & Sen, 2000). Oxygen radicals can oxidise low-density lipoproteins which can damage the endothelial wall and cause atherosclerotic changes (Nijveldt et al., 2001). Several studies have found that blackcurrant supplementation of approximately 240mg of anthocyanins can assist in reducing muscle damage and inflammation following exercise through reducing cytokine circulation (Hutchison, Flieller, Dillon, & Leverett, 2016; Lyall et al., 2009). This protection was most likely mediated through neutralising reaction oxygen species in damaged myofibres, halting further disruption to the sarcolemma and preventing additional leakage of cytokines (Hutchison et al., 2016).

In contrast, Braakhuis, Hopkins, and Lowe (2014) used an intervention of 300mg of anthocyanin and 15mg of vitamin C for three weeks while conducting hard training. Blackcurrant was found to have little effect on markers of oxidative stress, and it appears that vitamin C may have acted as a pro-oxidant, increasing markers of oxidative stress. Blackcurrant did appear to assist exercise performance possibly through changes in vascular function and proliferation of blood vessels (Ghosh & Scheepens, 2009). This effect only occurred in the high training load group which suggest that polyphenols enhance performance in combination with greater training loads. Murphy, Cook, and Willems (2017) also found that blackcurrant extract increased exercise performance resulting in faster performance times (placebo: 771 ± 60 seconds, New Zealand blackcurrant: 764 ± 56 seconds, $p = 0.034$). McKenna et al. (2006) hypothesised that N-acetylcysteine, an antioxidant compound, may affect exercise-induced oxidative stress through attenuating the maximal activity of the sodium-potassium pump and reduce skeletal muscle fatigue.

Protection of the cardiovascular system and vascular tone can result in an increase in exercise performance. Research into the beneficial effects of polyphenol the cardiovascular system has been ongoing for a number of years. These positive effects arises from polyphenols ability to scavenge free radicals, reduce vascular oxidative stress and stimulate endogenous antioxidant enzymes while inhibiting xanthine oxidase and NAD(P)H oxidase which generate large amounts of ROS (Nijveldt et al., 2001; Torel, Cillard, & Cillard, 1986). Nitric oxide (NO) alteration has been proposed to play a large role in the positive effects seen through polyphenol supplementation due to its role as a signalling molecule that can regulate vascular tone, mitochondrial respiration and skeletal muscle function (Behnia, Wheatley, Avolio, & Johnson, 2018).

A literature review completed by Schini-Kerth, Auger, Kim, Étienne-Selloum, and Chataigneau (2010) showed that polyphenols caused NO-mediated endothelium-dependent relaxations. There was also evidence of activation of endothelial NO synthase through polyphenol activity increasing intracellular calcium. Another signalling pathway affected was the PI3-kinase/Akt pathways which lead to the activation of endothelial NO synthase, which in turn increase the formation of NO (Edirisinghe, Burton-Freeman, & Kappagoda, 2008). The effect on eNOS is caused within minutes of polyphenol intake and persists for several hours. Diverse isoforms of flavanols all appear to have a beneficial effect on improving endothelial function in healthy and diseased humans. Flavanol intake can reduce blood pressure through improving endothelial function and increasing circulating NO, which causes vasodilation (Park, Kim, & Kang, 2004; Schroeter et al., 2006). This literature review highlights the potential of polyphenols that can ameliorate or restore vascular health through the formation of NO which reduces oxidative stress in the arterial wall, promoting anti-inflammatory and anti-thrombotic responses.

Blackcurrant profile and overall health benefits

Blackcurrants are well-known as a rich source of vitamin C and phenolics. They contain polyphenols that have antimicrobial, antibacterial and antiviral properties that protect and support organ functioning and digestive, nervous and circulatory systems (Krisch, Ördögh, Galgóczy, Papp, & Vágvölgyi, 2009; Tabart et al., 2012). Biochemical profiling of blackcurrants shows that they also contain polyunsaturated fatty acids, carbohydrates, tannins, non-volatile organic acids and stilbenoids (Gopalan et al., 2012). Randomised controlled trials show that blackcurrants may have the potential to change physiological parameters that can influence cognition and physical performance (Watson et al., 2018).

Anthocyanins comprise 80% of the phenolic content in blackcurrants which is made up of cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, delphinidin 3-O-glucoside and delphinidin 3-O-rutinoside (Kapasakalidis, Rastall, & Gordon, 2006). Anthocyanins vary in the number of hydroxyl groups, the methylation of these hydroxyl groups and the location of sugars and acids on the molecule. The more available hydroxyl groups, the more powerful the antioxidant due to its ability to scavenge free radicals. Free radicals are formed during normal metabolic processes or from UV radiation (Bonarska-Kujawa, Cyboran, Żyłka, Oszmiański, & Kleszczyńska, 2014). These often attack cell membranes which impair the structure and function of that cell, resulting in disease. Anthocyanins can bind and protect a cell due to their amphiphilic nature which allows them the structural similarity of membrane lipids. Animal studies show that consumptions of 1.5g per day of a polyphenol-rich blackcurrant extract causes beneficial modifications in antioxidant status, serum lipid and large-bowel function (Jurgoński, Juśkiewicz, Zduńczyk, Matusevicius, & Kołodziejczyk, 2014).

The Vitamin C content in blackcurrants acts as a potent antioxidant and free radical scavenger. Vitamin C content ranges from 70 to 280 mg/100g of blackcurrant fruit (Gopalan et al., 2012). Within its mineral composition, blackcurrant contains high levels of potassium, calcium, magnesium and iron. Ellagitannins and gallotannins are a class of polyphenols that are linked to polyol carbohydrate compounds, like glucose. These have been isolated in blackcurrant juice and augment its healing properties (Gopalan et al., 2012).

Blackcurrant has shown positive effects on both *in vitro* and *in vivo* experiments. *In vitro* experiments demonstrated that blackcurrants increased the expression of paraoxonase 1 which is anti-atherosclerotic, found in high-density lipoproteins (Rosenblat, Volkova, Attias, Mahamid, & Aviram, 2010). It was also shown that blackcurrant increased endothelial NO activation which dilates blood vessels (Edirisinghe, Banaszewski, Cappozzo, McCarthy, & Burton-Freeman, 2011). *In vivo* studies support increased vasodilation and blood flow to organs which decreases the risk of heart disease. In rat studies, blackcurrants GLA notably declined blood pressure levels when combined with fish and olive oil (Pregnolato et al., 1996). This combination reduced levels of serum thromboxane B2 which is an inactive metabolite of thromboxane A2. Thromboxane A2 is released by activated platelets and causes a pro-thrombotic effect, reducing any thrombus formation which decreases the development of cerebrovascular and cardiovascular events (Pregnolato et al., 1996). Eosinophilic-driven pulmonary inflammation is relieved through blackcurrant supplementation due to suppression of interleukin-4 induced CCL26 secretion which activates white blood cells (Hurst et al., 2010). The proanthocyanidin component of blackcurrants improved lung conditions associated with inflammation of the membranous lining (Garbacki, Tits, Angenot, & Damas, 2004). Metabolic by-products of proanthocyanidins contain anti-inflammatory properties that reduced the secretion of TNF-α, IL-1b and IL-6 within healthy blood cells in vitro (Monagas et al., 2010).

Blackcurrant supplementation can also directly alter the metabolic pathways of the nervous system. In diseases such as Alzheimer’s disease and other neurodegenerative and ageing diseases, vulnerability to oxidative stress is considered responsible for the decreases in cognitive and motor behaviours (Ghosh, McGhie, Fisher, & Joseph, 2007). Loss of calcium is an important factor in neuronal ageing and changes to learning and memory. A decline in calcium uptake can lead to excess intracellular Ca^{2+} that produces free-radical activity and continued cell degeneration. Sensitivity to oxidative stress increases parameters such as loss of glutathione, DNA fragmentation, striatal muscarinic receptor sensitivity, excitotoxic and astrocytic injury (Ghosh et al., 2007). Joseph, Fisher, and Carey (2004) conducted a study to test if excess amyloid and dopamine disrupt calcium regulation. They determined OS vulnerability by exposing transfected COS-7 cells (which had muscarinic receptors to dopamine) to amyloid-β and examined intracellular Ca^{2+}. These cells were unable to clear excess Ca^{2+} efficiently. However, when supplemented with blackcurrants, there was an increase in the recovery of calcium flux within the COS-7 cells. Blackcurrant has a protective effect against OS damage to DNA and also against insult by amyloid-β or excess dopamine.

Blackcurrant supplementation and cognitive performance

There has been a multitude of studies discussed above that show cognitive changes that arise from nutritional interventions. However it is unclear whether the polyphenols themselves are responsible for the change or the matrix within the flavonoid-rich foods causing the change.

A study completed by Shukitt-Hale et al. (2007) highlighted the different effects that various berry fruit has on areas in the brain and therefore the role each plays in cognitive changes. These authors suggested that the different flavonoids act in different brain regions to produce positive effects and that the food matrix within which the flavonoids are found, often contain other beneficial vitamins such as vitamin A, C and E which also contribute to brain health. The flavonoids found within berry fruit have been found to influence several aspects of memory and learning. Blueberry, blackberry and strawberry have been shown to reverse age-related deficits and improve short-term memory by activating the CA3-CA3 excitatory connections in the hippocampus (Spencer et al., 2009). Furthermore, the CA3 pathways transfer information to the dentate gyrus which is found in the hippocampus, the part of the brain most vulnerable to ageing and oxidative stress. Although there is no evidence that flavonoids alone cause positive benefits, consuming within the food matrix of which it is contained has separate benefits which increase general and cognitive health (Spencer, 2010).

Regarding the effects on humans, a symposium held by Lamport, Dye, Wightman, and Lawton (2012) compared the only ten studies conducted with human subjects. Long-term epidemiological trials indicated that consuming additional flavonoids can lead to increased cognitive functioning, especially in memory. Flavonoid intake within the general population is estimated to be approximated 1 gram/day (Kühnau, 1976). Plasma concentration rarely exceeds one µM, even when consuming a range of phenols from 10-100 mg (Scalbert & Williamson, 2000). However total plasma phenol concentration is most likely higher due to the metabolites produced by colonic microflora.

The quality of a supplement and its further benefits can depend on the mode of delivery into the human system. Watson et al. (2015) performed a double-blind, well-controlled trial of two blackcurrant extracts, a cold-pressed juice versus a freeze-dried powder. This intervention resulted in improved attention scores, compared to a sugar control, within a cognitively fatiguing battery of psychometric tests. However, there was no improvement observed for the Stroop tests or subjective measures of fatigue and mood. In this study, the blackcurrant supplement contained 483 mg of anthocyanins per 60 kg of bodyweight for the powder and 467 mg of anthocyanins per 60 kg of bodyweight for the juice. The juice also had more effect than the powder in decreasing monoamine oxidase (MAO) levels and attenuating blood glucose decline. These findings for the juice may highlight MAO inhibition and regulation of blood glucose as possible mechanisms of neurochemical change that are seen with blueberry anthocyanins. A reduction on MAO levels during attention and working memory tasks and have a positive correlation with performance on the task (Cox, Pipingas, & Scholey, 2015; Watson et al., 2015). Therefore, during cognitive tasks, MAO inhibition and slower glucose decline may be beneficial for monoaminergic neurotransmission resulting in improved attention. Another mechanism by which MAO inhibition may improve cognitive performance is through inhibiting MAO enzymes. These enzymes usually breakdown of serotonin, norepinephrine and dopamine, and their inhibition results in increased concentrations of these neurotransmitters, improving cognitive performance and outcomes (Schweitzer, 2000). This study highlights that the preparation of an intervention may affect those cognitive and physical outcomes due to different preparations and ratios of flavonoids within the mixes.

The matrix of the supplement being used for research is also important in determining other effects that may result from the food source matrix. Glucose is essential for all cell functioning, and cognitive improvements have been directly linked to glucose supplementation (Kennedy & Scholey, 2000). There appears to be a relationship between decreased glucose levels and cognitive performance, specifically when levels of cognitive demand are high (Kennedy & Scholey, 2000; Scholey et al., 2001). Attenuation in the decline of blood glucose concentration following cranberry and blackcurrant supplementation suggests that blood glucose regulations may affect executive functioning through increased availability of glucose over a longer period due to being rich in flavonoids (Bell, Lamport, Butler, & Williams, 2015). Therefore, it is important that during research, glucose within the matrix of the blackcurrant or supplement itself is taken into consideration as a possible mechanism for improved cognitive performance.

Gut health and cognition

The relationship between gut bacteria and health has been well-established by research in the last decade. Physical and mental well-being of the host can be attributed to modifying the types of bacteria found in the large intestine and colon to contain a higher proportion of beneficial probiotic bacteria, such as *Bifidobacterium* and *Lactobacillus*.

The brain-gut axis is a bidirectional relationship where the CNS, ANS, enteric nervous system (ENS) and hypothalamic-thalamic-pituitary-adrenal axis (HPA) affects peripheral intestinal function and vice versa (Carabotti, Scirocco, Maselli, & Severi, 2015). The axis uses endocrine, neural, immune and humoral pathways. Animal studies have shown that gut microbiota is vital to the development and maturation of the CNS and ENS. Neurotransmitter expression and turnover can be affected by an absence of beneficial bacteria along with alterations to gut sensory-motor sensors (Heijtz et al., 2011). The type of bacterial colonisation has been shown to affect maturation and function of microglia, modulate HPA axis activity and increase plasma tryptophan which is a precursor to serotonin (P. J. Kennedy et al., 2016).

Short-chain fatty acids are produced through bacterial metabolism and these can stimulate the sympathetic nervous system and release serotonin which affects memory and learning processes (Vecsey et al., 2007). *Lactobacillus* produces acetylcholine and both *Lactobacillus* and *Bifidobacterium* secrete GABA. (Lyte, 2011) These neurotransmitters play an essential role in brain functioning and behaviour, however, this finding is yet to be replicated in human trials.

Polyphenols have relatively low bioavailability compared to micro- and macro-nutrients. Depending on the polyphenols structure and polymerisation, it can be absorbed in the small intestine or the colon. Approximately 5-10% of polyphenols are absorbed in the small intestine with the remaining amount digested by colonic microflora (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013). Colonic microflora breaks down the polyphenol into low-molecular-weight phenolic metabolites that are more easily absorbed and may be responsible for the variety of health benefits reaped from berry fruit consumption (Duggan, Gannon, & Walker, 2002; Hervert-Hernandez & Goñi, 2011). There appears to be a symbiotic relationship between polyphenols and microbiota. Phenolic substrates modulate and cause fluctuations in the populations of colonic microbiota composition through antimicrobial and prebiotic effects on pathogenic gut bacteria (Gibson & Roberfroid, 1995). These beneficial bacteria provide the host with a range of benefits such as reducing serum cholesterol, protecting against gastrointestinal pathogens, aid in nutrient processing and reinforcing epithelial tight junctions (Vitali et al., 2010). Polyphenols can also stimulate cytokine release which increases mucus secretion and intestinal immunity. Bioefficacy and bioavailability of these polyphenols depend on individual differences in dietary intake and composition of gut microbiota (Duggan et al., 2002).

Anthocyanin fermentation can be a potential source of nutrients for gut bacteria. Anthocyanin bioavailability is determined by the matrix of the food from which it originates. Intact anthocyanins are poorly absorbed and hence are metabolised by gut microflora in the colon (Bingham, 2006). Absorption modulates the microbiota metabolism contributing to prebiotic activity, producing a significant increase in *Lactobacillus* spp and *Bifidobacterium* spp (Faria, Fernandes, Norberto, Mateus, & Calhau, 2014). *Lactobacillus* and *Bifidobacterium* contribute to human health through a variety of functions. The bacterium can stimulate the immune system, activate provitamins, modulate lipid metabolism, assist in restoring tight junction barriers, weaken HPA axis and ANS activities and prevent damage to hippocampal genes involved in synaptic plasticity (Carabotti et al., 2015; Gibson, 2008). Gut microbiota can also modulate afferent sensory nerves, inhibiting calcium-dependent potassium channel opening, which controls gut motility and pain perception (Kunze et al., 2009). *Lactobacilli* can convert nitrate and nitrite into nitric oxide which delivers some of the positive benefits seen in anthocyanin supplementation such as inhibition of pathogen growth, vasodilation and immunomodulation (Sobko et al., 2006).

Furthermore, beneficial gut bacteria can produce molecules that act as local neurotransmitters through generating an active form for catecholamines in the gut lumen which assimilate GABA, serotonin, histamine, melatonin and acetylcholine (Asano et al., 2012).

These neurotransmitters and hormones have functions in emotion regulation, sleep-wake cycles, motor activation, immune responses and neural health and growth (Blandina, Efoudebe, Cenni, Mannaioni, & Passani, 2004; Davis et al., 2000; Jean-Louis, Gizycki, & Zizi, 1998)

There is currently only one study that focusses on the effect of blackcurrant on gut microbiota. The paucity of the research in this area highlights the potential for future research to improve the understanding of the beneficial effects of blackcurrant. Molan, Liu, and Kruger (2010) conducted a study in rats, allocating them into four groups (n = 10): water, inulin, first leaf extract (containing 30 mg of anthocyanin per kg of body weight of blackcurrant powder, lactoferrin and lutein) and a Cassis Anthomix 30 group (containing 13.4mg of anthocyanin per kg of body weight of blackcurrant extract powder). Their results showed that the First Leaf and Cassis Anthomix 30 were effective at promoting the growth of *lactobacilli* and *bifidobacteria* and enhancing gut health by inhibiting pathogen growth. However, their study did not provide the concentration of anthocyanins contained in their products, and therefore it will be difficult to replicate using other blackcurrant supplements.

Studies exist which use other sources of polyphenols exist and can provide an idea of the similar effects that blackcurrant may have on gut microbiota and cognition. A study using red wine showed that supplementation of 50mg of anthocyanin per kg of body weight of polyphenols, found in blackcurrants, over a 2-16-week period change the composition of gut microbiota from a predominance in *Bacteroides*, *Clostridium* and *Propionibacterium* to a composition of *Bacteroides*, *Lactobacillus* and *Bifidobacterium* (Dolara et al., 2005).

Conducting gut microbiota studies in humans is difficult, so The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) was developed to allow long-term experiments on multiple, connected “parts” of the gastrointestinal tract which mimics a human’s digestive system (Marzorati et al., 2011). Using this model, gut microbiota was seen to increase in diversity when 0.15g/L of flavonoid supplementation was used (Huang et al., 2016). This new mode of assessing human gut microbiota and how certain polyphenols can interact with that will open up further research to investigate the possible effects in humans.

Methodological differences in previous research

Observed physical responses such as vasodilation have been consistently replicated in flavonoid supplementation studies. However, cognitive findings have not been as robust even with several studies claiming moderate to large effect sizes (Watson et al., 2015; Whyte, Schafer, & Williams, 2016). Methodological differences between studies are likely to partly explain the differences in cognitive observations. Studies often varied considerably in the concentration of phytochemicals used for interventions, type of exercise test and intensities along with different clinical populations. Direct comparisons between studies are difficult due to differences in flavonoid source (such as different berries, cocoa, gingko) and dose. Studies that had no control condition or baseline measures showed larger effect sizes on cognitive tests (Caldwell, Charlton, Roodenrys, & Jenner, 2015; Whyte & Williams, 2015). Studies that used an extensive list of psychometric tests also had one or two measures that proved significant. Using an array of tests at different times throughout the study increases the potential for type 1 error considerably.

Summary

Exciting research shows a relationship between nutrition, brain neurochemistry and exercise performance. Research has previously separated out the components of physical, cognitive and dietary health however it is now being understood how nutrition can affect cardiovascular and metabolic disease as well as cognitive functioning and prevention of mental disease (Van Praag, 2009). Phytochemical mechanisms can also influence exercise and recovery along with learning and memory (Van Praag, 2009).

These results support the premise that an increase in consumption and supplementation of a variety of flavonoids can increase overall cognition especially within the areas of learning and memory. Despite a wealth of information about the effect of flavonoids on cognition, few studies focus on the effects within humans. Further studies are needed to determine whether the strong positive benefit that berry fruit possess can be applied to human cognition and whether these arise from the specific phytochemicals or from the full matrix of the food. Identification of the precise brain processes and structures involved would lead to a better understanding of the cognitive changes that occur with phytochemical supplementation. Improving levels of bioactive compounds and identifying their mechanisms in humans are crucial components of understanding how berry fruits affect cognition and how we can maximise its effectiveness. Research surrounding gut bacteria and cognition is a growing area of interest and currently suggests that the promotion of “friendly” bacteria can affect cognition. This knowledge would create a strong basis for nutrition and supplementation to be considered when dealing with mental health, ageing diseases and overall cognitive health.

The Current Study

Previous research shows that phytochemicals have a positive effect on cognitive and exercise performance. The majority of research conducted on phytochemicals and supplementation have involved berry fruit such as blueberries. Blackcurrants are thought to have a strong influence on cognition due to anthocyanins ability to cross the blood-brain barrier. Reviews focussing on phytochemicals show a positive relationship between long-term consumption and cognitive performance (Macready et al., 2009). Short-term, high dose supplementation has not been so thoroughly researched. The below decisions regarding the methodology of the present study were guided by the literature review.

This study aimed to be a continuation of the work carried out by Harold (2016) and McKenzie (2017). The current study will investigate how a single dose of blackcurrant can affect a variety of different cognitive and physiological measures. This study was pre-registered at as.predicted.org prior to any data collection (Appendix A).

Participants

Sixty healthy participants, aged between 18 and 35 (Mean = 25.48, SD = 4.53) volunteered to participate in this study. The participants consisted of 35 female (Mean age= 24.83 , SD= 4.528) and 25 male (Mean age= 26.40, SD= 4.47). Participants were non-athletes to allow the research to be expanded beyond athlete populations.

Twenty-six of the participants identified as New Zealand European, five identified as Maori, two identified as Pacifica and ten identified as Asian ethnicity. Seventeen responded with ethnicities that were classified as other because of the low number of participants with these ethnicities. Participants had no known neurological, psychological or physical conditions that would impair their participant in strenuous exercise. All participants had normal or corrected-to-normal vision. Participants were recruited through flyers placed around Massey University Albany campus and local gyms, social media and word of mouth (see Appendix B).

Before testing, participants were provided with an Information Sheet, Standard Health Screening Questionnaire, Participant Consent Form and email prior to testing. These were supplied via email when the participant expressed interest and then read and signed before any procedures commenced (see Appendix C, D, E and F). Menstrual data was not included due to research that indicates no consistently shown statistically significant difference in fatigue or carbohydrate supplementation in different menstrual phases (Bailey, Zacher, & Mittleman, 2000; Oosthuyse & Bosch, 2010).

Each participant received a $30 Countdown voucher, a body composition analysis and a VO_2max report to compensate for their time throughout the research.

All procedures, forms and materials used for this project were approved by the Southern A Massey University Human Ethics Committee, application number SOA 17/11.

Data Analysis Plan

Hypothesis 1, 2, 3, 5, 6 and 7 were each assessed using a two factor ANOVA. This used a between subject's design and compared the control group with the blackcurrant intervention group. A within subject's design was also used to assess pre-exercise and post-exercise differences. Hypothesis 4 used a linear regression with dummy coded variable. This data analysis plan was pre-registered on aspredicted.org.

Hypothesis 1: A single dose of berry fruit juice will improve performance on cognitive tasks by ameliorating the effects of physical fatigue. Two factor ANOVA will be used (between subjects: placebo control and berry fruit; within subjects: pre-test and post-test). The primary parameter of interest is the interaction between test time and group. The control group is predicted to have an increased reaction time from pre- to post-test while the berry fruit group is predicted to have a lesser increase in reaction time from pre- to post-test.

Hypothesis 2: A single dose of berry fruit juice will reduce corrugator activity, the facial display of effort, during Stroop test. Two factor ANOVA will be used (between subjects: placebo control and berry fruit; within subjects: pre-test and post-test corrugator activity). The control group is predicted to have increased corrugator activity from pre- to post-test while the berry fruit group is predicted to have a lesser increase in corrugator activity from pre- to post-test.

Hypothesis 3: A single dose of berry fruit juice will reduce the facial display of effort for the same period of physical activity between session 1 and session 2. Two factor ANOVA will be used (between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score corrugator activity). The control group is predicted to have increased levels of perceived effort from session 1 to session 2. The berry fruit group is predicted to have a lesser increase of perceived effort from session 1 to session 2.

Hypothesis 4: As daily fruit and vegetable activity increases, mean Stroop reaction time decreases but only for berry fruit supplementation group. This will be measured using linear regression with dummy coded group variable

Hypothesis 5: A single dose of berry fruit juice will reduce heart rate for the same period of activity between session 1 and session 2. Two factor ANOVA will be used (between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score of heart rate). The control group is predicted to have a similar heart rate change score from session 1 to session 2. The berry fruit group is predicted to have a reduced heart rate change score from session 1 to session 2.

Hypothesis 6: A single dose of berry fruit juice will reduce the rating of perceived effort (RPE) for the same period of activity between session 1 and session 2. Two factor ANOVA will be used (between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score for RPE score). The control group is predicted to have a similar RPE change score from session 1 to session 2. The berry fruit group is predicted to have a reduced RPE change score from session 1 to session 2.

Hypothesis 7: A single dose of berry fruit juice will increase VO_2max for the same period of activity between session 1 and session 2. Two factor ANOVA will be used (between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score for VO2 max). The control group is predicted to have a similar VO_2max change score from session 1 to session 2. The berry fruit group is predicted to have a reduced VO_2max change score from session 1 to session 2.

Instruments

Cognitive Measure

One cognitive test, the Stroop Colour-Word Task, was used in this study (see Appendix G). This cognitive test was chosen as it measures selective attention, inhibition and cognitive flexibility. The Stroop effect demonstrates the difficulty of attending to the colour of the ink, rather than reading the word itself. This interrupts an automatic process of reading (MacLeod, 1991). This was a change from both McKenzie (2017) and Harold (2016) theses which involved a battery of neuropsychological tests which provided a higher chance of type I error occurring and the extended time testing could signify that the psychometric tests were no longer testing the effect of blackcurrant on fatigue but rather a participant's ability to sustain attention after long psychometric tests. A control group and baseline measures are also implemented to increase statistical power to reliably observe small changes in cognitive performance.

This test was administered on a laptop in front of the participant using a separate device (a modified E-prime Serial Response Box) mounted on the bike handles to record responses (see Appendix J and K for set-up and device). Technology is increasingly used to administer psychometric tests. Literature has shown that using technology has high test-retest reliability and was preferred by both the participants and researchers compared to other modalities

(Bush, Skopp, Smolenski, Crumpton, & Fairall, 2013; Schroeders & Wilhelm, 2010). Stroop reaction time was measured using EPrime 2.0 Professional software (Inc, 2012). There was a familiarisation session where participants completed the cognitive test five times to eliminate any practice effects. Elimination of practice effects is vital to obtain the true change between session scores that can be attributed to the intervention rather than practice. Lemay, Bédard, Rouleau, and Tremblay (2004) showed that the practice effect occurred in Stroop Interference but had settled with minimal improvement in reaction time after three testing blocks. Macleod (1998) tested participants using the Stroop task across five days and reported that reaction time decreased by 49%. Beglinger et al. (2005) demonstrated that participants showed improvements due to learning until session 4. In the current study, the number of trials was chosen based on this research and five familiarisation blocks with 16 trials in each block were implemented to reduce these practice effects as much as practically possible. Trial order was also randomised in every block to avoid memorisation.

The Stroop Colour-Word Task consisted of colour names (Red, Yellow, Green and Blue) or the letter "X" with each shown individually and centrally. The familiarisation trials contained a total of 80 trials separated into five sections. The pre- and post-exercise Stroop each contained 32 trials. The ink colour of the stimuli was either congruent or incongruent with the colour word. The "X" was used as the control and the participants would identify the ink colour.

There were three conditions:

Control: In this condition, the letter “X” will be presented with different ink colours. For example, when presented with X, the correct answer is “red”.

Congruent: In this condition, the colour name and the colour of the ink are the same. For example, when presented with **BLUE**, the correct answer is "blue." Participants can respond quickly because the word and the ink colour match and facilitate a faster response.

Incongruent: In this condition, the colour name and the colour of the ink differs. For example, when presented with **BLUE**, the correct answer is "red." Participants take longer to respond because the word and the ink colour do not match; reading the word interferes with identifying the ink colour.

Participants were instructed to push a button that corresponded to the ink colour of the word displayed on the screen. The participant used the index and middle finger of both hands that remained poised on the four buttons in front of them. They were instructed to answer as quickly and as accurately as possible. Participants could either sit on the bike or stand over the bike while completing the test based on which was more comfortable. Length of arms and dizziness after exercise determined which position the participant took. The position taken in the first session was recorded and replicated in the second session.

Reaction time and accuracy of responses were the dependent variables and were recorded for all stimuli.

Physical Task

Physical fatiguing was completed on the Monark cycle ergometer. Participants completed a Standard VO_2max Test. This involved cycling at 60 RPM for the duration of the experiment. This exercise was chosen as it is considered submaximal exercise until the participant can no longer complete the task and at that point becomes a maximal exercise test. Submaximal exercise was shown to affect the corrugator superciili muscle and elicit both physical and cognitive fatigue in participants (de Morree & Marcora, 2010).

The first two minutes of cycling involved no additional weight apart from the weight basket which has a weight of one kilogram. Every two minutes, the researcher would add 500 grams of resistance onto the ergometer until the participant admitted voluntary exhaustion or dropped below 60rpm for longer than 30 seconds.

At the two-minute interval, participants were asked to give a Rating of Perceived Exertion (RPE) score. The participants were asked to give a one-minute warning before they stopped cycling due to fatigue. At this one-minute warning, the researcher placed nose clips and the mouthpiece in their mouth to take VO_2 measures. If the participant could continue past the one-minute, they were instructed to do so until exhaustion. This task was first completed in the familiarisation session and completed again in session two.

Heart Rate

Heart rate was used to monitor and assess physical effort. Before cycling began, electrodes were placed under the right clavicle and next to the left hip bone. These pre-gelled sensors are comfortable and do not require the skin to be prepared in any way. These were then attached to leads which connected to a PowerLab system. Heart rate was monitored throughout the cognitive and physical tasks.

Facial Electromyography

Facial muscle electromyography was used to monitor facial activity. Electromyography (EMG) sensors detect the electrical activity that results from muscle activation. A ground electrode for the facial EMG and heart rate was placed in the centre of the forehead, below the hairline and 4mm Ag-AgCl electrodes filled with conductive gel were attached unilaterally to the corrugator superciili (muscle above the eyebrow). These were then attached to leads which connected to a PowerLab system. Before attachment, participants were asked to clean the areas of their face where the sensors were attached using facial cleanser and alcohol swabs so that the optimal level of electrode-skin adhesion was achieved. The experimenter abraded the skin at the facial electrode site and Signa Gel, which is hypoallergenic, was used on both the face and the electrodes. There were 5 electrodes used. Corrugator superciili activity was monitored throughout the cognitive and physical tasks.

Measurement reliability and validity can depend on the procedure used during facial EMG recording, signalling and cleaning of the data. Methodological choices for facial EMG data collection and analysis can affect the results and possible interpretation (Lajante et al., 2017). Data collection will use the same procedures outlined in the Pakiri lab manual developed by Dr Peter Cannon and Dr Michael Philipp (Appendix L). The sampling frequency of the raw fEMG signal was 2KHz and the data was filtered to remove power line frequency (50Hz) both low

(<30Hz) and high-frequency (>500Hz) artefacts, such as cable movement or RF interference, and was consistent across all participants.

Maximal Oxygen Consumption (VO_2max)

VO_2max was taken in the last minute of VO_2max cycling test. This used a Douglas bag and gas analyser in compliance with Health and Safety standards in the Sports and Exercise Laboratory at Oteha Rohe campus. The gas analyser was calibrated weekly by the Laboratory manager. VO_2 measurement involved the researcher placing a nose clip on the participants nose and the breathing piece into their mouth and breathing for 1 minute while still exercising. Nose clip and mouthpiece placement were practised before the start of the exercise test with each participant.

Rating of Perceived Exertion Scale

Rating of Perceived Exertion was used to determine self-assessed fatigue. Borg (1998) used peripheral and central nervous system fatigue information to develop this index. The scale begins at 6 and progresses until 20 (see Appendix H). An RPE index was taken at baseline (before cycling), every two minutes during the cycling task and a final index was taken immediately after cycling concluded.

Body Composition Analysis

Body composition was calculated using the InBody 230, a bioelectrical impedance analyser. BIA has an excellent relative agreement with Bodpod and DXA (Hurst et al., 2016). It is easy to use, portable and inexpensive compared to other body composition analysis methods.

The BIA is a non-invasive test simply involves standing on a scale-like device that has two electrodes plates. The participant was required to hold two arms which also have electrode plates. A low level, imperceptible electrical current was sent through the body which assessed the percentage of water, muscle and fat mass.

Food Diaries

Food diaries were collected from participants for both sessions. Food diaries were taken for two days, four days prior to each session. Participants were instructed to write all the food they had consumed within those two days (see Appendix F). The researcher then extracted the fruit and vegetable consumption in the food diaries and used FoodWorks 8 to complete the analysis.

Phytochemical Manipulation

The participants consumed either the blackcurrant or sugar control juice one hour prior to commencing the second session. This was served in a blue plastic cup to minimise participants recognising subtle differences in colour.

Blackcurrant

Participants who were allocated to the blackcurrant group were given a juice at the second session. The berry fruit extract used is a blackcurrant freeze-dried powder prepared by Plant and Food Research which has met regulations the extract is required to meet the standard outlined in the Dietary Supplements Regulations 1985, and the Food Act 1981. This juice contained 11grams of crushed blackcurrant powder (approximately 3.2mg anthocyanins/kg body weight) dissolved in 200mL of water.

Total anthocyanins: 2100mg/100g

Total Phenolics: 1.8g/100g

Total Vitamin C: 880mg/100g

Control

Sugar control drink containing equivalent sugars present in the blackcurrant drink. This contained glucose, fructose and sucrose dissolved in 200mL of water with a blackcurrant flavanol which contained no extra sugars. These sugars were equivalent to the sugar content within the blackcurrant manipulation drink.

Dietary Restrictions

Two days prior to the sessions, participants were requested to avoid a list of foods that have similar properties to blackcurrant (see Appendix I). The two days prior to the food restrictions, four days before the session, the participants were instructed to take food dairies for two days and eat as normally as possible throughout these days.

The day of the session, participants were asked not to eat two hours prior or drink one-hour prior to their time.

Procedure

There were two sessions and a follow up email. The first session determined baseline data such as fitness and psychometric scores. The second session involved the fruit extract intervention in the form of a juice and exercise followed by psychometric testing. The email was to determine if fruit extract can affect recovery from exercise.

Familiarisation Session

Participants were given an information sheet, standard health screening questionnaire, consent form and a reminder email (see Appendix C, D and E, F). This had previously been distributed through email to the participants. Body composition and height was taken using InBody 320 bioelectrical impedance analyser and stadiometer respectively.

Participants were then requested to wash their foreheads to start skin preparation for the facial electromyography electrodes. Electrodes for the electrocardiography were also placed. Once the electrodes were tested for functionality, the ergometer was adjusted and completed five blocks of the Stroop test for familiarisation. Each block consisted of 16 colour-word stimuli and in between each familiarisation trial, participants were prompted to take a small break and then press a key to continue. Participants could stay seated on the bike or stand if that was more comfortable.

After completing familiarisation, participants then completed pre-exercise Stroop which consisted of 32 colour-word stimuli. The researcher then removed the stimuli response board and participants completed a two-minute warm up. They were then asked to stop cycling, provide a baseline RPE score and then start the test. The cycling test was run to a Standard VO_2 max Protocol. This requires continuously cycling at 60rpm with 0.5kg resistance added every 2 minutes. During this time, facial electromyography and electrocardiography were used to monitor facial activity and heart rate. RPE index was requested every two minutes before the additional weight was added. VO_2max was collected when participants indicated they were in their last minute of exercise. At this stage, the researcher used encouragement to motivate participants to complete the final minute maintain 60rpm. After the minute is completed, final RPE index was taken and participants were given a drink of water. The stimuli response tray was returned to the bike handles where the participants were then instructed to complete post-exercise Stroop test which consisted of 32 colour-word items. Participants were then encouraged to warm-down and stretch and received water and were able to eat high carbohydrate confectionaries by the researcher.

Second Session

Participants had booked the same time slot, one week apart. For the second session, the participant arrived one hour earlier and consumed either the blackcurrant juice or control juice which was prepared earlier on the same day by the researcher. The participant could then relax in the next room for an hour until their appointment time. Participants were then instructed to clean their forehead with provided cleanser and their skin was prepared for the facial electromyography electrodes and electrocardiography electrodes were applied. The session then followed the same structure as the familiarisation session where participants would complete pre-exercise Stroop test, warm-up, Standard VO_2max test and post-exercise Stroop test. Data was collected separately to Session 1 data to avoid bias.

Once completed, the participant was debriefed, thanked for their time and received the $30 Countdown voucher and body composition analysis print out, with a brief explanation provided by the researcher. A report explaining the body composition analysis and VO_2max scores was then sent out to their email address.

Follow-up email

An email was sent out the morning after the participant's second session asking whether they felt more, less or the same amount of soreness and fatigue as their previous session.

Group Assignment and Statistical Power

The participants were randomly assigned to one of two conditions: blackcurrant or control group. There were 31 participants assigned the blackcurrant condition and 29 assigned to the control condition. This discrepancy occurred to randomisation. A *a priori* power analysis using the G*Power program was completed with a power of 0.80 and an alpha level of 0.05 (Faul, Erdfelder, Lang, & Buchner, 2007). It was estimated there would be a small effect size due to research completed by Harold (2016) and McKenzie (2017). The program required a sample size of at least 52 participants for the study to have adequate power. This was increased to 60 to allow for participant drop out.

Results

Preliminary ANOVA's indicated that there were no differences due to gender, time of day (am versus pm) differences. However, age indicated statistically significant effect on mean reaction time $F(1,286) = 7.85$, $p<.01$ respectively but not on mean change score of reaction time $F(1,286) = 0.63$, $p>.05$. This would be due to the participants controlling for themselves in the change score. Hypothesis 4 is the only hypothesis affected by mean reaction time and therefore the confounding effect of age.

Data was reviewed to ensure that participants had been physically fatigued from the exercise test.

Next the analysis looked at the effect of the blackcurrant supplement on cognitive and physical effects. The control group and blackcurrant group scores were compared on the Stroop test. Separate analyses were carried out on the Stroop scores: the reaction time differences between congruent, incongruent and control scores, sessions and groups. This study used all three types of Stroop analysis and therefore the comparison of these are reported.

Preliminary Tests

Statistical Assumptions

Before beginning each hypothesis, ANOVA and linear regression assumptions were tested and met. Each hypothesis reports the result of these assumptions.

Descriptive Statistics of Participants

Table 1. Descriptive Statistics of participants – Age, Gender, Group assignment and Ethnicity

	N	Minimum	Maximum	Mean	Std. Deviation
Age	60	18	35	25.48	4.534

		Frequency	Percent
Group	Control	29	48.3
	Blackcurrant	31	51.7
Gender	Female	35	58.3
	Male	25	41.7
Ethnicity	NZ European	26	43.3
	Maori/NZ	5	8.3
	Pacific Island	2	3.3
	Asian	10	16.7
	Other	17	28.3
	Total	60	100.0

Removal of Participants from Data Analysis

Twelve participants were removed from data analysis due to not completing the required testing and therefore having incomplete data sets. This was often due to dizziness experienced after the exercise component which lead to post-exercise Stroop not being taken or completed. Some of the hypotheses have additional participants removed to due data collection error. Data sample size will be specified in each hypothesis.

Hypothesis 1

The first hypothesis was tested by looking at the effects a blackcurrant juice would have on cognitive performance after the physically fatiguing participants. Earlier research conducted by Harold (2016) and McKenzie (2017) found that smaller doses of blackcurrant freeze-dried powder consumed in capsule form did not ameliorate the effects of physical fatigue on cognitive performance. The present study was a continuation of their work. Results showing no effect of blackcurrant supplementation would support both Harold and McKenzie's conclusions. For this hypothesis, 12 datasets were removed due to failure of participants to complete the testing. A total of 48 data sets were used with 22 participants in the control group and 26 participants in the blackcurrant group.

The present study examined the influence that blackcurrant supplementation would have on cognitive performance, measured by the Stroop task. It was expected that blackcurrant supplementation would decrease the fatiguing effects of exercise and that the change in reaction time between pre- and post-exercise of the blackcurrant group would be less than the control group. The change score was calculated by taking mean post-cycle Stroop reaction time and subtracting mean pre-cycle Stroop reaction time. A positive change score signifies that post-cycle reaction time was slower than pre-cycle reaction time while a negative change score signifies that the participant was faster in their post-cycle Stroop test than their pre-cycle test. A change score was used as it eliminates individual differences and participant's act as their own control.

An initial one-way ANOVA was conducted between Group and baseline change in Reaction Time and found no statistically significant difference between groups $F(1,142) = 1.25$, $p=.27$, $\eta^2_g =.009$. The Levene test, which tests the homogeneity of variance, demonstrated that the change score in Stroop reaction time variance was not significantly different in the two groups, p=.13. Mauchly's test for sphericity completed to assess the variances between the combinations of all conditions. In this case, there was no violation of the assumption with all p-values of the between-within conditions, all p>.05. A visual inspection of the plots (Appendix N) for the ANOVA assumptions revealed that all assumptions were met. Visual inspection of Q-Q plot revealed that the distribution of error was approximately normal.

In previous research conducted by Harold (2017) and McKenzie (2018), the main limitations were that practice effects were not assessed at the time of testing and therefore were not able to be controlled. For this study, five practice blocks of the Stroop test were conducted in both sessions prior to recording the data that would be used in the hypotheses. Figure 1 shows a significant reduction in reaction time across both groups between practice block one and two. Across the next four practice blocks the reaction time stabilises signifying that any change in reaction time during the pre- and post-cycle Stroop test can be attributed to the intervention rather than confounded through practice effects.

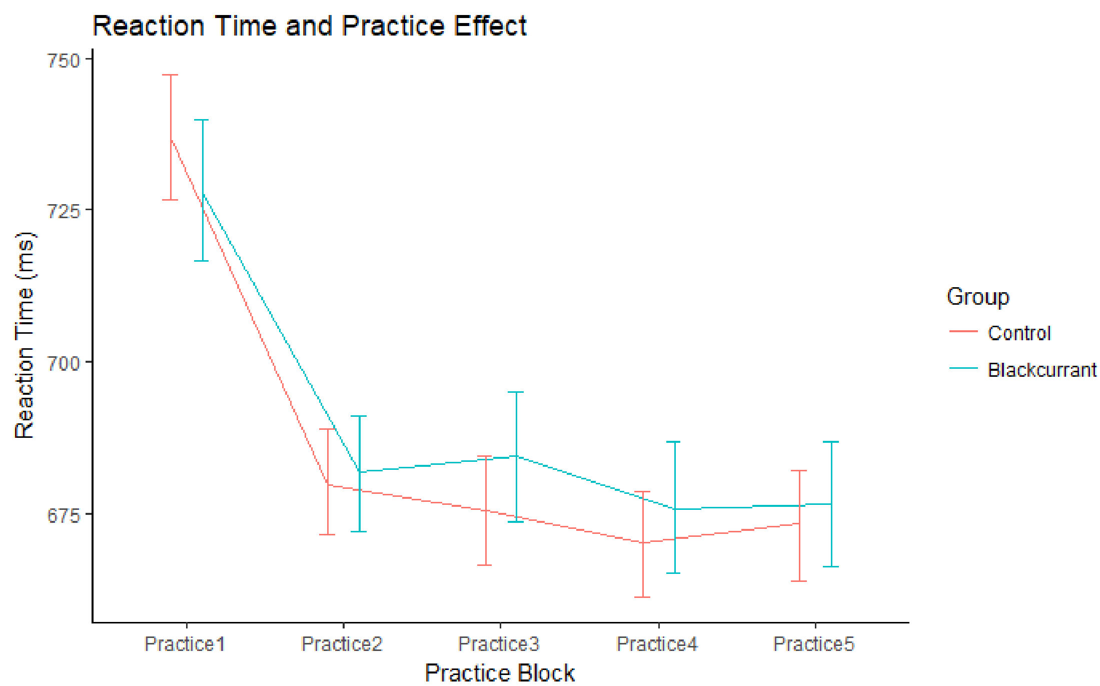

Figure 1. Line graph of Practice Stroop Blocks and Mean Reaction Time (ms). Error bars show 95% Confidence Interval

Separate analyses were conducted for the congruent, control and incongruent Stroop conditions. In particular, it was expected that the incongruent condition would be the most effected by the fatiguing exercise and that the blackcurrant group would reduce this impact compared to the control group.

The overall Stroop scores expected a lower value of the mean change score in the blackcurrant group compared to the control group. In session one, there was no intervention and both the blackcurrant and control group showed mean change scores that illustrated that they were faster in their post-cycle Stroop block compared to the pre-cycle Stroop block with change scores of -10.38 ms [-23.98, 3.22] and -22.66 ms [-40.21, -5.11] respectively.

In session two, there was an 11-millisecond difference in reaction time between the control and blackcurrant group. The blackcurrant group were faster in their post-cycle Stroop test with a change score of -1.63 ms [-13.49, 10.22] while the control group were slower with a mean change score of 9.44 ms [-6.16, 25.04]. As the confidence intervals for the mean difference included zero in both conditions, this suggests that overall the two groups' change scores were similar.

This appears to be contrary to the expected effect as both groups had faster reaction times in their session one Stroop blocks compared to their session two Stroop blocks. The blackcurrant group became 8.75 milliseconds slower while the control group became 32.10 milliseconds slower in session two compared to session one.

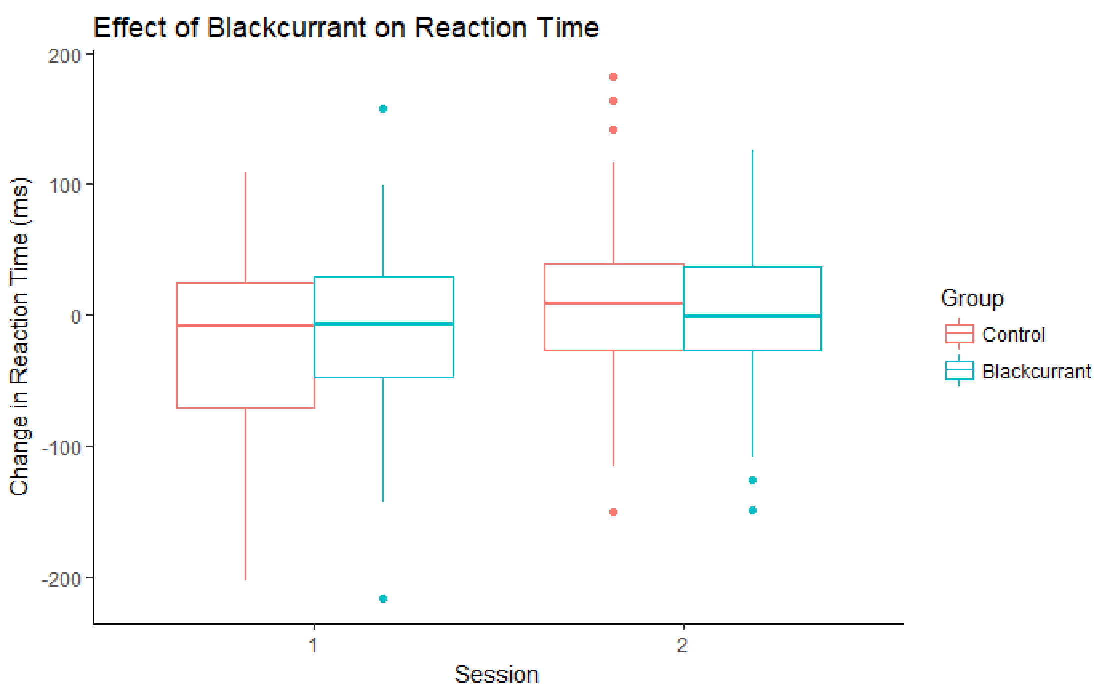

Figure 2. Boxplot showing Groups effect on Change in Reaction Time (milliseconds) across Sessions

Figure 2 gives a visual representation of the above statistics. This boxplot shows that groups did not differ greatly between sessions and that there was large variability in change scores of both groups.

An ANOVA analysis with repeated measures for between and within subject's data was run to investigate hypothesis one.

Table 2. Repeated Measures Between-within Subject's ANOVA Analysis

Predictor	df_{Num}	df_{Den}	*Epsilon*	*F*	*p*	η^2_g
(Intercept)	1.00	46.00		1.02	.318	.01
Group	1.00	46.00		0.00	.962	.00
Session	1.00	46.00		6.11	.017	.03
Group x Session	1.00	46.00		2.00	.164	.01
Stroop Condition	1.85	84.98	0.92	0.95	.386	.00
Group x Stroop Condition	1.85	84.98	0.92	0.49	.602	.00
Stroop Condition x Session	1.86	85.68	0.93	0.72	.479	.00
Group x Stroop Condition xSession	1.86	85.68	0.93	0.44	.632	.00

Note. df_{Num} indicates degrees of freedom numerator. df_{Den} indicates degrees of freedom denominator. Epsilon indicates Greenhouse-Geisser multiplier for degrees of freedom, *p*-values and degrees of freedom in the table incorporate this correction. η^2_g indicates generalized eta-squared.

Table 2 shows that the ANOVA revealed no significant effects for Group or Stroop Condition with both Fs <1. Session had a significant effect, $F(1,46)=6.11$, $p=.017$, $\eta^2_g = .03$, indicating a small effect size however the expected interaction of Group and Session did not occur. There was no significant interaction between Group and Stroop Condition or Group, Stroop Condition and Session. This was supported by the close to zero effect size representing the variance explained by the Group and Stroop Condition variables. A three-way robust ANOVA was also run and replicated the repeated measure ANOVA's results with a significant interaction of Session and Mean Change Score, $p=.02$ but no significant interactions between Group and Session, $p=.24$.

A planned contrast between Mean Change Score and Session confirmed that Session two had a significant effect on Change Score, $p=.006$ however this did not differ between groups, p=.11. As shown in the mean and confidence interval data above, this change was *contrary* to the hypothesised decrease in mean change score in session two and instead resulted in an increase in change scores across both groups.

These results suggest that the blackcurrant intervention had no statistically significant effect on the change score for reaction time in the Stroop test.

A post-hoc *Sensitivity* power analysis was run using the G*Power programme (Output can be viewed in Appendix O). Given a sample size of 48, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of f= .329, assuming statistical significance and such an effect size actually exists. As η^2_g =.00, there is mostly likely no effect that exists in this model.

Stroop Congruent Condition

In the Stroop Congruent task, the stimulus word matched the stimulus colour. The dependent variable measured was the time taken to respond by pressing the colour-coded button that matched the colour of the font of the word on the screen. The means and standard deviations for the Congruent Stroop condition are given in Table 3.

Table 3. Means and Standard Deviations for Change Score for the Stroop Congruent Task as a function of a 2(Session) X 2(Group) design

	Group			
	Control		Blackcurrant	
Session	*M*	*SD*	*M*	*SD*
1	-26.24	-0.11	-10.86	-0.05
2	7.58	0.03	6.55	0.03

Note. *M* and *SD* represent mean and standard deviation, respectively.

The change score for reaction time increased in session two in both the blackcurrant and the control group. Both groups had similar change in reaction times for session two. However, the control group had a great deterioration compared to their session one scores. The blackcurrant group were 17 milliseconds slower in session 2 while the control group were 34 seconds slower. Figure 3 shows the interaction between change in reaction time (ms) and group.

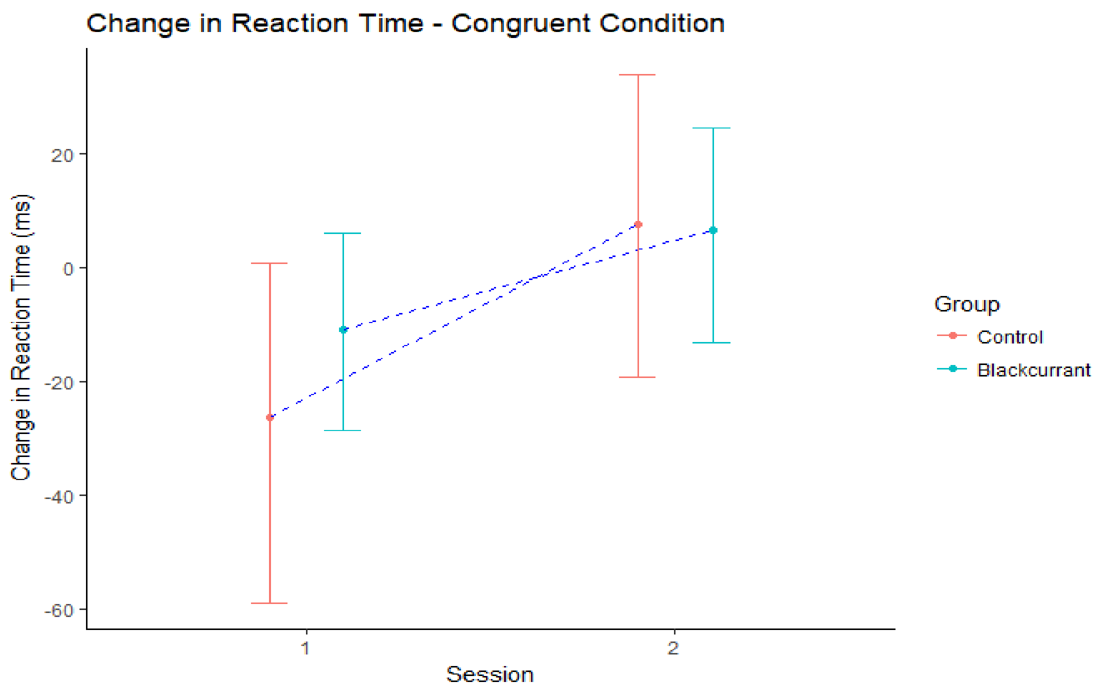

Figure 3. Mean Change in Reaction Time in the Stroop Congruent Condition across Sessions. Error bars show 95% Confidence Interval

Figure 3 shows that both groups reactions times were slower in session two. The hypothesis had predicted that the blackcurrant intervention group reaction time would have become faster as the blackcurrant would have eliminated the negative effects that maximal physical exercise has on cognition.

There was no significant interaction between Group and Congruent Stroop Condition $F(1,46)=.25$, $p=.62$, $\eta^2_g=.004$ however there was a significant effect of Session on the Congruent Stroop Condition, $F(1,46)=7.33$, $p=.009$, $\eta^2_g=.04$, confirming the previous result in the overall Stroop result section which illustrated an effect opposite to that hypothesised. There was a non-significant interaction effect between the Session and Group on the Congruent Stroop Condition, $F(1,46)=.75$, $p=.39$, $\eta^2_g=.005$. These results indicate that Group and Session did not affect the Congruent Stroop Condition and therefore did not confirm this hypothesis.

Stroop Control Condition

In the Stroop Control task, an "X" was presented with one of the four stimulus colours. The dependent variable measured was the time taken to respond by pressing the colour-coded button that matched the colour of the font of the "X" on the screen. The means and standard deviations for the Control Stroop condition are given in Table 4.

Table 4. Means and Standard Deviations for Change Score for the Stroop Control Task as a function of a 2(Session) X 2(Group) design

	Group			
	Control		Blackcurrant	
Session	*M*	*SD*	*M*	*SD*
1	-15.75	-0.07	-12.39	-0.05
2	16.41	0.07	2.64	0.01

Note. *M* and *SD* represent mean and standard deviation, respectively.

The change score for reaction time increased in session two in both the blackcurrant and the control group. However, the control group reaction time increased by 32 milliseconds in session two compared with the blackcurrant group that increased only 15 milliseconds. Figure 4 shows the interaction between change in reaction time (ms) and group.

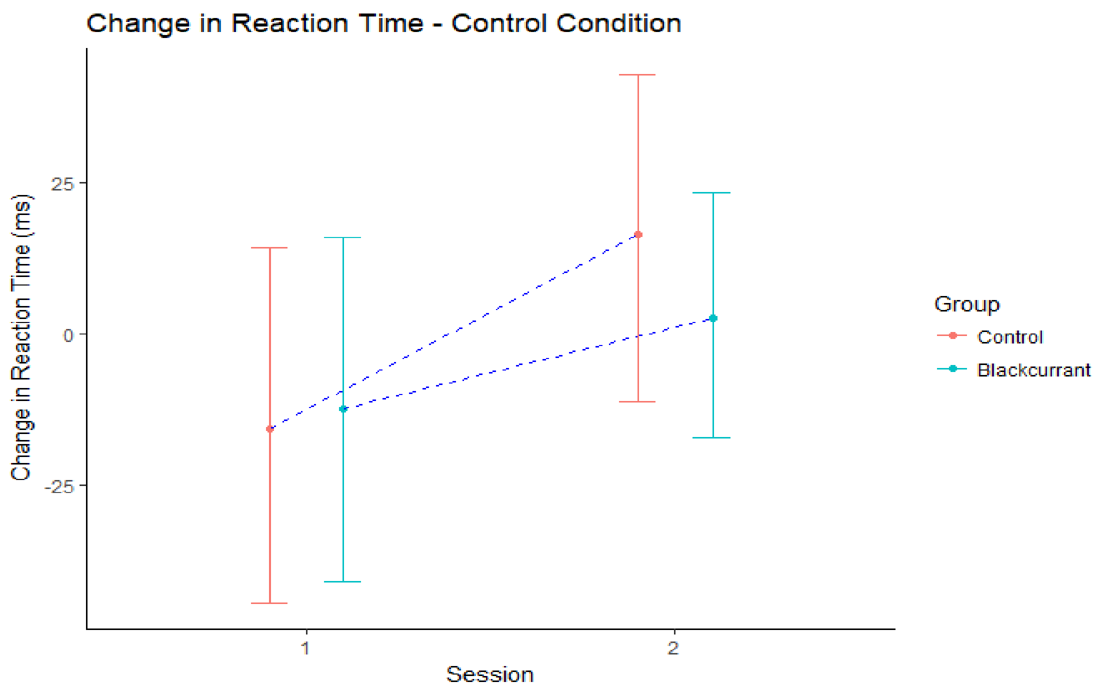

Figure 4. Mean Change in Reaction Time in the Stroop Control Condition across Sessions. Error bars show 95% Confidence Interval

Figure 4 shows that both groups reactions times were slower in session two. The hypothesis had predicted that the blackcurrant intervention group reaction time would have become faster as the blackcurrant would have eliminated the effects that maximal physical exercise has on cognition.

There was no significant interaction between Group and Control Stroop Condition $F(1,46)=.11$, $p=.74$, $\eta^2_g =.002$ or Session, $F(1,46)=3.76$, $p=.06$, $\eta^2_g =.03$. There was a non-significant interaction effect between the Session and Group on the Control Stroop Condition, $F(1,46)=.50$, $p=.48$, $\eta^2_g =.004$. These results indicate that Group and Session did not affect the Control Stroop Condition and therefore did not confirm this hypothesis.

Stroop Incongruent Condition

In the Stroop Incongruent task, the stimulus word did not match the stimulus colour. The dependent variable measured was the time taken to respond by pressing the colour-coded button that matched the colour of the font of the word on the screen and not the word itself. The means and standard deviations for the incongruent Stroop condition are given in Table 5.

Table 5. Means and Standard Deviations for Change Score for the Stroop Incongruent Task as a function of a 2(Session) X 2(Group) design

	Group			
	Control		Blackcurrant	
Session	*M*	*SD*	*M*	*SD*
1	-25.98	-0.11	-7.90	-0.03
2	4.32	0.02	-14.08	-0.06

Note. *M* and *SD* represent mean and standard deviation, respectively.

The change score for reaction time decreased in session two for the blackcurrant group and increased in the control group. This means that in session two, the blackcurrant group were 6 milliseconds faster in reaction time than their session one change in reaction time. The control group instead increased their reaction time by 30 milliseconds between session one and two. Figure 5 shows the interaction between change in reaction time (ms) and group.

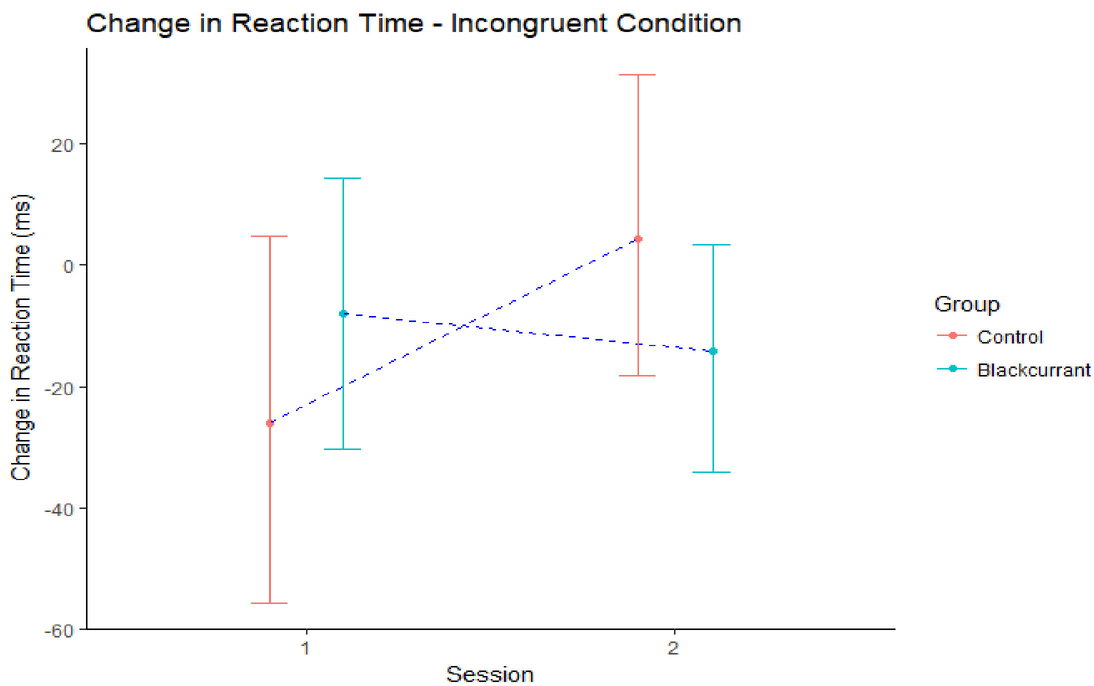

Figure 5. Mean Change in Reaction Time in the Stroop Incongruent Condition across Sessions. Error bars show 95% Confidence Interval

Figure 5 shows that the blackcurrant group had a faster reaction time in their post-cycle test compared to their pre-cycle test where the control group had a slower reaction time than the blackcurrant group and compared to their session one scores. The hypothesis had predicted that the blackcurrant intervention group reaction time would have become faster as the blackcurrant would have eliminated the effects that maximal physical exercise has on cognition. These results appear to show that the maximal exercise has had less effect on the blackcurrant group than the control group in session two.

There was no significant interaction between Group and Incongruent Stroop Condition $F(1,46)=.00$, $p=.99$, $\eta^2_g=.000$ or Session, $F(1,46)=1.27$, $p=.27$, $\eta^2_g=.000$. There was a non-significant interaction effect between the Session and Group on the Incongruent Stroop

Condition, $F(1,46)=2.89$, $p=.10$, $\eta^2_g = .000$. These results indicate that Group and Session did not affect the Incongruent Stroop Condition and therefore did not confirm this hypothesis.

Hypothesis 2

The second hypothesis was tested by attaching facial electrodes to the corrugator superciili muscle on the face throughout the Stroop test. This activity was separated into fixation periods which were half a second prior to the stimulus word-colour being presented on the screen and used a # to indicate a pause between stimuli. The corrugator data used for this analysis was the 500-millisecond period during the stimulus word-colour presentation and 1000-milliseconds after.

The present study examined the influence that blackcurrant supplementation would have on the facial display of effort during the Stroop task, measured by the corrugator superciili muscle. It was expected that blackcurrant supplementation would reduce the fatiguing effects of exercise and show reduced corrugator activity compared to the control group.

A change score was then calculated for every Stroop trial where the fixation (resting) data was used as a baseline and the stimuli activity was subtracted from that baseline for each trial. A new baseline was used for every trial as a person's resting face increases in frowning over time and so we used a change score to avoid confounding the Stroop corrugator activity. A score of zero signifies that the participants frowning has not changed between the fixation and stimuli presentation. A positive corrugator score indicates an increase in the corrugator superciili muscle activity compared to the fixation period. A negative number means that muscle activity is less than the fixation period which means that the muscle has relaxed. This activity was then averaged over the three different Stroop conditions and a positive high number signifies that participants frowned more while a negative value signifies that the participants relaxed their corrugator muscle. For this hypothesis, the previously mentioned 12 datasets were removed due to failure of participants to complete the testing and an additional 14 participants were removed due to the facial EMG data being incomplete, either through electrode dysfunction or removal during testing. A total of 34 data sets were used with 14 participants in the control group and 20 participants in the blackcurrant group.

An initial one-way ANOVA was conducted between Group and Mean Corrugator Activity and found no statistically significant difference between groups $F(1,202) = 2.00$, $p>.05$, $\eta^2_g = .09$. For the Levene test which tests the homogeneity of variance, corrugator superciili mean activity was not significantly different in the two groups, $p=.129$. Mauchly's test for sphericity was completed to assess the variances between the combinations of all conditions. In this case, there was no violation of the assumption with all p-values of the between-within conditions $p>.05$. A visual inspection of the plots (Appendix N) for the ANOVA assumptions revealed that all assumptions were met. Visual inspection of Q-Q plot revealed that the distribution of error was approximately normal.

Separate analyses were conducted for the congruent, control and incongruent Stroop conditions. In particular, it was expected that the incongruent condition would be the most effected by the fatiguing exercise and that the blackcurrant group would reduce this impact more compared to the control group.

The overall Stroop scores expected a lower amount of corrugator activity in the blackcurrant group compared to the control group. In session one, there was no intervention and the both the blackcurrant and control group showed corrugator activity around 0 µV with -0.02 µV [-0.10, 0.06] and 0.06 µV [-0.01, 0.14] respectively. These results show small change from the resting fixation period in response to the stimuli presentation.

In session two, there was a 0.14 µV difference between the control group and the blackcurrant group. The blackcurrant group had a mean activity of 0.09 µV [0.00, 0.18] which indicates very small frowning muscle contraction while the control group had a mean activity of -0.05 µV [-0.20, 0.09]. As the confidence intervals for the mean difference included zero, this suggests that the two groups muscle activity was similar.

These results are in contrast to the hypothesised effect as the blackcurrant group had greater contraction of the superciili muscle, and therefore more facial displays of effort compared to the control group.

An ANOVA analysis with repeated measures for between and within subject's data was run.

Table 6. Repeated Measures Between-within Subject's ANOVA Analysis

Predictor	df_{Num}	df_{Den}	*Epsilon*	*F*	*p*	η^2_g
(Intercept)	1.00	32.00		0.23	.637	.00
Group	1.00	32.00		0.14	.712	.00
Session	1.00	32.00		0.00	.966	.00
Stroop Block	1.00	32.00		0.50	.485	.00
Group x Session	1.00	32.00		3.24	.081	.01
Group x Stroop Block	1.00	32.00		2.45	.127	.01

Session x Stroop Block	1.00	32.00		0.01	.911	.00
Group x Session x Stroop Block	1.00	32.00		0.07	.795	.00
Stroop Condition	1.80	57.74	0.90	0.85	.421	.00
Group x Stroop Condition	1.80	57.74	0.90	0.76	.461	.00
Stroop Condition x Session	1.77	56.61	0.88	1.89	.164	.01
Stroop Condition x Stroop Block	1.68	53.84	0.84	0.35	.672	.00
Group x S Stroop Condition x Session	1.77	56.61	0.88	0.01	.991	.00
Group x Stroop Condition x Stroop Block	1.68	53.84	0.84	1.59	.216	.01
Stroop Condition x Session x Stroop Block	1.93	61.63	0.96	0.82	.440	.00
Group x Stroop Condition x Session x Stroop Block	1.93	61.63	0.96	1.80	.175	.01

Note. df_{Num} indicates degrees of freedom numerator. df_{Den} indicates degrees of freedom denominator. Epsilon indicates Greenhouse-Geisser multiplier for degrees of freedom, *p*-values and degrees of freedom in the table incorporate this correction. η^2_g indicates generalized eta-squared.

Table 6 shows that the ANOVA revealed no significant effects for Group, Stroop Condition, Stroop Block or Session all with F values <1. The expected interaction of Group and Session also did not occur. This was also replicated in the robust ANOVA with no significant interaction between variables, $p>.05$. There was no significant interaction between Group and Stroop Condition or Group, Stroop Condition, Stroop Block and Session. This shows that no reliable difference between the groups was found and this was supported by the close to zero effect size representing the variance explained by the Stroop Condition and Block, Group and Session variables.

A post-hoc *sensitivity* power analysis was run using the G*Power programme (Output can be viewed in Appendix O). Given a sample size of 34, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of *f*= .397, assuming statistical significance and such an effect size exists. As $\eta^2 < .01$ between Stroop condition, session and group, there is mostly likely no effect that exists in this model.

Stroop Congruent Condition

The Stroop Congruent condition should require the least amount of effort out of the three conditions as the response is facilitated by the colour and the word matching, therefore having a faster reaction time and requiring less cognitive effort. The lesser amount of cognitive effort should be reflected in the corrugator activity, with a zero or negative value. The means and standard deviations of corrugator superciili activity are given in Table 7 and Table 8 for Session one and two receptively.

Table 7. Means and Standard Deviations of Corrugator Superciili Activity during the Stroop Congruent Task for Session One

	Session 1					
	Pre-Cycle			Post-Cycle		
Group	*M*	*M* 95% CI [LL, UL]	*SD*	*M*	*M* 95% CI [LL, UL]	*SD*
Control	0.10	[-0.17, 0.37]	0.46	0.04	[-0.09, 0.17]	0.23
Blackcurrant	0.02	[-0.14, 0.18]	0.34	0.02	[-0.20, 0.24]	0.47

Note. *M* and *SD* represent mean and standard deviation, respectively. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval for the mean, respectively.

Table 8. Means and Standard Deviations of Corrugator Superciili Activity during the Stroop Congruent ask for Session Two

	Session 2					
	Pre-Cycle			Post-Cycle		
Group	*M*	*M* 95% CI [LL, UL]	*SD*	*M*	*M* 95% CI [LL, UL]	*SD*
Control	-0.26	[-0.91, 0.38]	1.11	-0.04	[-0.09, 0.17]	0.38
Blackcurrant	0.00	[-0.15, 0.15]	0.32	0.05	[-0.20, 0.24]	0.34

Note. *M* and *SD* represent mean and standard deviation, respectively. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval for the mean, respectively.

Both groups across both sessions had high levels of variations in their corrugator superciili muscle activity. In session two, the control group showed a relaxation of the muscle with negative corrugator scores in Stroop blocks pre- and post-cycling. Meanwhile the blackcurrant group showed no difference in corrugator muscle activity in response to the Stroop blocks prior to starting exercise and a slight increase in corrugator muscle activity – more frowning - in the post-cycle Stroop blocks. It is important to note that in both groups has the following restrictions: the mean value is so small and close to zero, the standard deviations are large, and the confidence intervals for the mean difference included zero which suggests that overall the two groups’ activity were similar. This highlights that there was no significant difference between the two groups, as shown by the ANOVA in Table 6 and the close to zero effect size.

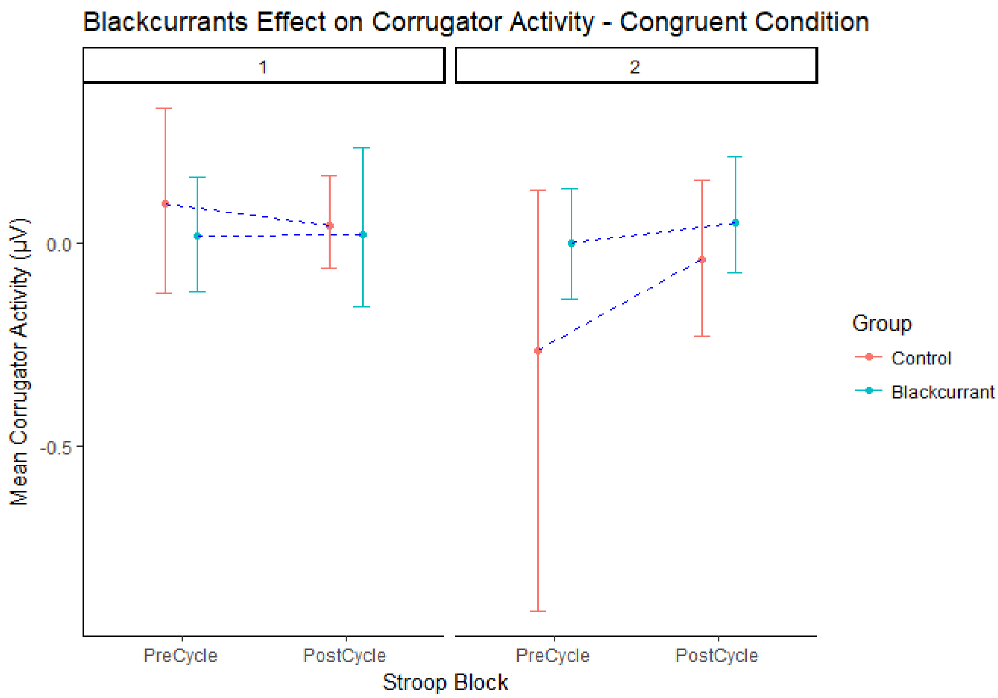

Figure 6. Corrugator Activity for the Congruent Stroop Condition across Sessions. Error bars show 95% Confidence Interval

Figure 6 shows that the blackcurrant group had a small increase in corrugator superciili activity in session two suggesting that effort and negative affect increased in the post-cycle Stroop block. The control group corrugator activity decreased in session two however their pre-cycle Stroop block scores were highly variable.

There was no significant interaction between Group and Congruent Stroop Condition $F(1,32)=.38$, $p=.54$, $\eta^2_g=.004$ or Session, $F(1,32)=2.07$, $p=.16$, $\eta^2_g=.01$, or Stroop Block, $F(1,32)=.45$, $p=.51$, $\eta^2_g=.003$. There was a non-significant interaction effect between the Session, Group and Stroop Block on the Congruent Stroop Condition, $F(1,32)=.46$, $p=.50$, η^2_g

=.003. These results indicate that Group, Session and Stroop Block did not affect the Congruent Stroop Condition and therefore did not confirm this hypothesis.

This suggests that blackcurrant did not reduce corrugator activity, which is the physiological marker of effort, during the congruent component of the Stroop task.

Stroop Control Condition

The Stroop Control condition should require more effort than the congruent condition but less than the incongruent condition. This is due to the participant still needing to attend to the colour rather than the "X" but does not have to inhibit the natural response to read the word and look at the colour. Therefore, corrugator activity should be slightly more positive than the congruent condition but less than the incongruent condition as per the Stroop effect. The means and standard deviations of corrugator superciili activity are given in Table 9 and Table 10, showing session one and session two data respectively.

Table 9. Means and Standard Deviations of Corrugator Superciili Activity during the Stroop Control Task for Session One

	Session 1					
	Pre-Cycle			Post-Cycle		
Group	*M*	*M* 95% CI [LL, UL]	*SD*	*M*	*M* 95% CI [LL, UL]	*SD*
Control	0.12	[-0.07, 0.30]	0.32	0.10	[-0.04, 0.23]	0.24
Blackcurrant	-0.08	[-0.24, 0.08]	0.34	0.00	[-0.25, 0.25]	0.54

Note. *M* and *SD* represent mean and standard deviation, respectively. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval for the mean, respectively.

Table 10. Means and Standard Deviations of Corrugator Superciili Activity during the Stroop Control Task for Session Two

	Session 2					
	Pre-Cycle			Post-Cycle		
Group	*M*	*M* 95% CI [LL, UL]	*SD*	*M*	*M* 95% CI [LL, UL]	*SD*
Control	0.17	[-0.10, 0.44]	0.46	-0.14	[-0.63, 0.35]	0.85
Blackcurrant	0.01	[-0.28, 0.30]	0.62	0.20	[-0.09, 0.50]	0.63

Note. *M* and *SD* represent mean and standard deviation, respectively. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval for the mean, respectively.

In the second session, the control group had a higher activation of the corrugator muscle by 0.157 µV in the pre-cycle Stroop block. However, in the post-exercise block, the blackcurrant condition had higher activation of the corrugator muscle by 0.342 µV signifying that the blackcurrant group exerted more effort in the post-cycle Stroop test in session two compared to the control group. Similarly to the congruent condition, both groups had large variation, values close to zero and confidence intervals overlapping zero which signify no change in corrugator activity between the resting period and the stimuli presentation and therefore no physiological marker of perceived effort.

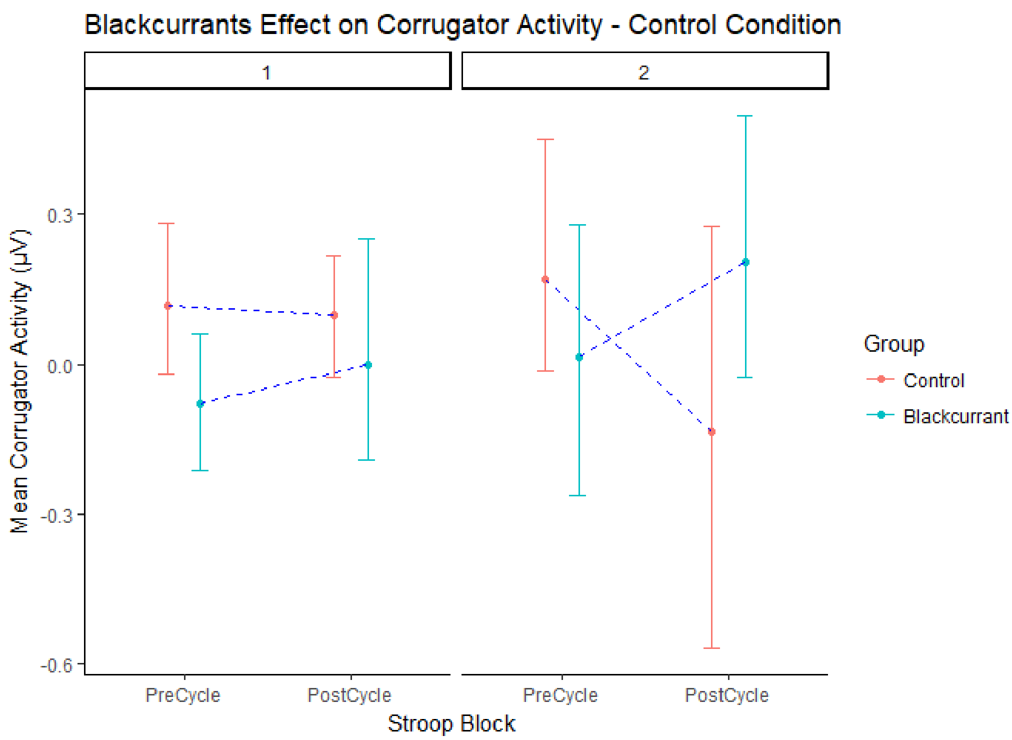

Figure 7. Corrugator Activity for the Control Stroop Condition across Sessions. Error bars show 95% Confidence Interval

Figure 7 shows that the blackcurrant group had low corrugator muscle activity during the Stroop test before exercising which largely increased after exercising in session two. This suggests that the post-cycle Stroop test required more effort than the pre-cycle Stroop test. The control group instead maintained similar corrugator activity across pre- and post-cycle Stroop tests.

There was no significant interaction between Group and Control Stroop Condition $F(1,32)=.07$, $p=.79$, $\eta^2_g=.001$ or Session, $F(1,32)=.07$, $p=.78$, $\eta^2_g=.001$, or Stroop Block, $F(1,32)=.03$, $p=.86$, $\eta^2_g=.0002$. There was a non-significant interaction effect between the Session, Group and Stroop Block on the Control Stroop Condition, $F(1,32)=1.51$, $p=.22$, η^2_g

=.008. These results indicate that Group, Session and Stroop Block did not affect the Control Stroop Condition and therefore did not confirm this hypothesis.

This suggests that blackcurrant did not reduce corrugator activity, which is the physiological marker of effort, during the control Stroop task.

Stroop Incongruent Condition

The Stroop Incongruent condition should require the most amount of cognitive effort out of the three conditions as the response required is unnatural as the participant must inhibit the natural response to read and respond to the word stimulus, and instead attend the colour of the ink and respond appropriately. As these don't match, the participants reaction time is slower and due to requiring more cognitive effort. The higher amount of cognitive effort should be reflected in the higher positive values of corrugator activity. The means and standard deviations of corrugator superciili activity during the two sessions are given in Table 11 and Table 12.

Table 11. Means and Standard Deviations of Corrugator Superciili Activity during the Stroop Incongruent Task for Session One

	Session 1					
	Pre-Cycle			Post-Cycle		
Group	*M*	*M* 95% CI [LL, UL]	*SD*	*M*	*M* 95% CI [LL, UL]	*SD*
Control	0.07	[-0.05, 0.20]	0.22	-0.05	[-0.32, 0.22]	0.47
Blackcurrant	-0.18	[-0.38, 0.01]	0.41	0.12	[-0.12, 0.36]	0.52

Note. *M* and *SD* represent mean and standard deviation, respectively. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval for the mean, respectively.

Table 12. Means and Standard Deviations of Corrugator Superciili Activity during the Stroop Incongruent Task for Session Two

	Session 2					
	Pre-Cycle			Post-Cycle		
Group	*M*	*M* 95% CI [LL, UL]	*SD*	*M*	*M* 95% CI [LL, UL]	*SD*
Control	-0.03	[-0.41, 0.34]	0.65	-0.02	[-0.25, 0.21]	0.40
Blackcurrant	0.10	[-0.07, 0.27]	0.36	0.20	[-0.10, 0.49]	0.64

Note. *M* and *SD* represent mean and standard deviation, respectively. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval for the mean, respectively.

The control group shows more corrugator muscle relaxation in session two prior to cycling and after completion compared to the blackcurrant group. The blackcurrant group had a higher corrugator muscle activation in the pre-cycle Stroop blocks by 0.131 µV and also higher muscle activation in post-cycle Stroop blocks by 0.212 µV. This demonstrates that the blackcurrant group exerted more effort in both Stroop blocks, resulting in higher frowning and therefore perceived effort exerted, compared to the control group. Similarly to other Stroop groups, both groups had high variance seen through large standard deviations and confidence intervals that include zero. All the corrugator superciili values are close to zero and signify no change to very small change in corrugator activity between the resting period and the stimuli presentation and therefore no physiological marker of perceived effort. This is consistent with the ANOVA results in Table 6 and the zero effect scores.

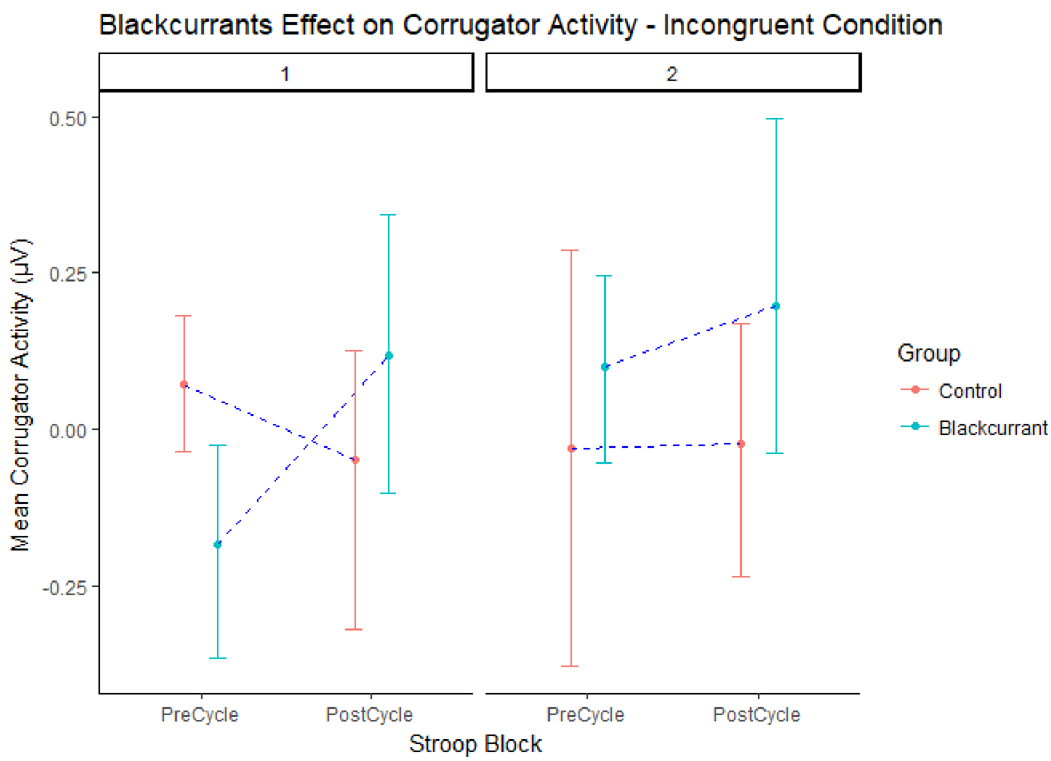

Figure 8. Corrugator Activity for the Incongruent Stroop Condition across Sessions. Error bars show 95% Confidence Interval

Figure 8 shows that the blackcurrant group had higher corrugator muscle activity during the Stroop test before and after exercising compared to the control group in session two. The blackcurrant group corrugator activity also increased largely after exercising in session two compared to the control group mean. However, the control group had larger variability compared to the blackcurrant group. This suggests that the post-cycle Stroop test required more effort than the pre-cycle Stroop test and that the pre-cycle Stroop also required more effort compared to the congruent and control conditions.

There was no significant interaction between Group and Incongruent Stroop Condition $F(1,32)=.44$, $p=.51$, $\eta^2_g=.003$ or Session, $F(1,32)=.92$, $p=.34$, $\eta^2_g=.005$, or Stroop Block, $F(1,32)=.72$, $p=.40$, $\eta^2_g=.006$. There was a non-significant interaction effect between the Session, Group and Stroop Block on the Incongruent Stroop Condition, $F(1,32)=1.18$, $p=.28$, $\eta^2_g=.007$. These results indicate that Group, Session and Stroop Block did not affect the Incongruent Stroop Condition and therefore did not confirm this hypothesis.

This suggests that blackcurrant did not reduce corrugator activity, which is the physiological marker of effort, during the incongruent Stroop task.

Hypothesis 3

The third hypothesis was tested by attaching facial electrodes to the corrugator muscle on the face throughout the VO2max test (the physical exercise). The corrugator superciili muscle is associated with frowning and contracts significantly at high power outputs that occur at the stage of exhaustion in both females and males (Huang et al., 2014). Facial muscle EMG activity appears to be more sensitive at higher intensity exercise levels. For this hypothesis, the previously mentioned 12 datasets were removed due to failure of participants to complete the testing and an additional 12 participants were removed due to the facial EMG data being incomplete, either through electrode dysfunction or removal during testing. A total of 36 data sets were used with 14 participants in the control group and 22 participants in the blackcurrant group.

The present study examined the influence that blackcurrant supplementation would have on the facial display of effort during exercise, measured by the corrugator superciili muscle. It was expected that blackcurrant supplementation would reduce the fatiguing effects of exercise and show reduced corrugator activity during exercise compared to the control group.

An initial one-way ANOVA was conducted between Group and Mean Corrugator Activity and found no statistically significant difference between groups $F(1,33) = 0.01$, $p=.95$, $\eta^2_g=.012$. The Levene test was used to test the homogeneity of variance, an assumption of ANOVA analysis. This Levene test found that corrugator mean activity during cycling was not significantly different in the two groups, $p=.324$. A visual inspection of the plots (Appendix N) for the ANOVA assumptions revealed that all assumptions were met. Visual inspection of Q-Q plot revealed that the distribution of error was approximately normal.

The mean corrugator activity was similar in session one between the control group, -0.07 µV [-2.71, 2.58], and the blackcurrant group 0.01 µV [-1.13, 1.16]. In session two, the control group had less corrugator activity, -1.73 µV [-5.37, 1.91], then the blackcurrant group 0.89 µV [-1.37, 3.15]. This difference demonstrates that the control group had much less frowning activity in session two compared to their session one values, showing a 1.66 µV decrease in corrugator superciili muscle activity. This signifies a decrease in corrugator activity between the resting period and the stimuli presentation and therefore less perceived effort during the Stroop stimuli presentation. Meanwhile the blackcurrant group had an increase in corrugator superciili activity of 0.88 µV illustrating that this group had higher levels of perceived effort during the Stroop stimuli presentation compared to the resting period. As seen in Figure 9 below, the difference between the two groups in session two is much larger than their scores in session one. The control group had muscle relaxation on average 2.54 µV less than the blackcurrant group. This contradicts the hypothesis where it was predicted that the blackcurrant group would have less muscle activation and more muscle relaxation compared to the control group.

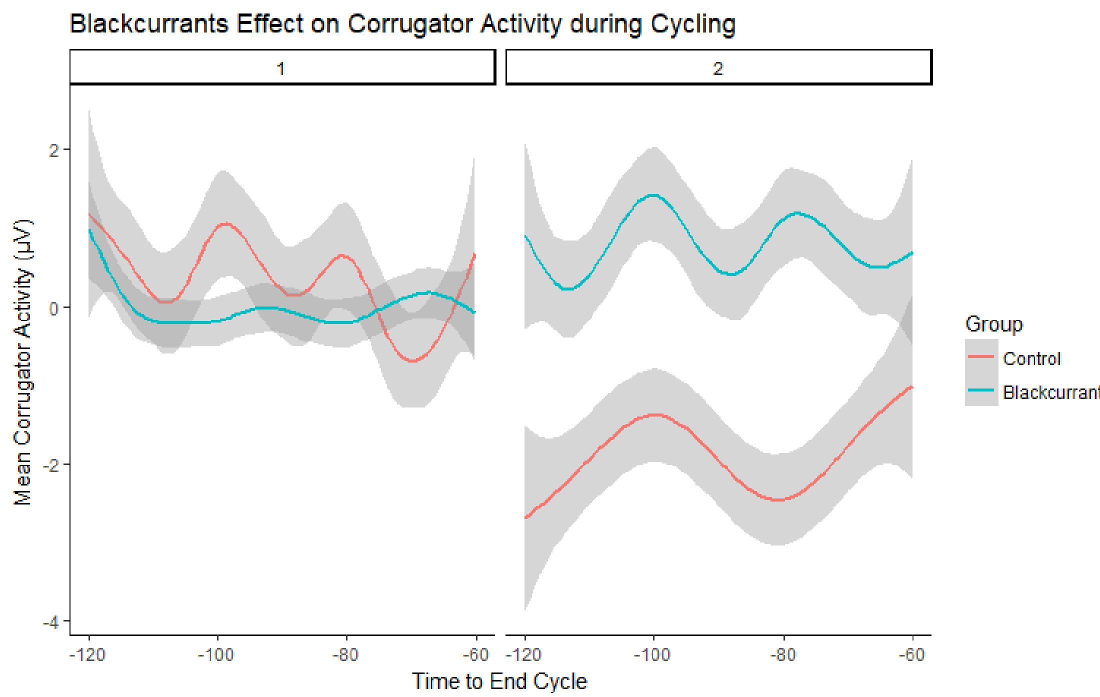

Figure 9. Smoothed line graph of Corrugator Activity (µV) for the time period one to two minutes prior to end cycle. Grey shade indicates 95% Confidence Interval

Figure 9 illustrates the above statistics. This smoothed line graph uses the minute prior to VO_2 collection which is two minutes prior to exercise completion and would be considered submaximal exercise. Figure 9 shows that both groups fluctuate in corrugator superciili activity as the cycling duration continues. This is due to the maximal test becoming harder with more weight added onto the ergometer and therefore more effort being exerted to maintain rpm. In session two, the blackcurrant group has higher corrugator superciili contraction, indicating more perceived effort than their previous session one scores and compared to the control group. The control group in session two show more muscle relaxation but this increases during the cycling duration toward slightly more corrugator muscle contraction. Appendix M shows several individual smoothed corrugator activity curves during exercise.

An ANOVA with repeated measures for between and within subjects was used to analyse this data.

Table 13. Repeated Measures Between-within Subject's ANOVA Analysis

Predictor	df_{Num}	df_{Den}	F	p	η^2_g
(Intercept)	1	33	0.15	.705	.00
Group	1	33	1.33	.258	.02
Session	1	33	0.14	.712	.00
Group x Session	1	33	1.43	.240	.02

Note. df_{Num} indicates degrees of freedom numerator. df_{Den} indicates degrees of freedom denominator. η^2_g indicates generalized eta-squared.

In the repeated-measures ANOVA shown in Table 13, there was no significant main effects of Session with an F value less than 1. Group also did not have a significant effect and the expected interaction of Group and Session on Corrugator Superciili activity during exercise did not occur. This was also replicated in the robust two-way ANOVA with Group, Session and the interaction between Group and Session all at $p>.05$.

Even though there appears to be a difference between the two groups, the ANOVA in Table 13 illustrates that there was no reliable difference between the two groups. This null effect is supported by the close to zero effect size, representing the variance explained by the group and session, and the confidence intervals for the mean difference including zero in both conditions, suggesting that overall the two groups' corrugator superciili activity were similar.

Blackcurrant has not had a statistically significant effect on reducing corrugator muscle activity during exercise and did not reduce the appearance of perceived effort.

A *post-hoc* power analysis was conducted using G*Power (All output can be viewed in Appendix O). With an alpha level of .05, a sample size of 36, and a small effect size of *f*=.14 (Cohen, 1992), achieved power for the study was .128. A post-hoc *Sensitivity* power analysis was run with a sample size of 36, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of *f*= .495, assuming statistical significance and such an effect size actually exists. A post-hoc *a priori* test was also completed using the effect size of *f*=.14 found in this study to determine the sample size for future research. With an alpha level of .05, and minimum power of .80 (Cohen, 1992), 390 participants would be necessary to find a statically significant effect in the model.

Hypothesis 4

The fourth hypothesis was tested through using FoodWorks 8 to analyse the food diaries provided by the participants. Four days' worth of food diaries was collected and fruit and vegetable intake was calculated as portions per day and combined for this analysis. Combined Intake refers to the combined intake of fruit and vegetables. For this hypothesis, only mean reaction time for session two was used. The total number of participants for this hypothesis was 46, 21 in the control group and 25 in the blackcurrant group. An additional two participants were excluded as they did not hand in food diaries and therefore their fruit and vegetable consumption could not be completed.

The present study examined the relationship between blackcurrant supplementation and fruit and vegetable consumption on mean reaction time, measured by the Stroop task. It was expected that higher levels of fruit and vegetable intake would increase the effects of the blackcurrant supplementation through beneficial gut microbiota having been established and would decrease the fatiguing effects of exercise. It was expected the blackcurrant group would have faster reaction times compared to the control group.

An initial one-way ANOVA was conducted between Group and Combined Fruit and Vegetable Intake and found no statistically significant difference in fruit and vegetable consumption, as provided by food diaries, between groups $F(1,44) = .18$, $p=.67$, $\eta^2_g =.06$. The data did not violate any assumptions and regression assumption graphs can be viewed in Appendix N.

Figure 10 below shows that reaction time does not vary across combined fruit and vegetable intake. Contrary to the hypothesis, reaction time in the blackcurrant group increased as fruit and vegetable intake increased while the control group had a decreased reaction time as fruit and vegetable intake increased in both sessions.

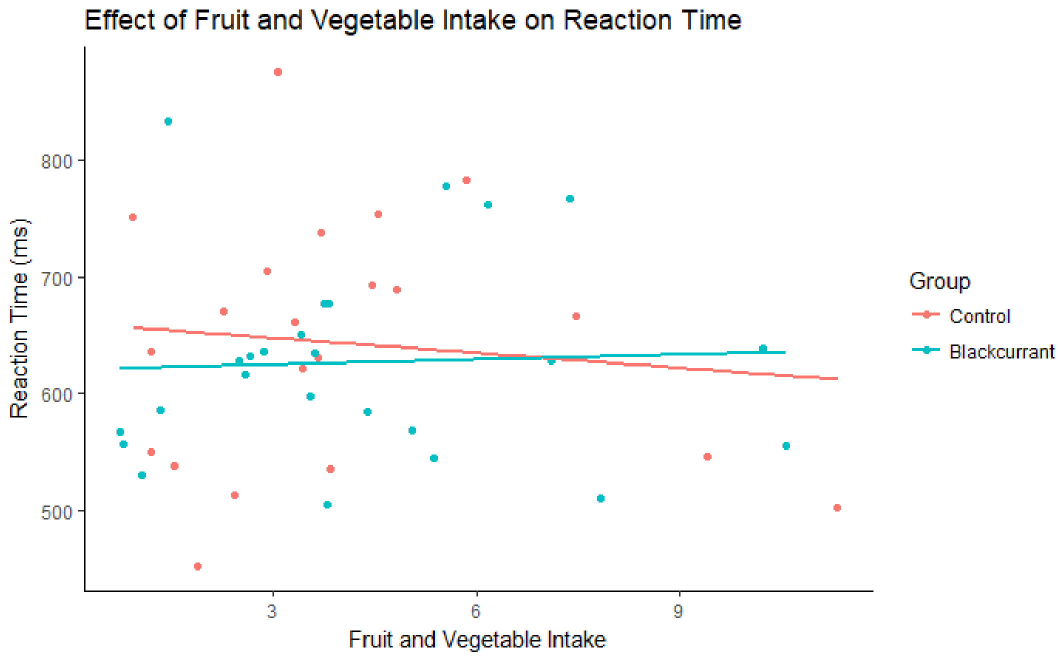

Figure 10. Combined Fruit and Vegetable Intake on Stroop Reaction Time

A linear regression was run as per the hypothesis and the results can be viewed in Table 14 and Table 15.

Table 14. Regression results using Mean Reaction Time as the criterion for the Blackcurrant Group

		Blackcurrant		*Group*		
Predictor	*b*	*b* 95% CI [LL, UL]	*beta*	*beta* 95% CI [LL, UL]	*r*	Fit
(Intercept)	636.95**	[606.14, 667.75]				
Combined	1.19	[-4.90, 7.27]	0.03	[-0.13, 0.19]	.03	
						R^2 = .001 95% CI[.00,.03]

Note. A significant *b*-weight indicates the semi-partial correlation is also significant. *b* represents unstandardized regression weights. sr^2 represents the semi-partial correlation squared. *LL* and *UL* indicate the lower and upper limits of a confidence interval, respectively.
* indicates p < .05. ** indicates p < .01.

Table 14 shows that Combined Intake did not significantly predict Mean Reaction Time in the Blackcurrant Group. The fit of the model of R^2 =.001 indicates the Combined Intake accounts for 0.01% of the variation in Mean Reaction Time. The *b* value indicates that as Combined Intake of Fruit and Vegetables increases by one portion, Reaction Time increases by 1.19 milliseconds. The bootstrap confidence interval based on 5000 replications is from -4.62 to 6.34. The above confidence intervals in Table 14 are very close to the bootstrapped confidence intervals, suggesting that normal distribution was met. As the confidence intervals include zero, this demonstrates that Combined Intake does not predict Mean Reaction Time.

Table 15. Regression results using Mean Reaction Time as the criterion for the Control Group

			Control	*Group*		
Predictor	*b*	*b* 95% CI [LL, UL]	*beta*	*beta* 95% CI [LL, UL]	*r*	Fit
(Intercept)	649.75**	[608.99, 690.51]				
Combined	-1.36	[-9.94, 7.21]	-0.03	[-0.21, 0.15]	-.03	
						R^2 = .001 95% CI[.00,.04]

Note. A significant *b*-weight indicates the beta-weight and semi-partial correlation are also significant. *b* represents unstandardized regression weights. *beta* indicates the standardized regression weights. sr^2 represents the semi-partial correlation squared. *r* represents the zero-order correlation. *LL* and *UL* indicate the lower and upper limits of a confidence interval, respectively. * indicates $p < .05$. ** indicates $p < .01$.

Table 15 shows that Combined Intake did not significantly predict Mean Reaction Time in the Control Group. The fit of the model of R^2 =.001 indicates the Combined Intake accounts for 0.01% of the variation in Mean Reaction Time. The *b* value indicates that as Combined

Intake of Fruit and Vegetables increases by one portion, Reaction Time decreases by 1.36 milliseconds. This effect was contrary to the hypothesis as the control group had a decrease in reaction time as fruit and vegetable intake increased while the blackcurrant group had an increase in reaction time. However, these results are in milliseconds and the difference is not large and has been found insignificant.

The bootstrap confidence interval based on 5000 replications is from -8.33 to 7.70. The above confidence intervals in Table 15 are very close to the bootstrapped confidence intervals, suggesting that normal distribution was met. As the confidence intervals include zero, this demonstrates that Combined Intake does not predict Mean Reaction Time.

These results show that daily fruit and vegetable consumption, combined with a blackcurrant supplement, has no statistically significant effect on reaction time.

A *post-hoc* power analysis was conducted using G*Power (All output can be viewed in Appendix O). With an alpha level of .05, a sample size of 46, and a small effect size of .036 (Cohen, 1992), achieved power for the study was .35. A post-hoc *sensitivity* power analysis was run with a sample size of 46, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of f= .139, assuming statistical significance and such an effect size actually exists. A post-hoc *a priori* test was also completed using the effect size of .036 found in this study to determine the sample size for future research. With an alpha level of .05, and minimum power of .80 (Cohen, 1992), 172 participants would be necessary to find a statically significant effect in the model.

Exploratory analysis: Using change score of reaction time instead of mean reaction time.

This exploratory analysis is to examine how using mean change score, calculated in hypothesis one, instead of the mean reaction time may relate to combined fruit and vegetable intake. This also allows for the elimination for the confounding variable of age, which had a statistically significant effect on reaction time. This is due to the change score allowing participants act as a control for themselves. Similarly, a linear regression model was used. The results can be viewed in Table 16 and Table 17.

Table 16. Regression results using Mean Change Score as the criterion for the Blackcurrant Group

		Blackcurrant		*Group*		
Predictor	*b*	*b* 95% CI [LL, UL]	*beta*	*beta* 95% CI [LL, UL]	*r*	Fit
(Intercept)	-1.98	[-19.43, 15.47]				
Combined	-0.95	[-4.40, 2.50]	-0.04	[-0.21, 0.12]	-.04	
						R^2 = .002 95% CI[.00,.04]

Note. A significant *b*-weight indicates the beta-weight and semi-partial correlation are also significant. *b* represents unstandardized regression weights. *beta* indicates the standardized regression weights. sr^2 represents the semi-partial correlation squared. *r* represents the zero-order correlation. *LL* and *UL* indicate the lower and upper limits of a confidence interval, respectively. * indicates $p < .05$. ** indicates $p < .01$.

Table 16 shows that Combined Intake did not significantly predict Mean Change Score in the Blackcurrant Group. The fit of the model of R^2 =.002 indicates the Combined Intake accounts for 0.02% of the variation in Change Score. The *b* value indicates that as Combined

Intake of Fruit and Vegetables increases by one portion, Change Score decreased by 0.95 milliseconds. This indicates that the blackcurrant had faster reaction times in their post-cycle Stroop block compared to the pre-cycle Stroop block, ameliorating the effects of physical fatigue. The bootstrap confidence interval based on 5000 replications is from -4.38 to 2.40. The above confidence intervals in Table 16 are very close to the bootstrapped confidence intervals, suggesting that normal distribution was met. As the confidence intervals include zero, this demonstrates that Combined Intake does not predict Mean Change Score.

Table 17. Regression results using Mean Change Score as the criterion for the Control Group

			Control	*Group*		
Predictor	*b*	*b* 95% CI [LL, UL]	*beta*	*beta* 95% CI [LL, UL]	*r*	Fit
(Intercept)	-44.01**	[-72.96, -15.06]				
Combined	6.30*	[0.21, 12.39]	0.18	[0.01, 0.36]	.18*	
						R^2 = .033* 95% CI[.00,.11]

Note. A significant *b*-weight indicates the beta-weight and semi-partial correlation are also significant. *b* represents unstandardized regression weights. *beta* indicates the standardized regression weights. sr^2 represents the semi-partial correlation squared. *r* represents the zero-order correlation. *LL* and *UL* indicate the lower and upper limits of a confidence interval, respectively. * indicates $p < .05$. ** indicates $p < .01$.

Table 17 shows that Combined Intake did significantly predict Mean Change Score in the Control Group, $p<.05$. The fit of the model of R^2 =.033 indicates the Combined Intake accounts for 0.33% of the variation in Change Score. The *b* value indicates that as Combined Intake of Fruit and Vegetables increases by one portion, Change Score increases by 6.30 milliseconds.

This indicates that the control had statically significant slower reaction times in their post-cycle Stroop block compared to the pre-cycle Stroop block. There was a small positive correlation *r*=.18 to support these statistics showing that as fruit and vegetable intake increase, so does change score in the control group. The exploratory hypothesis focussed on the potential decrease of change score in response to an increased fruit and vegetable intake but did not clarify what the expected effect would be to the control group. Furthermore, it was unexpected that fruit and vegetable intake would cause an increase in change score, insinuating that fruit and vegetable intake is debilitating to cognitive functioning. The bootstrap confidence interval based on 5000 replications is from 1.54 to 12.80. The above confidence intervals in Table 17 are very close to the bootstrapped confidence intervals, suggesting that normal distribution was met. As the confidence intervals doesn't include zero, this demonstrates that Combined Intake predicts Mean Change Score.

However, it is important to recognise that although statistically significant, these results are in milliseconds and therefore the change is not large.

Hypothesis 5

The fifth hypothesis was tested through attaching ECG electrodes under the right collarbone and by the left hip bone for the duration of the Stroop task and the exercise. The heart rate was then calculated in beats per minute (bpm) and the data used was from the time period of two minutes to one minute prior to completing exercise, which was considered the peak of the participant's performance. For this hypothesis, the previously mentioned 12 datasets were removed due to failure of participants to complete the testing and an additional

13 participants were removed due to electrodes falling off during exercise or excessive noise in the wires that could not be removed. A total of 35 data sets were used with 13 participants in the control group and 22 participants in the blackcurrant group.

The present study examined the influence that blackcurrant supplementation would have on physical effort, measured by heart rate. It was expected that blackcurrant supplementation would decrease the fatiguing effects of exercise and that heart rate during exercise would be lower than the control group.

An initial one-way ANOVA was conducted between group and mean corrugator activity and found no statistically significant difference between groups $F(1,33) = 0.78$, $p=.38$, $\eta^2_g=.15$. For the Levene test which tests the homogeneity of variance, heart rate mean activity was not significantly different in the two groups, $p=.09$. A visual inspection of the plots (Appendix N) for the ANOVA assumptions revealed that all assumptions were met. Visual inspection of Q-Q plot revealed that the distribution of error was approximately normal.

In session one, the blackcurrant group control had a heart rate of 177 bpm [173, 181] and the control group had 172 bpm [164, 180]. In session two, both groups decreased their heart rates by 2 bpm compared to their session one scores with the blackcurrant group having a heart rate of 175 bpm [171, 178] and the control group with a heart rate of 170 bpm [160, 180]. These results did not confirm the hypothesis as both groups had the same effect, most likely from either increased familiarisation with the exercise task to be completed, having high circulating sugars due to the intervention or due to exerting less effort than their previous session one trial.

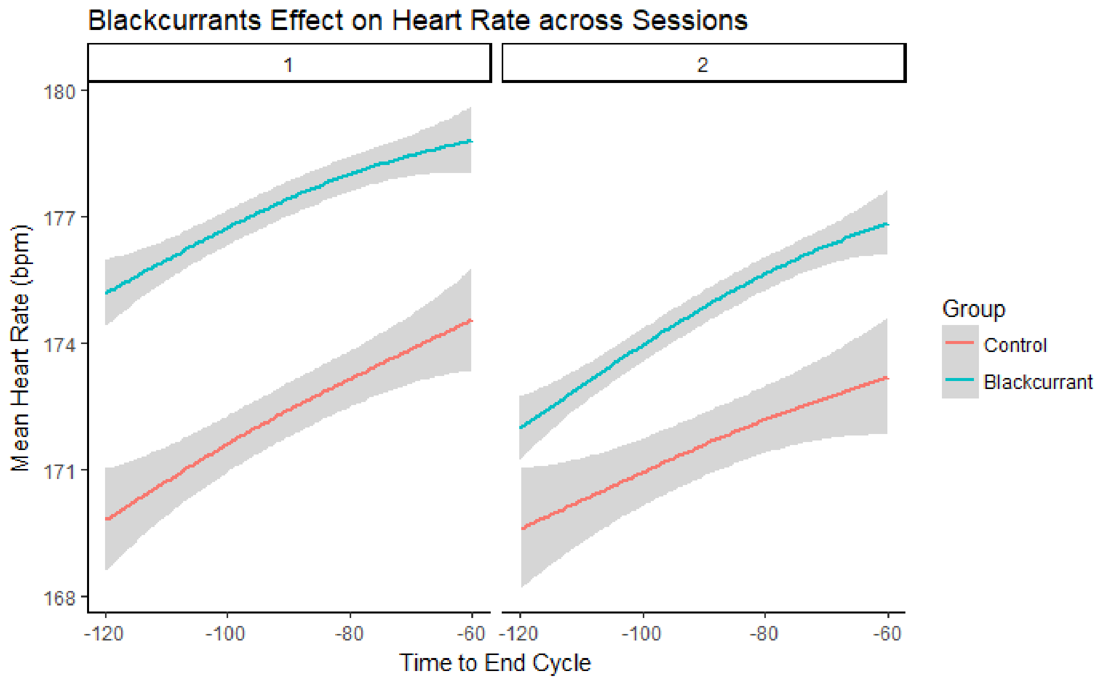

Figure 11. Mean Heart Rate during time period of two minutes to one minute prior to cycle end. Error bars show 95% Confidence Interval.

Figure 11 shows that the mean heart rate during the submaximal peak in exercise performance increases throughout the duration of testing for both groups across both sessions. The blackcurrant group had a higher mean heart rate than the control group and this difference carried across into session two.

Table 18. Repeated Measure Between-within Subject's ANOVA Analysis

Predictor	df_{Num}	df_{Den}	F	p	η^2_g
(Intercept)	1	33	7847.04	.000	1.00
Group	1	33	1.58	.217	.04
Session	1	33	3.14	.086	.01
Group x Session	1	33	0.03	.858	.00

Note. df_{Num} indicates degrees of freedom numerator. df_{Den} indicates degrees of freedom denominator. η^2_g indicates generalized eta-squared.

The repeated measures ANOVA in Table 18 showed that there was not the hypothesised effect of Group and Session on Heart Rate. This indicates that the Blackcurrant and Control Groups did not differ across Sessions. This was also replicated in the robust two-way ANOVA with Group, Session and the interaction between Group and Session which showed that none of the variables or interactions had significant p-values or effect sizes.

A post-hoc *sensitivity* power analysis was run using the G*Power programme (All output can be viewed in Appendix O). Given a sample size of 35, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of f= .50, assuming statistical significance and such an effect size exists. As the η^2_g=0.00, there is mostly likely no significant effect to be found in this model.

Hypothesis 6

This hypothesis is unable to be tested as pre-registered and hypothesed prior to data collection as it was not a strong enough hypothesis at time of data analysis. As most participants started their rating between 6 and 9 and ended their ratings between 18-20, this would not have provided any useful measure of the progression of fatigue for the same period of time which is needed to compare sessions. Due to this, an exploratory analysis will be completed and must be treated with caution. Whatever is found from this cannot be taken as something that can be safely generalised for but can be used to guide future research.

Exploratory analysis

The present study conducted an exploratory analysis to determine the influence that blackcurrant supplementation would have on perceived exertion, measured by RPE scores. It was expected that blackcurrant supplementation would decrease the fatiguing effects of exercise and that the blackcurrant group would have lower RPE scores compared to than the control group. The midpoint RPE score was used as it was the best representation of the individual's fatigue. The midpoint RPE score was deduced by dividing cycle time in half and then the RPE score given closest to that time was used. Time was rounded according to international rounding standards. For example, the mid-point of the cycle time is 5 minutes and 22 seconds. As an RPE score is taken for every two minutes during exercise so therefore the RPE score for the 6^{th} minute was used for data analysis.

There were 48 complete data sets for this hypothesis, 20 in the control group and 28 in the blackcurrant intervention group. An initial one-way ANOVA was conducted between group and mean corrugator activity and found no statistically significant difference between groups $F(1,50) = 0.59$, $p=.45$, $\eta^2_g = .12$. For the Levene test which tests the homogeneity of variance, RPE midpoint was not significantly different in the two groups, p=0.95.

In session one, the blackcurrant group had a mean RPE midpoint of 12 [12, 13] and the control group had a score of 13 [12, 13]. In session two, there was no notable difference between the two groups with blackcurrant having a mean midpoint RPE score of 13 [12, 13] and the control group of 12 [12, 13]. As this is a self-rated scale, the difference of one RPE score is notable as it is between score 11 which is given the descriptor “Light” and 13 which is given the descriptor “Somewhat Hard” (Appendix H). At the midpoint stage of their cycling, most participant in both groups were finding the exercise somewhat hard or close to somewhat hard and due to the slight change between groups, it is difficult to determine whether the one score difference is due to the intervention or through the nature of self-rated scales.

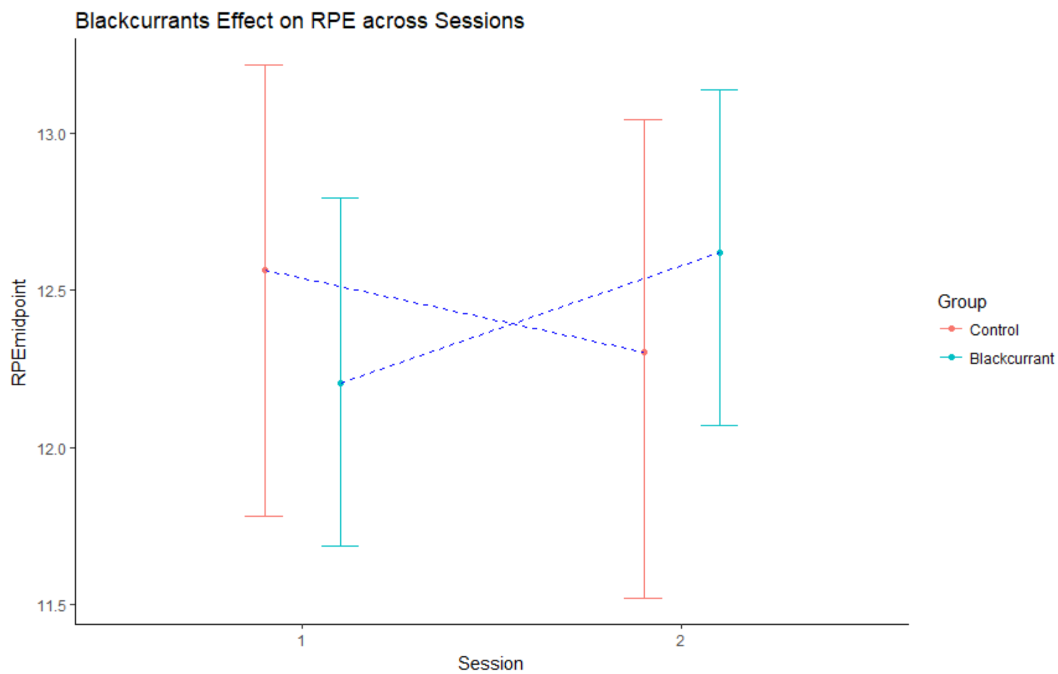

Figure 12. Mean RPE Midpoint score across Sessions. Error bars show 95% Confidence Interval.

Figure 12 gives a visual representation of the difference in mean RPE midpoint scores across sessions. This shows that the control group had a decrease in self-rated effort while the blackcurrant group had an increase in their perception of effort in session two. However, these changes are small being only one RPE point apart.

An ANOVA analysis with repeated measures for between and within subject's data was run to explore hypothesis six.

Table 19. Repeated Measures Between-within subject's ANOVA Analysis

Predictor	df_{Num}	df_{Den}	F	p	η^2_g
(Intercept)	1	50	4053.78	.000	.98
Group	1	50	0.00	.957	.00
Session	1	50	0.08	.775	.00
Group x Session	1	50	1.61	.211	.01

Note. df_{Num} indicates degrees of freedom numerator. df_{Den} indicates degrees of freedom denominator. η^2_g indicates generalized eta-squared.

The ANOVA in Table 19 showed that the expected interaction between Group, Session and RPE Midpoint Score was not statistically significant. There was no reliable difference between the Groups and this was supported by the close to zero effect size, representing the variance explained by Group and Session. This was also replicated in the robust two-way ANOVA with Group, Session and the interaction between Group and Session all at $p>.05$. These results suggest that blackcurrant does not affect perceived self-rated effort during submaximal exercise.

A *post-hoc* power analysis was conducted using G*Power (All output can be viewed in Appendix O). With an alpha level of .05, a sample size of 48, and a small effect size of .10 (Cohen, 1992), achieved power for the study was .10. A post-hoc *sensitivity* power analysis was run with a sample size of 48, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of f= .422, assuming statistical significance and such an effect size actually exists. A post-hoc *a priori test* was also completed using the effect size of .10 found in this study to determine the sample size for future research. With an alpha level of .05, and minimum power of .80 (Cohen, 1992), 782 participants would be necessary to find a statically significant effect in the model.

Hypothesis 7

The seventh hypothesis was tested through collecting expired air in the last minute of the exercise test. This is due to effect that previous literature has demonstrated where blackcurrant and other berry fruit can cause increased vasodilation which would increase the delivering of glucose, oxygen and other metabolites to the heart, lungs and muscles to improve aerobic ability (Wallace, 2011).

A total of 48 participants were used for this hypothesis, 20 in the control group and 28 in the blackcurrant group.

The present study examined the influence that blackcurrant supplementation would have on aerobic capacity and oxygen consumption, measured by VO_2max. It was expected that blackcurrant supplementation would decrease the fatiguing effects of exercise and that the blackcurrant group would have an increase in aerobic capacity and oxygen consumption compared to the control group.

An initial one-way ANOVA was conducted between group and mean corrugator activity and found no statistically significant difference between groups $F(1,46)=0.19$, p=66, $\eta^2_g=.06$. For the Levene test which tests the homogeneity of variance, the VO_2 value was not significantly different between the two groups, $p=0.15$.

In session one, the blackcurrant group has a VO_2max score 40.64 ml/kg/min [35.25, 46.08] while the control group had a lower score of 37.28 ml/kg/min [32.56, 42.00]. In session two, the intervention did not affect the blackcurrant group with the VO_2max score staying almost the same with 40.24 ml/kg/min [36.21, 44.27] while the control group had an improvement of 1.79 ml/kg/min to a VO_2max score of 39.07 ml/kg/min [35.81, 42.33]. However this increase is so small that it is most likely caused by the increased circulating sugars after the sugar-controlled drink which can result in an increase of VO_2max (Newell, Wallis, Hunter, Tipton, & Galloway, 2018).

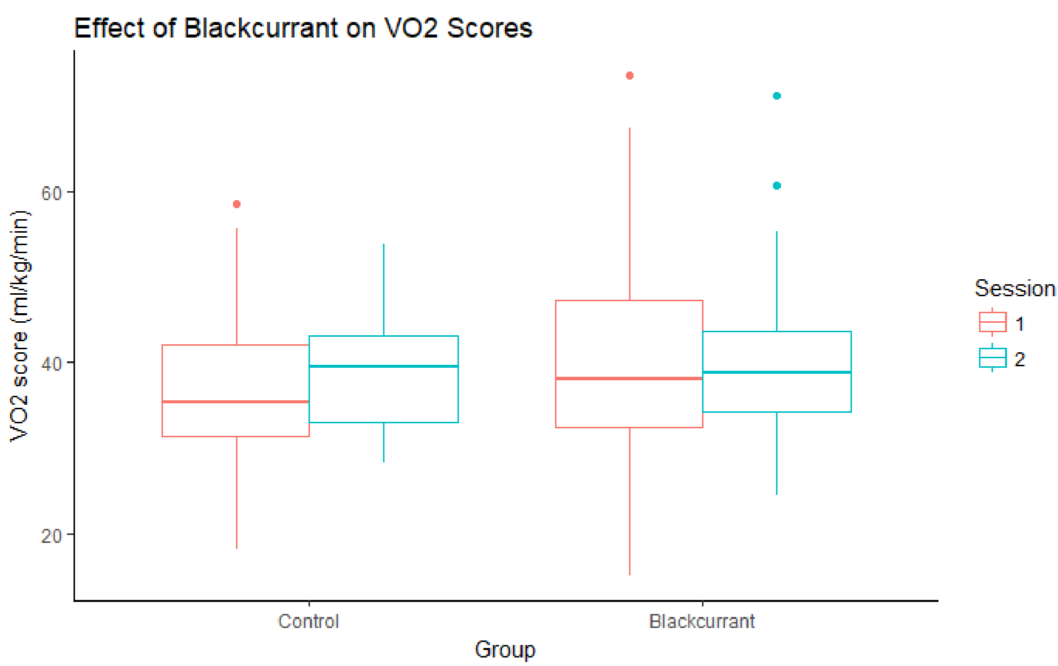

Figure 13. Boxplot showing VO_2max score across sessions.

Figure 13 shows that the control group had a slight improvement in VO_2max scores, indicating an improvement in aerobic capacity. However, the boxplot also shows that the control group had more variation in the VO_2max scores compared to the blackcurrant group, therefore the slight increase on VO_2max between session one and session two is most likely a result from the higher variation in session two scores.

An ANOVA analysis with repeated measures for between and within subject's data was run to investigate hypothesis seven.

Table 20. Repeated Measures Between-within Subject's ANOVA Analysis

Predictor	df_{Num}	df_{Den}	F	p	η^2_g
(Intercept)	1	46	684.58	.000	.93
Group	1	46	0.58	.452	.01
Session	1	46	0.37	.547	.00
Group x Session	1	46	0.98	.327	.00

Note. df_{Num} indicates degrees of freedom numerator. df_{Den} indicates degrees of freedom denominator. η^2_g indicates generalized eta-squared.

As indicated by Table 20. Repeated Measures Between-within Subject's ANOVA, there was no significant interaction between VO_2max score and Group or Session, or an interaction between Group and Session and VO_2max score. This was also replicated in the robust two-way ANOVA with Group, Session and the interaction between Group and Session all at $p > .05$. These results illustrate that one-dose of blackcurrant supplement did not influence VO_2 capacity.

A post-hoc *Sensitivity* power analysis was conducted using G*Power (All output can be viewed in Appendix O). Given a sample size of 48, an alpha level of .05, and minimum power of .80 (Cohen, 1992), there is an 80% chance of detecting an effect size of f= .422, assuming statistical significance and such an effect size exists. As the η^2_g =0.00, there is most likely no significant effect to be found in this model.

Secondary exploratory hypothesis

The secondary hypothesis was intended to investigate whether a relationship existed between RPE, heart rate and corrugator superciili activity. The initiative behind this hypothesis is that heart rate is typically used as an identifier of physical exertion while RPE is a scale that was developed to identify the person's rating of exertion. The Borg's RPE scale is well-validated in literature as an accurate predictor of physiological exertion (Bousquet et al., 2000). Corrugator superciili activity has also been observed to be a representation of physical exertion and the facial display of perceived effort (de Morree & Marcora, 2010). This hypothesis was aiming to test whether perceived and physical effort correlate and whether corrugator superciili activity may be used in future research as a dynamic form of testing physical exertion and perceived effort.

The data used for this hypothesis was a minute worth of exercise data that was taken from two minutes prior to one minute prior to the competition of exercise. The corrugator superciili activity and heart rate data was averaged to a mean for that time and the RPE score was the nearest score given by the participant in that minute time period.

A Pearson's correlation was run to investigate the interaction between corrugator activity, heart rate and RPE score.

Table 21. Means, Standard Deviations, and Correlations with Confidence Intervals

Variable	*M*	*SD*	1	2
1. Heart Rate	174.50	11.57		
2. Corrugator	-0.04	6.02	.03** [.01, .05]	
3. RPE score	16.59	1.44	.05** [.03, .07]	.03** [.02, .05]

Note. *M* and *SD* are used to represent mean and standard deviation, respectively. Values in square brackets indicate the 95% confidence interval for each correlation. * indicates $p < .05$. ** indicates $p < .01$.

Table 21 shows that a small, statistically significant correlation between all variables. Heart rate has a small positive correlation with Corrugator indicating that as Heart Rate increases, Corrugator activity increases. RPE score had a small positive correlation with Corrugator demonstrating that as RPE score increases, Corrugator increases. RPE score also had a small positive correlation with Heart Rate, indicating that as RPE score increases, Heart Rate increases.

The below graphs show the relationships between heart rate, RPE score and corrugator superciili activity individually.

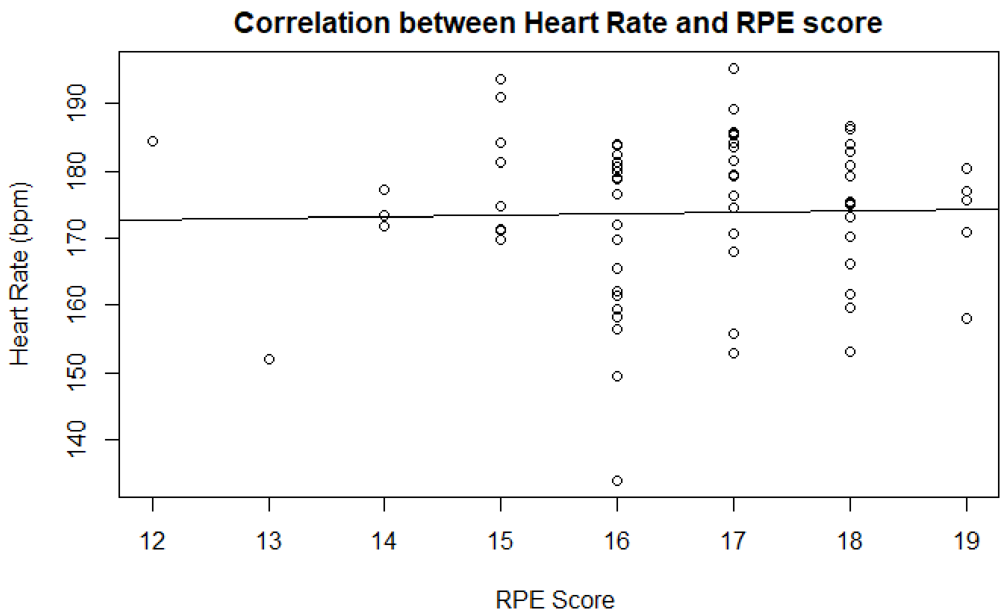

Figure 14. Scatter plot with regression line for Heart Rate and RPE score in duration of two to one minute prior to end cycle.

Figure 14 shows the small positive correlation between heart rate, the physiological marker of effort and RPE score, the self-rated marker of perceived effort.

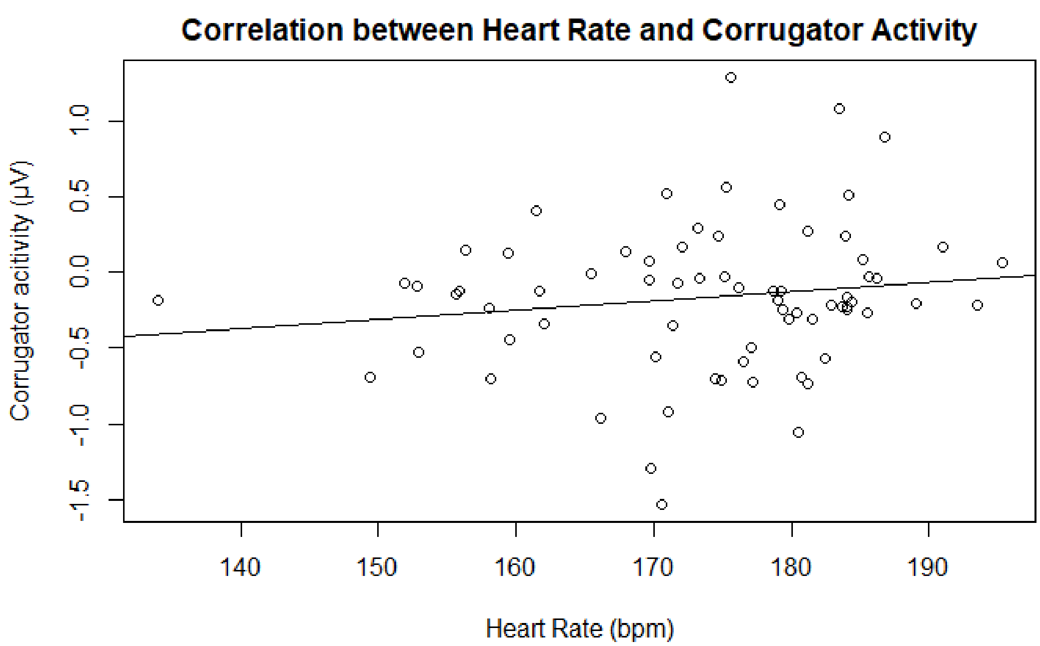

Figure 15. Scatter plot with regression line for heart rate and corrugator superciili activity in duration of two to one minute prior to end cycle.

Figure 15 shows the small positive relationship between heart rate, the physiological marker of effort and corrugator superciili activity, the physiological marker of perceived effort.

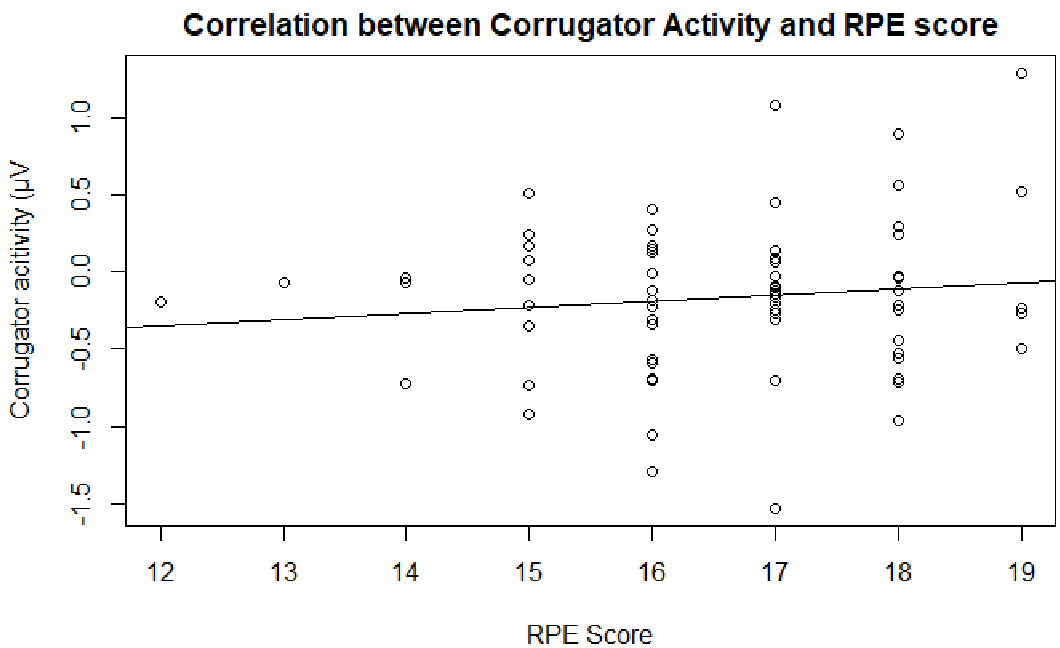

Figure 16. Scatter plot with regression line for Corrugator Superciili activity and RPE score in duration of two to one minute prior to end cycle.

Figure 16 shows the small positive relationship between RPE score, the self-rated marker of perceived effort and corrugator superciili activity, the physiological marker of perceived effort.

Discussion

The present study aimed to continue the research conducted by Harold (2016) and McKenzie (2017) investigating the effect of blackcurrant supplementation on cognitive performance. Results support findings from these previous studies that no effect on cognition was found from single dose blackcurrant supplementation. A limitation for both of Harold (2016) and McKenzie (2017) studies was possible confounding caused by practice effects during the cognitive tests. In the current study, practice effects were ameliorated by having participants complete five practice blocks at the beginning of each session. Conducting such practice sessions in this way meant that reaction time data was representative of the blackcurrant intervention itself rather than a change in reaction time attributable to practice effects.

Another change from the Harold (2016) and McKenzie (2017) studies was the pre- and post-cycle Stroop test were completed while stationed at the ergometer, instead of moving participants across the room to a computer. The current study allowed the participants to complete the Stroop test as close to the end of cycling as possible, allowing the test to assess immediate effects of fatigue and eliminate the opportunity to recover. The current study only used one psychometric test, the Stroop test, to reduce demand and participant confusion regarding multiple tests and instructions. Using one test prevented type I error occurring where if multiple cognitive tests are used, there is an increased chance of finding a significant false positive effect in one of the tests. Using the Stroop test assessed also allowed the measurement of deterioration in reaction time to be directly associated with the physical fatigue of the exercise and subsequent cognitive fatigue. Prolonged use of attentional resources is fatiguing in itself and causes further cognitive fatigue which could potentially confound the results (Hockey, Baddeley, & Weiskrantz, 1993). As the hypotheses in the current study aims to focus on blackcurrants amelioration of the effects of physical fatigue on cognition, using one psychometric test allows this to be assessed without these potential confounds.

Physical exercise was used to induce cognitive fatigue, therefore, it is important that the intensity and type of exercise are discussed. The Yerkes and Dodson (1908) U-hypothesis states that intense exercise has an inverted U-effect on cognitive performance. This implies that cognitive performance is negatively influenced by high-intensity exercise (HIIT). HIIT induces stress on physiological and biochemical systems. High intensity exercise narrows attention, and relevant cues are processed with increased effort (Brisswalter et al., 2002). HIIT is defined as maintaining 75-95% of maximal heart rate (Milanović, Sporiš, & Weston, 2015). HIIT has been found to be an effective and time-efficient method to induce fatigue similar to traditional endurance training (Skelly et al., 2014). Fatigue was monitored using heart rate, VO_2 and RPE. Inducing cognitive fatigue was essential to the current study. However, individual differences and the complexity of cognition make it difficult to establish the level of physical exercise required to produce cognitive fatigue, and large variability was seen in participant’s ability to complete the maximal test.

The current study based its hypothesis off the assumption that the VO_2 maximal exercise test would instigate fatigue and physiological processes that would in turn affect cognitive functioning. Several previous studies have used physical fatigue to induce cognitive fatigue and often supported the well-known U-hypothesis that states intense exercise has an inverted U-effect on cognitive performance (Lambourne & Tomporowski, 2010). High intensity exercise also affects the physiological state through increased blood lactate, fat oxidation and carbohydrate metabolism (Milanović et al., 2015). This in turn, affects the CNS and narrows attention to the relevant cues with increased effort (Brisswalter et al., 2002). Therefore, the intense exercise used in this study should have negatively affected cognitive performance. RPE scores were used as an indicator of exhaustion at the time of testing and the majority of the participants reached the maximum RPE score, indicating extreme exhaustion. Following data analysis, mean maximal heart rate was high in both the intervention and control groups across both sessions, indicating high effort. Participants consistently ended on self-rated RPE scores of 18 – 20 indicating "extremely fatigued" (Borg, 1971). However, it is difficult to understand whether this physical fatigue transferred to affect the cognitive domain. If cognitive performance was not sufficiently affected, small changes due to blackcurrant supplementation would not have been detected.

The hypotheses will be discussed separately and drawn together at the end of the Discussion section.

Hypothesis 1: A single dose of berry fruit juice will improve performance on Stroop test by ameliorating the effects of physical fatigue.

The first hypothesis was tested by investigating the effects of a single dose of blackcurrant supplement on cognitive performance in physically fatigued participants. Previous research conducted by Harold (2016) and McKenzie (2017) found that 240 mg of anthocyanin-rich blackcurrant freeze dried powder did not ameliorate the effects of physical fatigue and improve cognitive performance. The current study partially replicated Harold's and McKenzie's research but refined the analysis to one psychometric test, the Stroop test.

Previous research has demonstrated that anthocyanins benefit cognition in numerous ways. Polyphenols have been found to stimulate, enhance and protect neurons and neuronal signalling, subsequently promoting memory, learning and general cognitive performance (Spencer, 2008). The majority of human studies in this area focus on polyphenols benefits on preventing neurocognitive decline. However, animal studies have shown that anthocyanins can cross the blood-brain barrier and directly affect cognition. The majority of studies show a positive relationship between polyphenol consumption and cognitive performance (Macready et al., 2009). Long term studies also show that high intake of flavonoids is linked with improved cognitive functioning compared to baseline scores (Letenneur et al., 2007).

The present study examined the effects of flavonoids on the Stroop test with three conditions: congruent, control and incongruent. It was expected that the blackcurrant intervention group would have a faster reaction times compared to a sugar-controlled group and therefore a lower change score compared to the control group. However, no statistically significant interaction between group, session and change in reaction time was found. There was a significant effect of session on change in reaction time which occurred in session two however this did not differ between groups. Both groups had an increase in reaction time in session two. This result was possibly due to awareness around the intensity of the exercise test following session one, and as a result, greater feelings of fatigue post cycle. When broken down into the separate Stroop conditions, both groups had increased change scores in the congruent and control group. However, the blackcurrant group performed better than the control group in the session two incongruent condition, considered the more cognitively difficult out of the three conditions. Although not statistically significant, this suggests that the maximal exercise had less effect on the blackcurrant group than the control group in session two incongruent condition. It is interesting to note that this difference was observed only within the most difficult of the three conditions. This might be attributed to the complexity of the particular condition which was able to utilise high cognitive processing skills. This in turn may have made the incongruent condition more susceptible to fatigue and therefore the subsequent improvement in the blackcurrant group versus the control group was observed. Another explanation can be attributed to Paas and Adam (1991) who found unexpected beneficial effects on decision task making during high cycling workloads. The authors attributed this benefit to participants investing more processing ability and resources into the more complex decisional tasks. An unconscious or conscious decision of the participants to invest more effort into their performance may be due to motivational variables or effects of expectancy. This may also explain the improved performance of the blackcurrant group in the incongruent condition but not in the simpler congruent or control conditions.

Hypothesis 2: A single dose of berry fruit juice will show reduced corrugator activity, the facial display of effort, during Stroop test.

The corrugator superciili is a dynamic form of assessing effort during cognitive and physical tests. Cognitive effort is known to increase with time during prolonged exercise and vice versa with mental fatigue causing an increase in perception of effort during submaximal exercise (de Morree & Marcora, 2012).

This hypothesis was developed to further research on whether corrugator superciili monitoring can be used within research as a form of assessing in-the-moment effort of participants. Activity of the corrugator superciili muscle is associated with negative affect and emotion and its activation is thought to be linked to the immobilisation of mental resources, task engagement and error processing (de Morree & Marcora, 2010; Elkins-Brown et al., 2016; Silvestrini & Gendolla, 2009).

Blackcurrant extract has been previously shown to improve cognitive performance through multiple mechanisms, including anti-inflammatory and antioxidant responses, improvements in neural sign calling and MAO inhibitory effects to reduce oxidative stress (Watson et al., 2015). This improved cognitive performance results in speeding up recovery rate after exercise, increasing vasodilation to improve blood glucose and oxygen transportation and scavenging free radicals which damage cells. In the current study, it was investigated whether blackcurrant supplementation would decrease the facial display of effort during the Stroop test. To the author's knowledge, this is the first study of its kind where supplementation may influence the corrugator muscle and the participants perception of effort.

The expectation was that the blackcurrant group would show less positive corrugator superciili muscle activation, signifying higher contraction levels and therefore perceived effort. However, there was no significant difference between session, group, Stroop condition and mean corrugator activity. Within the separate Stroop conditions, participants in the blackcurrant intervention group did not have any relaxation in corrugator muscle activity. Within the congruent condition, both groups had similar activation (~0 µV) meaning that no change in muscle activation occurred between the resting period and the presentation of the stimuli. Within the control condition, the control group performed better in session two with a large relaxation of the corrugator muscle from pre-cycle to post-cycle while the blackcurrant group increased their activity and therefore frowning with perceived effort. Within the incongruent condition, the most difficult condition, both groups did not change significantly in the second session. It is also important to note that all of the scores within both groups had large variability between points, making analysis difficult. Individual factors were controlled through the corrugator activity being processed as a change score between the previous resting period and the subsequent stimuli presentation. Therefore, variability cannot be attributed to individual differences. The average values also did not vary significantly in any Stroop condition or session. This might signify that the physically fatiguing exercise did not translate into adequate cognitive fatigue to affect participant’s perception of effort when completing the post-cycle Stroop tasks.

Hypothesis 3: A single dose of berry fruit juice will show reduced facial display of effort for the same period of physical activity between session 1 and session 2.

Frowning muscle activity, as measured by facial electromyography, reflects the perception of effort during incremental workload cycling. Corrugator superciili activity is associated with RPE scores which led the current study to investigating whether supplementing with blackcurrant will reduce corrugator activity and therefore, the perception of effort (Blanchfield et al., 2014; de Morree & Marcora, 2010, 2012). Corrugator activity is dynamic and can provide insight into in-the-moment perception of effort while RPE scores remain static and are associated with the disadvantages of self-rated scales. Perception of effort reflects central motor commands during movement execution, and motor overflow may explain why facial muscles are recruited with increased effort (Marcora et al., 2009).

Blackcurrant extract has been shown to ameliorate the effects of physical fatigue and increase recovery. The physiological effects such as increasing peripheral blood flow and anti-inflammatory and antioxidant affects assist in removing by-products of exercise metabolism and providing glucose and oxygen to the muscles (Pires et al., 2011). This hypothesis combined two theories to assess whether the antioxidant effects of blackcurrant could ameliorate muscle fatigue and therefore cause a reduction in corrugator activity.

The results from hypothesis three found that there were no statistically significant effects between group, session or the interaction of these variables. Figure 9 showed that during submaximal exercise, corrugator activity did not increase proportionally to time as expected and that there was no difference between groups or the participants baseline session. This suggests that blackcurrant had no effect on corrugator superciili activity, the psychophysiological marker of effort, during submaximal exercise.

Hypothesis 4: As daily fruit and vegetable intake increases, mean Stroop reaction time decreases but only for berry fruit supplementation group.

High levels of fruit and vegetable consumption have been shown to change the composition of gut bacteria, encouraging the growth of bacteria such as b*ifidobacterium* and *lactobacillus* which can improve both physical and mental well-being. Having large populations of these types of bacteria can affect the gut-brain axis and adjust microglia functioning, HPA axis activity and serotonin and GABA production which effect memory, learning emotion and pain perception (P. J. Kennedy et al., 2016). The present study attempted to investigate whether there was a link between habitual fruit and vegetable consumption and the effect the blackcurrant supplement would have on reaction time in the Stroop task. It was expected that participants who had a higher regular intake of fruit and vegetables would have larger populations of beneficial bacteria which assist the host in maintaining physical and cognitive health. It was also expected that due to the larger populations of these bacteria, when the blackcurrant supplement was digested, it would have a larger effect due to existing bacteria that may utilise the by-products of its metabolism. Using the reaction time as the dependent variable, there was no statistically significant interaction between combined intake, group and session. However, the mean reaction time was found to be confounded by participant's age.

An exploratory analysis was run using the mean change score of reaction time which allows the participants to act as their own control, and removes age as a confounding variable. Using the change score, the analysis showed a statistically significant correlation between combined intake and group, as well as a statistically significant, small correlation between combined intake, group and session. Due to the exploratory nature of the analysis, this result can only be used as a guide for future research and cannot be used to conclude that fruit and vegetable intake has a significant effect on change in reaction time when paired with the blackcurrant supplement. Liu (2003) suggested that a synergistic and additive effect of different combinations of antioxidants in fruits and vegetables were responsible for their antioxidant properties. However, these results did not support Liu's proposal as there was no difference in reaction time as fruit and vegetable intake increased. Therefore, the conclusion for this hypothesis is that fruit and vegetable intake does not have a significant effect on reaction time when paired a blackcurrant supplement.

It is possible that the one hour waiting period between consumption of the supplement and starting the testing protocol was insufficient to fully digest the blackcurrant flavonoids. It therefore seems unlikely that any change in neuronal functioning following the flavonoid ingestion would have been seen. Cognitive improvements have been seen at later time points for flavonoid ingestion (Bell et al., 2015) and therefore the reason for the null finding may be attributed to inadequate time postprandially for the blackcurrant to exert its effects on cognitive performance.

Hypothesis 5: A single dose of berry fruit juice will show reduced heart rate for the same period of activity between session 1 and session 2.

During exercise, heart rate increases in response to the body pumping blood to the relevant exercising muscles. Exercise duration of longer than 15 minutes should induce a heart rate of approximately 160 bpm. Studies have shown that high maximal heart rate correlates with performance decrements in both acute and chronic exercise and can be used as a marker of fatigue or exercise recovery (Nelson et al., 2017). Blackcurrant supplementation contains anthocyanins that might alter cardiovascular function through decreased peripheral resistance and increased cardiac output (Cook, Myers, Gault, Edwards, & Willems, 2017). This hypothesis investigated whether blackcurrant supplementation would reduce heart rate during submaximal exercise due to the physiological effects that anthocyanins may possess. The present study found no statistically significant difference in heart rate between groups or sessions. This lack of effect could be attributed to the expectation of physiological changes after a single dose of blackcurrant. Willems, Myers, Gault, and Cook (2015) used a daily blackcurrant supplement containing 136mg of anthocyanins for seven days. During this time, stroke volume and cardiac output increased by 25% and 26%, respectively, and peripheral resistance decreased by 16%. Therefore, it is possible that the effect size of any change may have been too small for the statistical power of this study, or that a longer supplementation period is needed to see the previously cited benefits of blackcurrant on cardiovascular measures.

Hypothesis 6: A single dose of berry fruit juice will show reduced rating of perceived effort (RPE) for the same period of activity between session 1 and session 2.

RPE scales are the most consistently used form of assessing participant exertion across studies and therefore enables comparability of the current study with past literature. RPE represents a person's perception of the intensity of exercise, providing information that is equally as important as physiological measures (McAuley, 1992). It also removes the error that can be obtained from physiological measures. For example, heart rate can be affected by other emotive or anxiety type responses that can cause an increase in heart rate not related to the exercise being performed. RPE scores have been well-validated with high efficacy (McAuley, 1992). Due to the previously discussed physiological effect of blackcurrant on decreasing recovery time and improving physical performance, the current study investigated the effect that blackcurrant supplementation would have on participant's rating of their perceived exertion during a VO_2max test. This hypothesis was exploratory as it was not a strong hypothesis at the time of data collection. The reason being, that every participant started at about the same RPE score of 6-9 and ended at around 18-20. As the VO_2max test was a maximal test and participants continued to cycle in both session until they could not cycle anymore, this distribution of RPE scores was expected. A more informative indicator of the participant fatigue could be tested using the RPE score at the midpoint of their cycling time to determine whether participants felt more or less fatigued during the same period of activity. However, the expected interaction between group, session and RPE midpoint was not statistically significant. Post-hoc power analyses were conducted and found that the achieved power of the study was 0.10, which is very small. For future research to identify a statistically significant effect, a very large sample size of 782 participants would be required, suggesting that most likely there is no substantial effect.

A possible reason for the insignificant effects can be attributed to expectancy effects. RPE and other subjective scales of effort may not be reliable or valid measures of participant's actual effort. Participants may consciously or unconsciously invest more effort in tasks through motivational or expectancy effects (Tomporowski, 2003). This means that participants may overcome possible effects of fatigue through the knowledge that their performance is being measured or through motivational variables such as reimbursement.

Hypothesis 7: A single dose of berry fruit juice will show increased VO_2max for the same period of activity between session 1 and session 2.

VO_2max scores are a useful form of measuring aerobic capacity and endurance. As muscles need oxygenated blood for exercise, the more oxygen the body can use reflects the greater amount of energy the body can produce to continue exercise (Bentley & McNaughton, 2003). Blackcurrant contains anthocyanins which improve vasodilation and endothelial function, and reduces blood pressure (Schini-Kerth et al., 2010). The current study aimed to observe if blackcurrant supplementation could increase VO_2max scores through its physiological effects. This would signify an improvement in cardiorespiratory fitness. The present study found no statistically significant effect on VO_2max score by group or session or in the interaction between group and session. Previous research conducted by Braakhuis et al. (2014) found that 240mg of blackcurrant anthocyanin supplementation before and after exercise decreases cytokine secretion. The dose provided by Braakhuis et al. (2014) was a similar dosage of the current study, but in that study, participants were trained athletes. However, all other studies that have shown physiological effects of blackcurrant on physical markers supplemented with similar dosages of blackcurrant anthocyanin but were over a longer period of time, varying from one week to three weeks supplementation (Cook, Myers, Blacker, & Willems, 2015; Hutchison et al., 2016; Willems et al., 2015).

Secondary exploratory hypothesis: Is there a positive correlation between heart rate, RPE and corrugator superciili activity?

Heart rate, corrugator superciili activity and RPE scores are all methods of assessing effort during physical exercise. Each measure provides its own insight into overall exerted effort and perceived effort by the participant. However, each of these measures also has its own disadvantages through conscious or unconscious processes. The current study aimed to find a positive correlation between each of these variable, to validate the current methods of measuring exertion, heart rate and RPE scores and to introduce to the literature the corrugator superciili muscle as a dynamic measure for measuring physical exertion. There was a very small, significant, positive correlation seen between corrugator activity, heart rate and RPE score demonstrating that these variables can all measure physical and perceived fatigue. However, the individual data points of participants were highly variable. Within exercise literature, heart rate and RPE scores are often paired as a useful measure to guide exercise intensity (Faulkner, Parfitt, & Eston, 2007). Previous research has found strong correlations between heart rate and RPE scores (range from $r = 0.54$ to $r = 0.78$) which highlights this study as an outlier within current research knowledge (Halson, 2014). de Morree and Marcora (2010) also found a strong correlation between RPE scores and corrugator superciili activity during leg-extensor exercise (range from $r = 0.61$ to $r = 0.71$). The present study's results are in line with current research on the interaction between heart rate and RPE scores also indicates that the corrugator muscle can be used in line with these other, well-researched forms of measuring effort.

The null findings in this study may be due to the concentration of anthocyanins within the powder used to make the blackcurrant supplementation. Although the participants were given 11 g of the powder, the total anthocyanin concentration would have been 231 mg which may have been too low a dose to see short-term effects. Previous research showing a medium effect size of flavonoid supplementation on cognitive performance have used 483- 579 mg flavonoid doses (Dodd, 2012; Watson et al., 2015). Harold (2016) and McKenzie (2017) used doses of 240 -250 mg and had found not significant effect. However, research conducted by Watson et al. (2015) showed an improvement in cognitive performance, especially in areas of sustained attention, in groups consuming an anthocyanin enriched blackcurrant extract, containing 552 mg or 142 ml of a cold-pressed blackcurrant fruit juice which contained an average anthocyanin dose of 571 mg. This research by Watson et al. (2015) raises the question as to whether the dose used in previous and the current research was adequate for any effects to be observed, particularly in single-dose studies where doses may need to be larger to see any immediate effect. Furthermore, this study used a freeze-dried powder of blackcurrant extract versus a sugar controlled placebo, containing free-sugars. Watson et al. (2015) found that their freeze-dried blackcurrant extract powder increased blood plasma anthocyanin levels compared to the blackcurrant juice treatment. This significant difference may relate to different metabolism of the extracts where juice is more bioavailable than powdered or rehydrated extracts.

This could pose a question towards the current study as to whether benefits seen in the control group may have been attributed to the glucose, fructose and sucrose that was readily available and therefore the body was able to use as an energy source. In comparison, the freeze-dried powder in the blackcurrant group may have taken longer to metabolise and therefore the anthocyanin and sugar bioavailability may not have been absorbed at the time of the post-cycle cognitive testing.

These points about bioavailability of extracts highlight the need for future research to emphasise how the food matrix may impact anthocyanin absorption. It is also important to consider how the food matrix may have affected participant's reaction time and perceived and physiological exertion. The presence of polyphenols can influence the glycaemic index of foods and therefore the post-prandial glucose concentration through inhibiting sugar metabolising enzymes and transporters (Williamson, 2013).

In relation to this study, if the polyphenols within the blackcurrant supplementation reduced the bioavailability and glucose absorption within the blackcurrant group, this may have contributed to some of the group differences seen in the majority of this study. Due to the muscle glycogen depletion that would have occurred during the exercise test, the bioavailability of simple sugars would have affected muscle and central fatigue. Coyle (1991) found that carbohydrate and electrolyte ingestion during prolonged submaximal exercise can delay the onset of fatigue and improve physical and cognition performance, attenuating exertion perception during exercise. Again, it would be difficult to determine whether the control group, which had equal sugar content albeit free sugars, versus the freeze-dried blackcurrant powder, where the sugars were within a food matrix, may have contributed to group differences in RPE scores, corrugator activity and reaction time.

To date, methodological differences in research pertaining phytochemicals and their benefits on ameliorating the effects of fatigue on cognitive performance have been present. Differences in exercise protocols, including intensity, duration and regime along with cognitive testing during or following the exercise can alter the physiological and the cognitive outcomes being tested. Moreover, the cognitive tests themselves have differed widely through assessing different cognitive domains. The population base of participants has also ranged from athletes to sedentary participants meaning that most studies are incomparable. Therefore, while the current study failed to support the hypotheses stated, findings are consistent with some previous literature (McKenzie, 2017; Tomporowski, 2003) but inconsistent with other areas (Cook et al., 2015; Watson et al., 2015). The current study had refined the methodology of previous studies, using only one psychometric test which used higher levels of information processing and inhibition that are more sensitive to fatigue.

Cognitive tests that are used in nutritional studies, including the current study, may not be producing any effects due to a lack of understanding of how phytochemicals can affect cognition. Macready et al. (2009) reviewed 39 studies, 121 of which used cognitive tests that investigated the effects of micronutrients and found that none of the cognitive tests were designed to assess specific nutritional interventions. The Stroop colour-word task is popular in literature due to its ability to assess different cognitive domains however it is inconclusive as to whether this test is sensitive to chronic supplementation. As nutritional interventions often result in small changes to physical and mental health, psychometric tests may not be sensitive enough to detect these changes. It is also suggested that the psychometrics may not be assessing the regions of the brain that are being influenced by phytochemicals.

Conclusions and Recommendations for Future Research

At the conclusion of data collection, it was evident there were some limitations in this study. Firstly, the sample size was much smaller than expected (*n*=48) due to the physical ability of participants. Although during participant recruitment it was stated that participants had to be physical fit, twelve participants were unable to complete the post-cycle Stroop component and therefore none of their collected data could be used. Several data sets also had to be eliminated due to errors in collection (e.g. mouthpieces of VO_2 falling out or problems with the heart rate and facial EMG electrodes). *A priori* power analysis required 52 participants to have adequate statistical power, however this was not reached. Therefore, it is unable to be known whether the blackcurrant supplement had no effect on the variables or whether the effect size was smaller than our statistical power allowed. Future studies should use larger sample sizes as the effect size is most likely small. However, a small effect size results in the need for hundreds of participants which is often beyond the capacity for many researchers.

Another limitation may have been the social communicative role of corrugator activity and other effort related responses. As the researcher was present during both the Stroop task and the exercise task, however was always located behind the participant out of sight-line, the presence of the researcher may have altered the participants response to RPE scores, where the participant may have wanted to have been seen as exerting less effort than their actual effort. Corrugator activity may also have varied due to increased comfort with the researcher and therefore increased social communication of effort exertion or simply the participants found it harder or exerted more effort than their previous trail. Future studies using self-rated and facial EMG should aim for participants to have as little contact with the research team as possible to ensure that social communication or perception does not confound the participants results.

Lastly, a limitation concerning the exercise component of this research was that there was no familiarisation session for the participants. This methodology was chosen due to the restraints that the current study had on time and participant commitment. Three sessions of intense exercise may have resulted in higher drop out or inability to recruit adequately due to the population being normal adults and not trained athletes. Future research, if resources allow, should have familiarisation sessions for both the psychometric tests and the exercise test being performed.

Although the current study failed to support the proposed hypotheses that a single dose of blackcurrant would improve various physical and cognitive domains, it does add to the body of knowledge in this area and may guide future related research.

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Appendix A – Pre-registration on as.predicted.org

AsPredicted
Pre-Registration made easy

You are logged in as: **natalie_peart@hotmail.co.nz** (Log out)

HOME | See List | Make Suggestion | Change my email

As Predicted: *"Fruit modulation of the effects of fatigue on cognitive performance"* (#5111)

Created: 08/09/2017 02:05 AM (PT)

Author(s)
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1) Have any data been collected for this study already?
No, no data have been collected for this study yet

2) What's the main question being asked or hypothesis being tested in this study?
The aim of this project is to evaluate if a fruit extract reduces the impairment in cognitive performance associated with exhaustion as assessed using a traditional executive functioning task as well as facial muscle activity (frowning).

Hypothesis 1: A single dose of berry fruit juice will improve performance on Stroop test by ameliorating the effects of physical fatigue.

Hypothesis 3: A single dose of berry fruit juice will result in reduced facial display of effort for the same period of submaximal physical activity between session 1 and session 2.

Hypothesis 4: As daily fruit and vegetable intake increases, mean Stroop reaction time will decrease but only for berry fruit supplementation group.

Hypothesis 5: A single dose of berry fruit juice will reduce heart rate for the same period of submaximal physical activity between session 1 and session 2.

Hypothesis 6: A single dose of berry fruit juice will reduce rating of perceived effort (RPE) for the same period of submaximal physical activity between session 1 and session 2.

Hypothesis 7: A single dose of berry fruit juice will increase VO2 max for the same period of submaximal physical activity between session 1 and session 2.

3) Describe the key dependent variable(s) specifying how they will be measured.
Stroop reaction time measured using EPrime 2.0 Professional software of the Stroop task. Psychology Software Tools, Inc. [E-Prime 2.0]. (2012). Retrieved from http://www.pstnet.com.

Frowning using the muscle Corrugator Supercilii. Activity is measured using Facial Electromyography with bipolar electrodes placed on the skin surface above bilateral corrugator supercilii.

Heart rate using electrocardiography

VO2 max measured using Douglas bag and gas analyser

4) How many and which conditions will participants be assigned to?
Two conditions with 30 participants in each condition: Berry fruit intervention group and glucose-controlled placebo group. Assignment to groups will be randomised. Data collection will cease once 30 participants are recruited into each group.

5) Specify exactly which analyses you will conduct to examine the main question/hypothesis.
Hypothesis 1: Two factor ANOVA (Between subjects: placebo control and berry fruit; within subjects: pre-test and post-test). The primary parameter of interest is the interaction between test time and group. The control group is predicted to have an increased reaction time from pre to post-test while the berry fruit group is predicted to have a lesser increase in reaction time from pre to post-test.

Hypothesis 2: Two factor ANOVA (Between subjects: placebo control and berry fruit; within subjects: pre-test and post-test post-test corrugator activity). The control group is predicted to have increased corrugator activity from pre to post-test while the berry fruit group is predicted to have a lesser increase in corrugator activity from pre to post-test.

Hypothesis 3: Two factor ANOVA (Between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score corrugator activity). The control group is predicted to have increased levels of perceived effort from session 1 to session 2. The berry fruit group is predicted to have a lesser increase of perceived effort from session 1 to session 2.

Hypothesis 4: Linear Regression with dummy coded group variable

Hypothesis 5: Two factor ANOVA (Between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score of heart rate). The control group is predicted to have a similar heart rate change score from session 1 to session 2. The berry fruit group is predicted to have a reduced heart rate change score from session 1 to session 2.

Hypothesis 6: Two factor ANOVA (Between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 change score for RPE score). The control group is predicted to have a similar RPE change score from session 1 to session 2. The berry fruit group is predicted to have a reduced RPE change score from session 1 to session 2.

Hypothesis 7: Two factor ANOVA (Between subjects: placebo control and berry fruit; within subjects: session 1 change score and session 2 score for VO2 max). The control group is predicted to have a similar VO2 max score from session 1 to session 2. The berry fruit group is predicted to have a reduced VO2 max score from session 1 to session 2.

6) Any secondary analyses?
A positive correlation between submaximal physical activity evoked heart rate, RPE and corrugator supercilii activity.

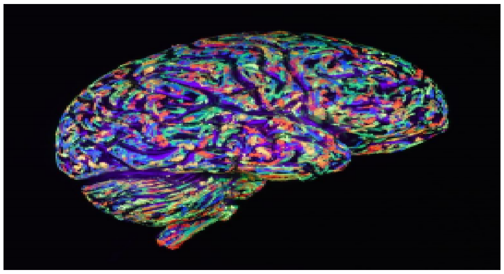
7) How many observations will be collected or what will determine sample size?
No need to justify decision, but be precise about exactly how the number will be determined.
Based on a power analysis using the G*Power program, we estimated the effect size was small (0.2). We want a power of 0.80 and an alpha level of 0.05 and therefore require 52 participants for our study to have adequate power.
We aim to recruit 60 participants to allow for participant drop out.

8) Anything else you would like to pre-register?
(e.g., data exclusions, variables collected for exploratory purposes, unusual analyses planned?)
No

Appendix B – Recruitment Poster

RESEARCH PARTICIPANTS NEEDED

CAN BERRY FRUIT SUPPLEMENTS MAKE YOU THINK BETTER AFTER EXERCISE?



Who: YOU! If you are aged between 18–35, of good general health and do cardio/resistance training 3x a week

Where: Massey University Albany

What's in it for me: You will get a body composition analysis (worth $50), a VO^2 max test (worth $150) and a $30 Countdown voucher for your time

What am I doing? You will be doing cycling test with resistance and com–pleting a psychometric test

Interested? Contact na–talie.masseyuni@gmail.com

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Appendix C – Information Sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Fruit modulation of the effects of fatigue on cognitive performance

INFORMATION SHEET

You are invited to take part in a study on Fruit Modulation of the Effects of Fatigue on Cognitive Performance. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason. If you do want to take part now, but change your mind during the study, then you can withdraw your participation and your data will be deleted. Because your study data is not linked to your identity, once you complete participation then your data cannot be removed from the study.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. You may also bring whānau or friends with you to the sessions.

If you agree to take part in this study, you will be asked to sign the Consent Form. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is four pages long. The Consent form will then be provided which is two pages long. Please make sure you have read and understood all the pages.

Researchers Introduction

My name is Natalie Peart and I am a student working towards my Masters of Science majoring in Psychology. This project interests me as I am also completing my Postgraduate Diploma of Science majoring in Human Nutrition. The interaction between diet and how it can affect us psychologically is important to study for furthering product development and health benefits for New Zealand.

Dr Peter Cannon conducts research into emotion at the School of Psychology, Massey University and is Natalie's postdoctoral supervisor.

Project Description and Invitation

The aim of this project is to discover if a fruit extract may be able to reduce the decrease in cognitive performance associated with exhaustion. This will be assessed by traditional psychometric evaluation as well as facial muscle activity.

If you are interested in participating this project, please read the following and contact us.

Participant Identification and Recruitment

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Participants for this study are being recruited from nearby gyms, sports areas or through word of mouth. We are looking for 60 participants to complete this study.

To participate in this study, you will need to be:
- 18-35 years old
- Good health and fitness
- No past or current heart or lung problems
- No allergies or intolerances to berries
- No current injuries or injuries that are exacerbated by exercise

Participants names will not be collected or disclosed. A number will be assigned to each participant to maintain confidentiality.

This study involves intense exercise for a maximum of 20 minutes. This may cause some normal physical discomfort associated with exercise.

Project Procedures

There will be two sessions and follow up phone call/email. The first session will determine baseline data such as fitness and a psychometric score. Baseline measures will also involve body mass being calculated using a bioelectrical impedance analyser. The bioelectrical impedance analyser is a non-invasive test simply involves standing on a scale and holding a pair of arms. A low level, imperceptible electrical current is sent through your body which assess the percentage of water and fat mass. It looks like the following:

We will also be collecting your weight, height, and heart rate. The first session will also compromise of an exercise test (more information below) and a psychometric test.

There will be some foods we ask you to avoid 48 hours prior to each session. These are listed in Appendix A at the end of page 4. We will also collect two days' worth of food diaries at the start of each of the two sessions which will be taken on days where there are no food restrictions. This will involve recording the amount and type of food you eat for two days. Please eat as normally as possible on these days. Before each of the two sessions, we will ask you not to eat for 2 hours prior to coming in to the session.

The project will involve cycling for approximately 20 minutes, or until you are exhausted with an additional 5-minute warm up using a Standard VO^2 Max Protocol. This requires continuously cycling at 60rpm with 0.5kg resistance added every 2 minutes. During this time, we will be using facial electromyography to monitor facial activity and electrocardiography to measure heart rate. A facial electromyography will require five electrodes that are stuck onto your forehead using a hypoallergenic gel and adhesive tape. We will also be collecting VO2

max which will involve you breathing into a tube for a total of 1 minute throughout your exercise.
After you have indicated that you are tired, you will then complete a psychometric test. The electrodes and heart rate monitor will stay attached throughout the psychometric test.

The second session will occur a week after the first session. This will involve a berry fruit extract intervention in the form of a freeze-dried powder and water mix an hour prior to the cycling test. This will then be followed by a psychometric test. We will also collect the food diaries.

The day after the second session, you will receive a phone call or email to determine if fruit extract can affect recovery from exercise.
This should only require a maximum of five hours of your time.
There is no conflict of financial interest within this project.

Data Management

Data will be collected by measuring weight, height and heart rate. Data will then be collected using baseline fitness and psychometric tests to assess cognitive functioning and level of fatigue after exercising.

Clinical health information relating to an identifiable individual must be retained for at least 10 years (this does not include your health screening questionnaire).
As this is student research, Dr Peter Cannon will be responsible for the archiving of these data. Deidentified data will be archived in an online repository.

All data will be coded using a participant number allocated to your data. No names or identifiable data will be used.

A summary of the project findings will be emailed to you at the completion of data analyses. If you wish to receive this summary then please give your email address to the experimenter.

Participant's Rights

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

- decline to answer any particular question;
- stop any activity at any time;
- withdraw from the study;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Project Contacts

If you have any questions or concerns, please feel free to contact us at any time about the project.

Natalie Peart

[redacted]

Peter Cannon
p.r.cannon@massey.ac.nz
09414 0800 **Ext:** 43102

Declarations

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 17/11. If you have any concerns about the conduct of this research, please contact Dr Lesley Batten, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 356 9099 x 85094, email humanethicsoutha@massey.ac.nz .

Compensation for Injury

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

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

Appendix A

Please avoid eating these foods two days before each session.

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)

Appendix D – Health Screening Questionnaire

Pre-Exercise Health Screening Questionnaire

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TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Pre-Exercise Health Screening Questionnaire

Name:____

Address: ____

Phone: ______________________________

Age: _______________

Emergency Contact

Name:____

Phone: ______________________________

Please read the following questions carefully. If you have any difficulty, please advise the researcher who is conducting the exercise test.

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

This questionnaire has been designed to identify the small number of persons (15-69 years of age) for whom physical activity might be inappropriate. The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

Qu 6. Do you take any medication?

Yes ☐ No ☐

What is the medication for?

Qu 7. Do you have a bone or joint problem that could be made worse by vigorous exercise?

Yes ☐ No ☐

Qu 9. Do you know of any other reason why you should not do physical activity?

Yes ☐ No ☐

Qu 10. Have any immediate family had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 11. Are you now, or have you been recently pregnant?

Yes ☐ No ☐

Qu 12. Have you been hospitalised recently?

Yes ☐ No ☐

Qu 13. Do you have any chronic injury or are you currently recovering from an injury?

Yes ☐ No ☐

Qu 14. Do you have any eye sight injuries or require glasses for computer/reading work?

Yes ☐ No ☐

Qu 15. Do you have any allergies or intolerances to berries?

Yes ☐ No ☐

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise. Though extremely rare, such cases can occur in people with an undiagnosed heart condition. If you have any reason to suspect that you may have a heart condition that will put you at risk during exercise, you should seek advice from a medical practitioner before undertaking an exercise test.

I have read, understood and completed this questionnaire.

Signature: ______________________________________ Date: __________________

References

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

Appendix E – Participant Consent Form

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TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Fruit modulation of the effects of fatigue on cognitive performance

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree/do not agree to the being contacted about the results of this study.

I know who to contact if I have any questions about the study in general.

I understand the compensation provisions in case of injury during the study.

I consent to the research staff collecting and processing my information, including information about my health.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time.

I have been given sufficient time to consider whether or not to participate in this study.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

I understand that my participant in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: .... **Date:** ....

Full Name - printed ....

Declaration by member of research team:

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

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

Signature: .... **Date:** ....

Full Name - printed ....

Appendix F – On the Day

Intervention day

Thank you for taking part in this study! Your help is greatly appreciated! Below are parking tips, and information about the sessions.

On your last session you will receive your $30 voucher, a body analysis report and I will also send you a report of the analysis from your VO2 max test to your email address.

My contact details are:

[redacted] Or [redacted]

If you have any troubles or cannot make a session, please let me know as soon as possible.

Directions to Oteha Rohe Campus:

This campus is on Albany Highway. For the first session, the best parking would be in the Student Parking through Gate 5. Please make sure you use the student parking spots and not the staff or visitors. Appropriate parking areas are marked SP on the map below. All the buildings on this campus are a maximum 5 minute walk so wherever you park will not be far from the buildings that we will be using.

Please note that we will go through all of this on the day also so don't feel pressured to memorise it.

What to wear:

Please wear comfortable gym clothes that you can cycle in. The heart rate monitor will be attached to your chest and hip so flexible clothing will be required. We will have a hypoallergenic cleanser (Cetaphil) to allow you to wash your face before we stick on the electrodes.

Food Diaries:

Please take two days worth of food diaries before the intervention and either give me the paper on the day or send it to me via email. A food diary is just a note of what food you have eaten that day.

Please do not take food diaries on the below two restriction days.

Eg: Monday:

2 weetbix

only continue for one more minute and at this stage I will give you nose clips and put a tube in your mouth to measure your VO2 max capacity. We will practice this at the start so that you know what it will feel like.

After you have stopped cycling you will complete the psychometric test again.

There are showers and a toilet in the Lab so feel free to have a shower when you have finished.

Second Session

For the second session, we will be in the same building - Sport and Exercise lab at Building 60 (Gate 5). You will drink a berry fruit juice and then wait for an hour before completing the rest of the test. During this time, you can leave or study/hang out on facebook.

Once the hour has passed we will then attach all the monitors and complete a psychometric test. Then the cycling will be completed again and another psychometric test.

Follow up email

The next day I will send you a follow up email and ask you how you feel after the exercise.

200mL standard milk

45g chocolate

1 banana

150g yoghurt

90g chicken

1 cup of mixed vegetables (etc)

Please try to eat what you would normally eat.

Two days before your session:

Please try to limit your intake or avoid (preferably) all foods with the following:

> **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)

Please do not take food diaries on these two restriction days.

First Session:

On the day, it would be appreciated if you would arrive 5 minutes early at Building 60 (see below map). Please do not eat anything two hours before your session time.

The first thing we will do is the body analysis composition. If you could avoid any drinks in the hour beforehand and use the bathroom (either at home or there is one in the building), this would make your analysis more accurate.

We will then attach the electrodes for the facial electromyography and heart rate monitor. You will then complete a psychometric test a couple of times to get used to the test and what it asks of you. After that we will begin the cycling which will have resistance of 0.5kg added every 2 minutes. I will ask you every two minutes how you are feeling. You will indicate when you can

Appendix G – Stroop Test

Appendix H – Borg Rating of Perceived Exertion Scale

Borg Rating of Perceived Exertion

6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix I – Dietary Restrictions

Please avoid eating these foods two days before each session.

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.

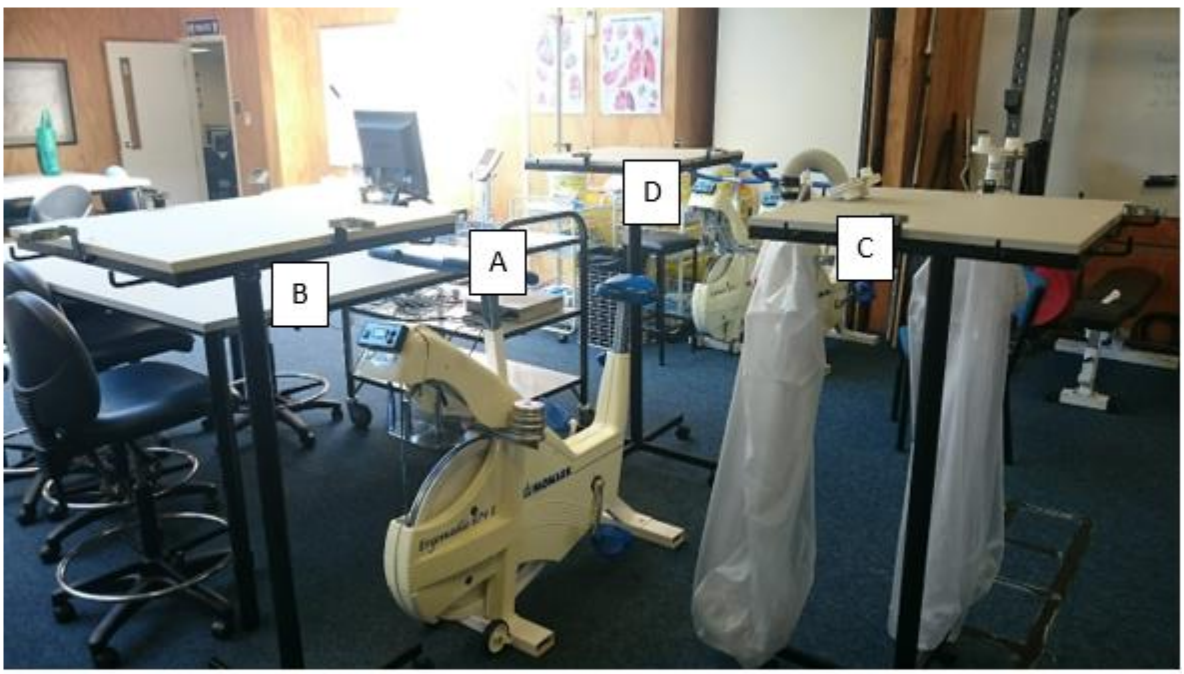
Vegetables and Vegetable Juice: Aubergine, Beans (red and kidney), Potato/Sweet Potato (red and purple), Onion (red), Cabbage (red), Broccoli (purple), Beetroot, Corn (purple), Olives (black), and Avocado.

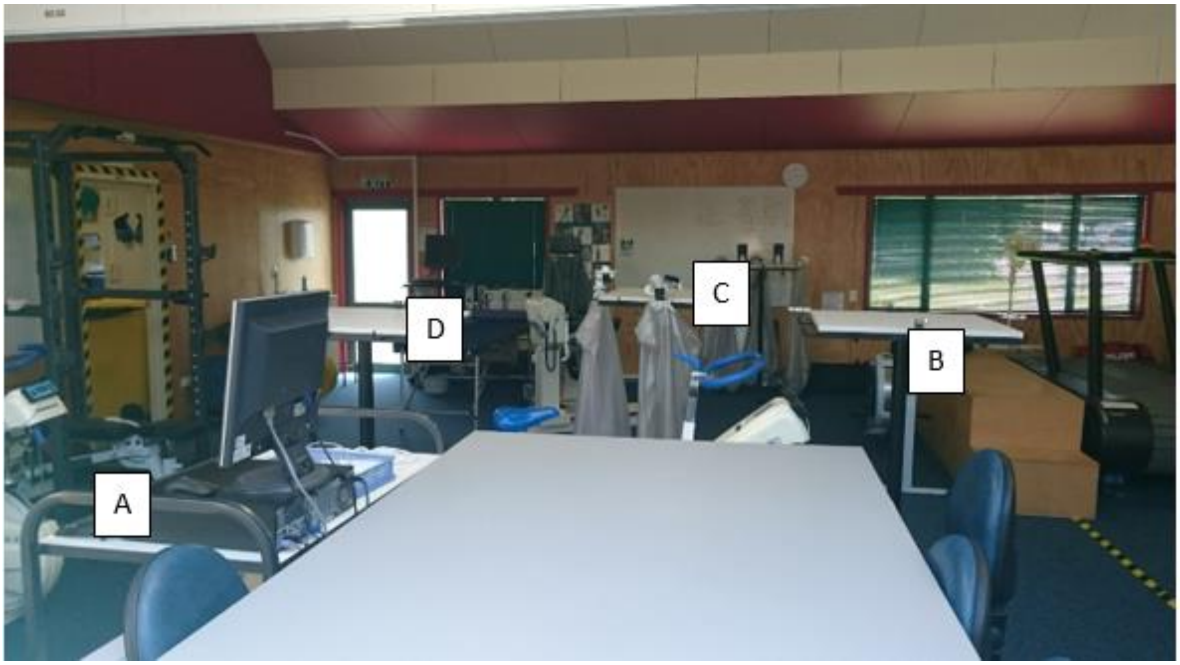
Miscellaneous: Wine (red), Coffee, Chocolate (dark), and Tea (black and green).

Dietary Supplements: (e.g. all supplements containing berries, vitamin C and Vitamin E)

Appendix J – Lab set up

Computer was based on a rolling shelf (A) for easy transportation. This contained all materials needed for the study, screen for the researcher to monitor heart rate and facial EMG and the Powerlab. Three rolling high tables were used. One to hold the laptop screen in front of the participants on the bike (B). One to hold the Douglas bags which was placed next to the participant on the bike (C). One to hold the cord from the Powerlab that connected to the electrodes (D). This could be moved easily if the participant wanted to sit or stand.





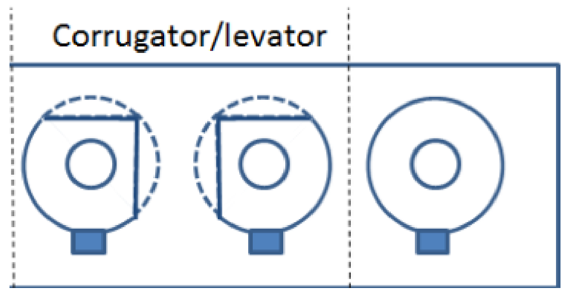
The below pictures are the Gas analyser used and stadiometer and BIA machine.

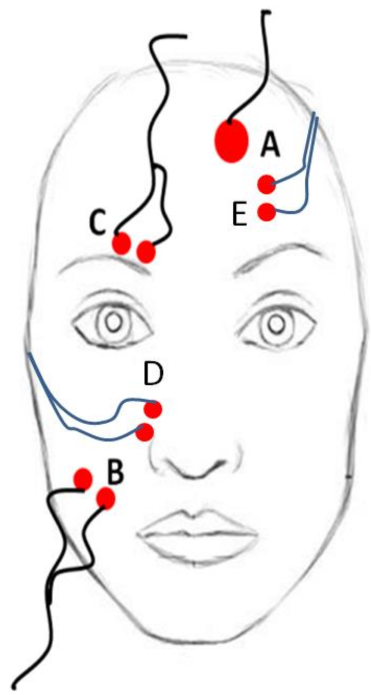

Human Metabolic Pathways
ANATOMY OF THE HEART
THE RESPIRATORY SYSTEM


Harvard
DRY GAS METER

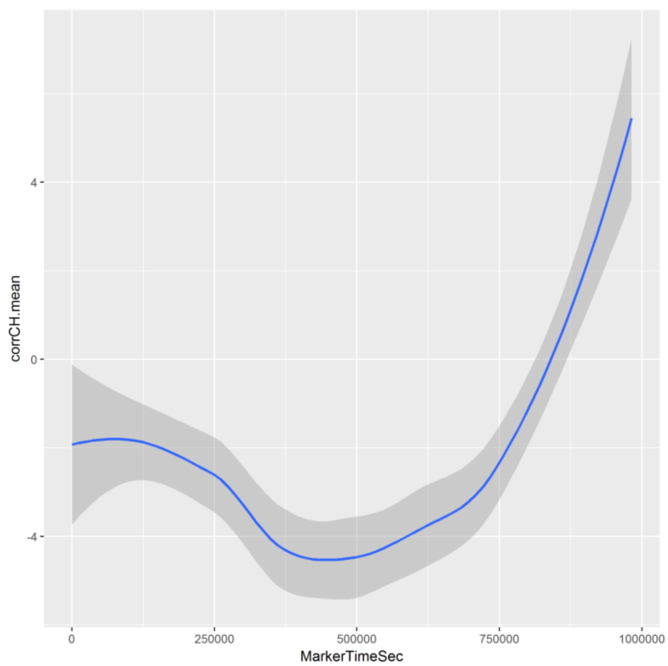
Appendix K – Device used for Reaction Time

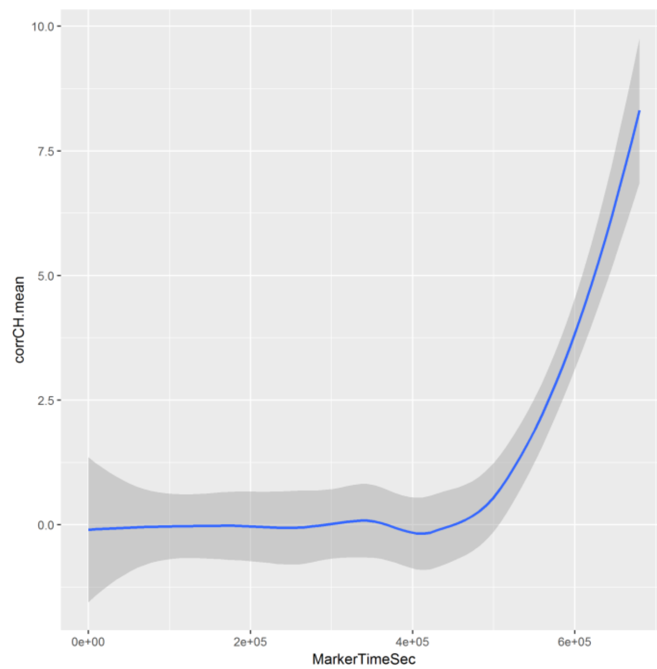
Appendix L – Pakiri Lab Manual

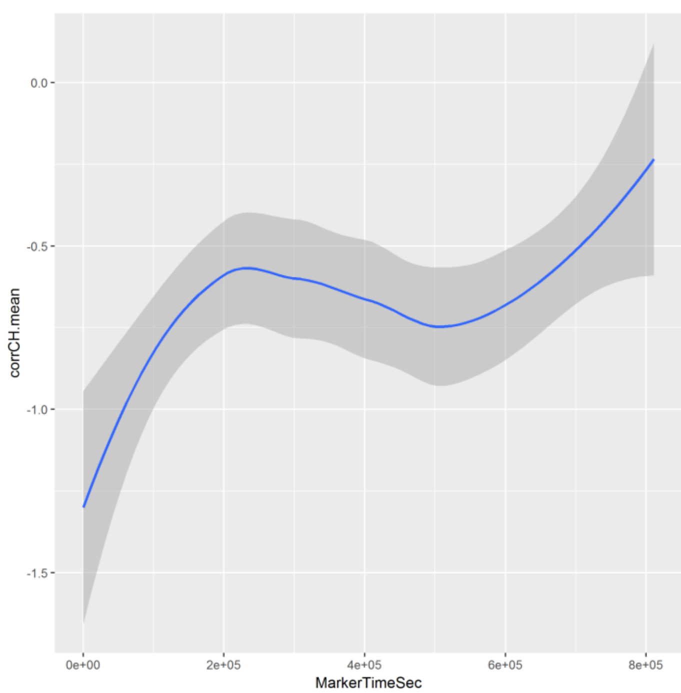


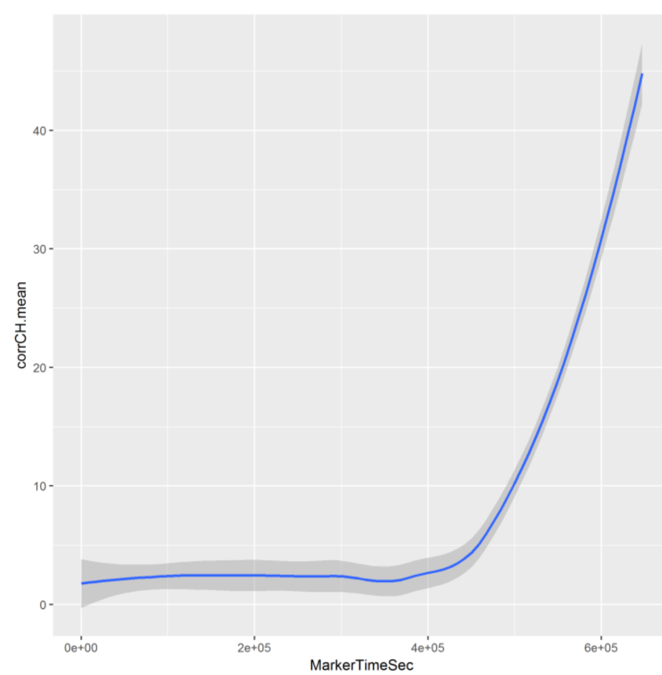


Appendix M – Individual Smoothed Curves for Hypothesis 3



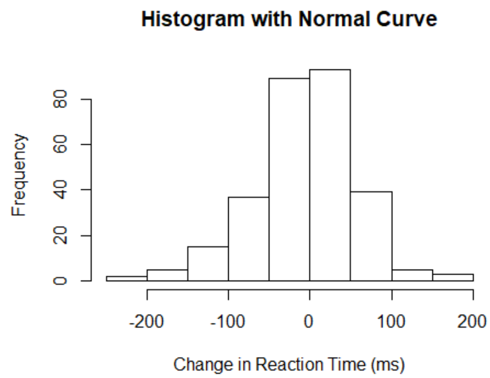


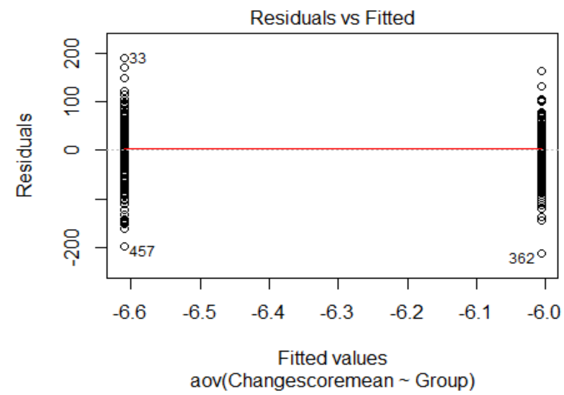


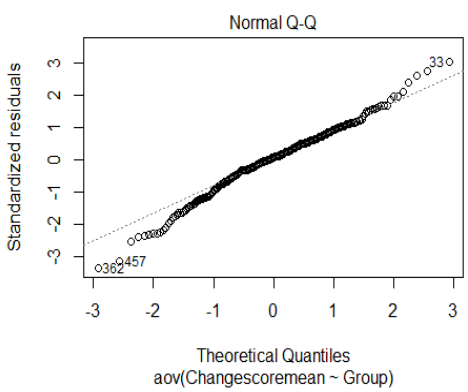


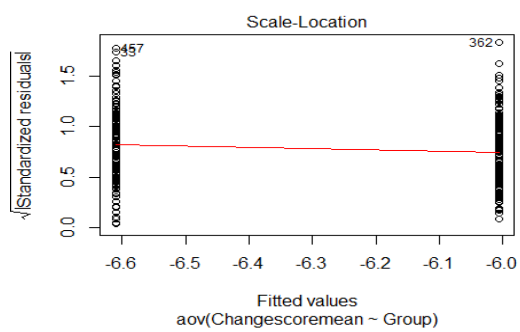
Appendix N – Q-Q Plots and other assumptions

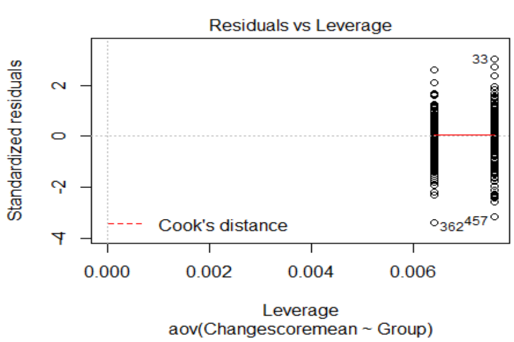
Hypothesis One



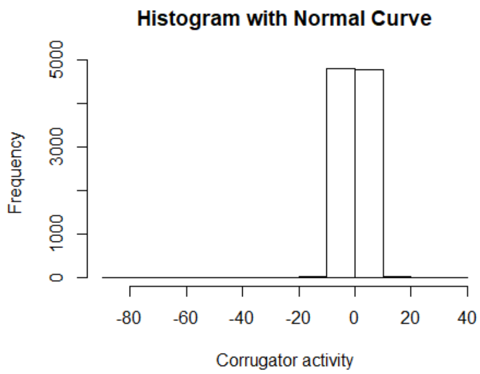


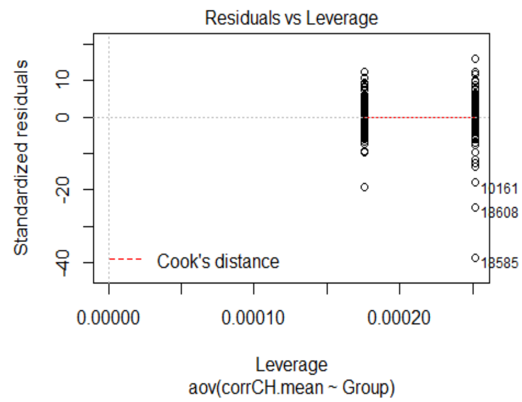


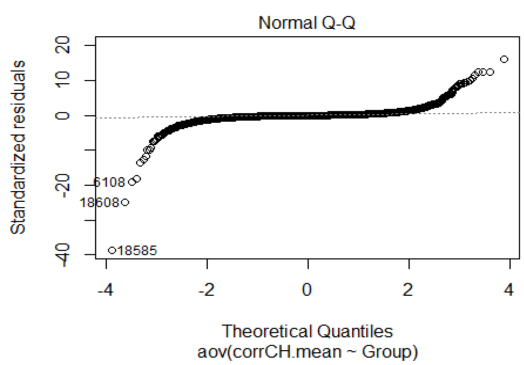


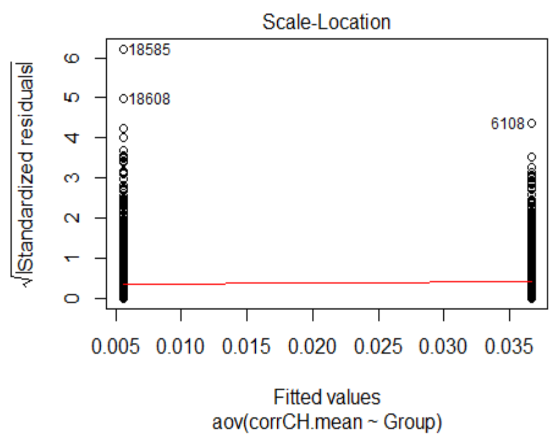


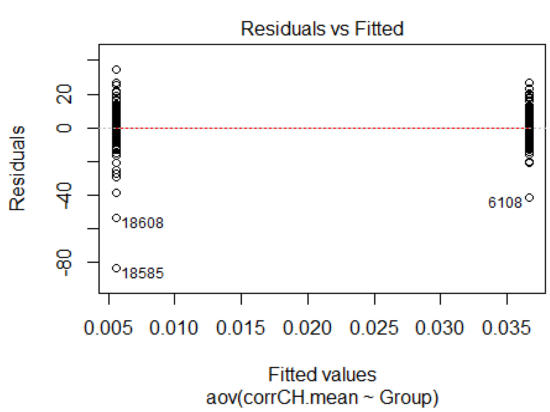
Hypothesis Two



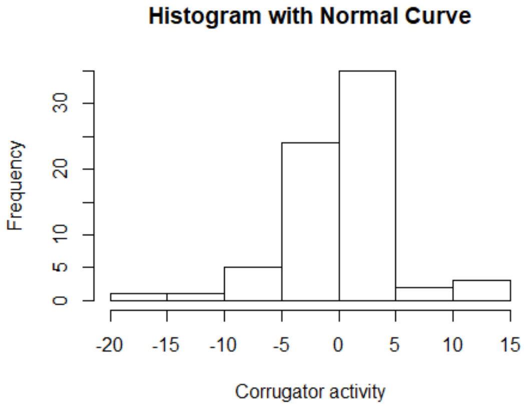


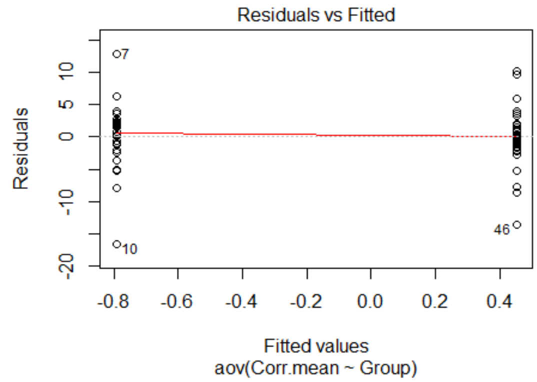


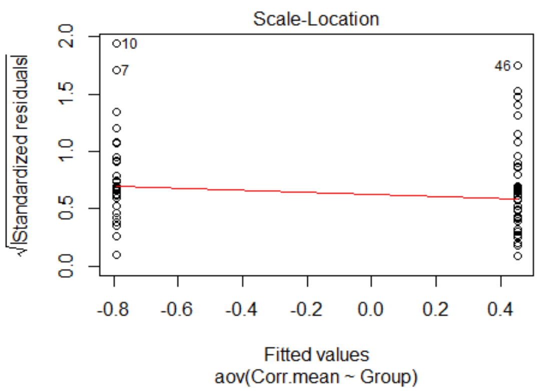


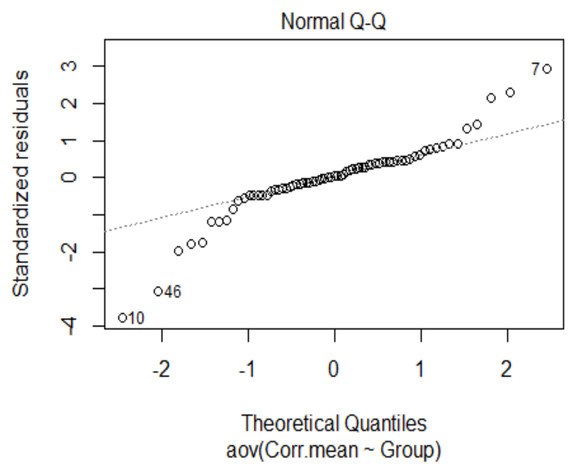


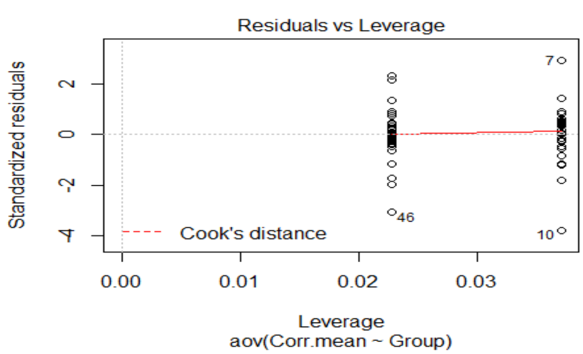
Hypothesis Three



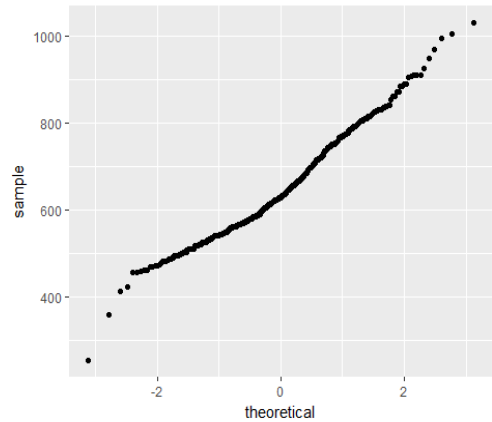


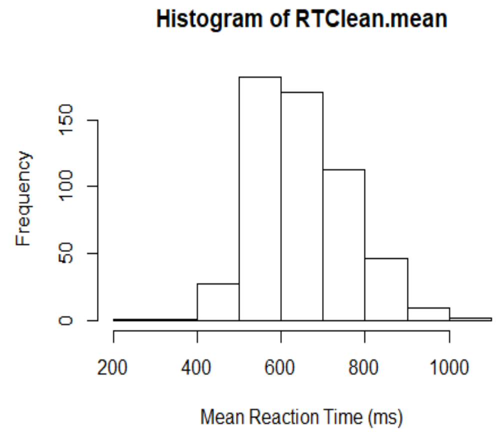


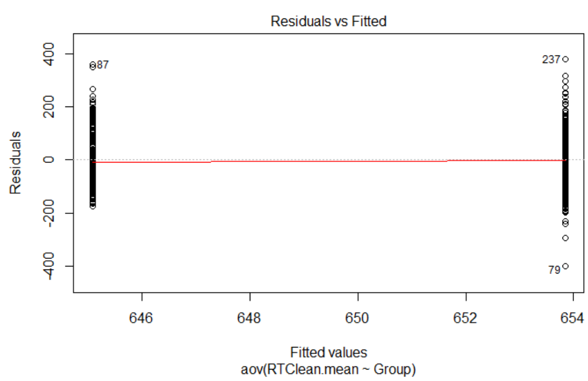


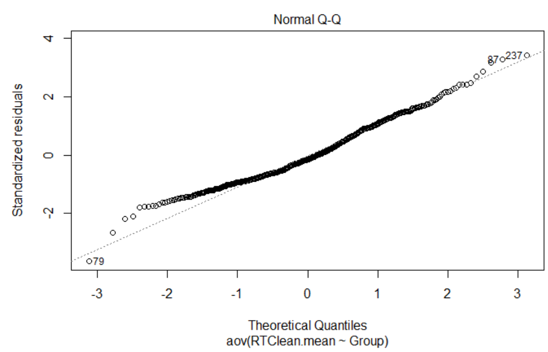


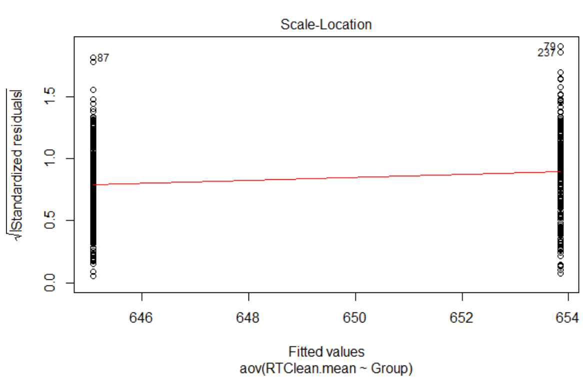
Hypothesis Four

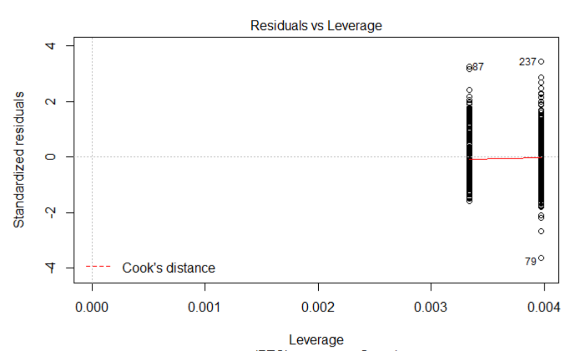




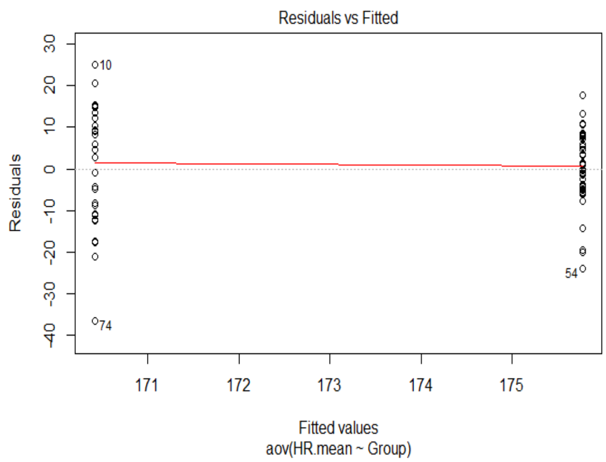


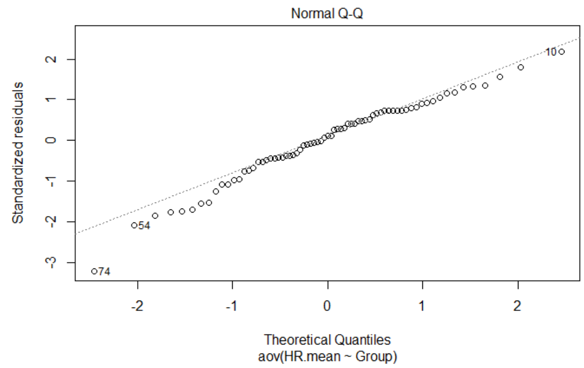


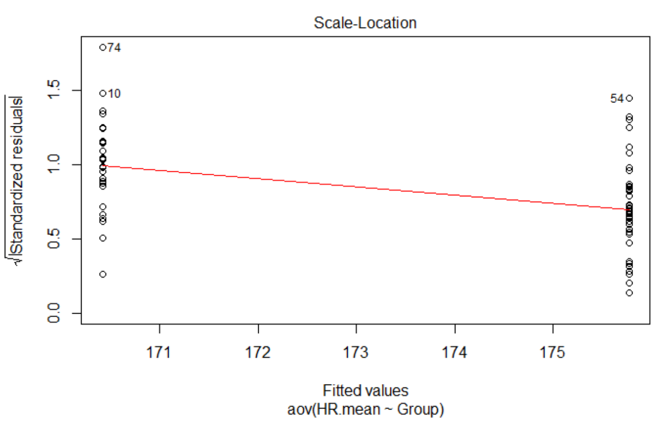


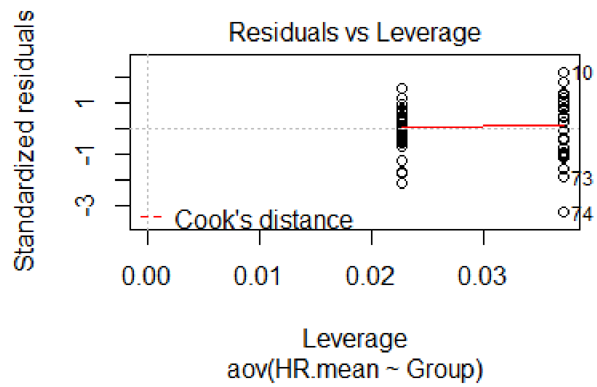


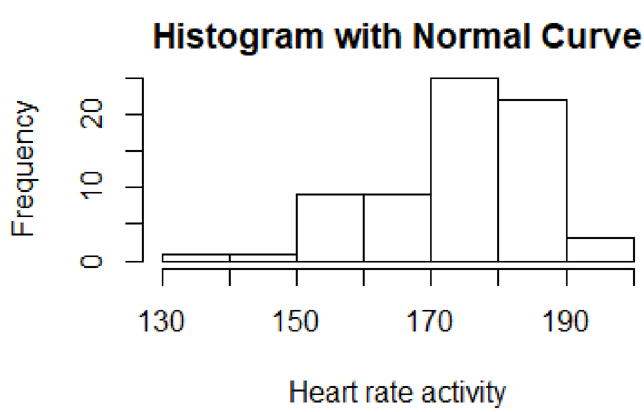
Hypothesis Five



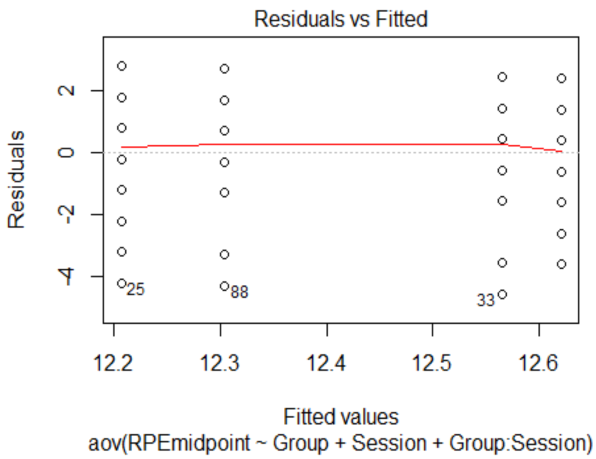


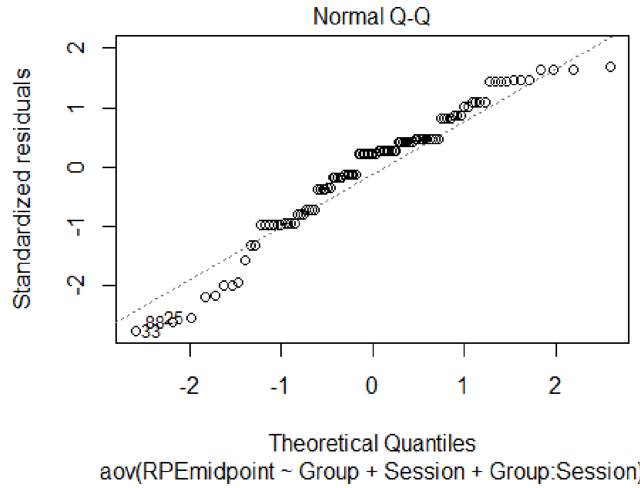


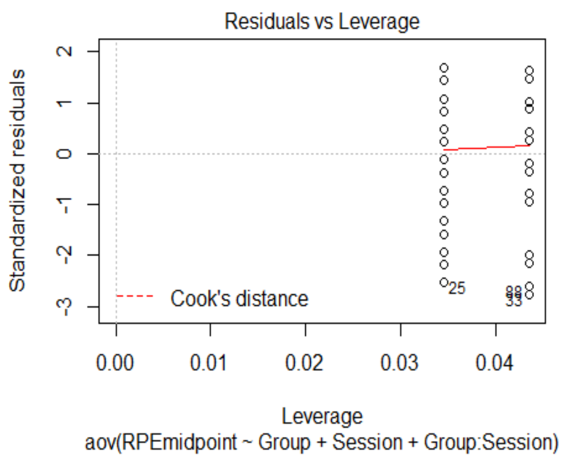


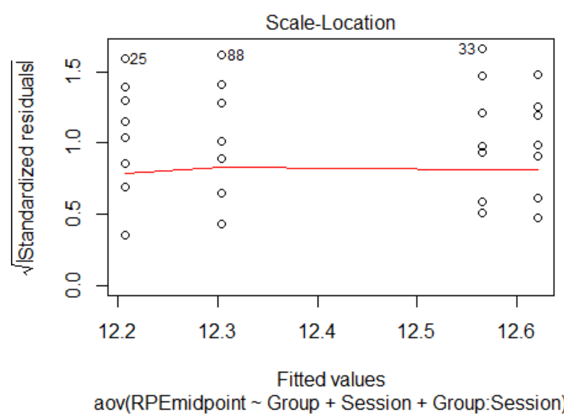


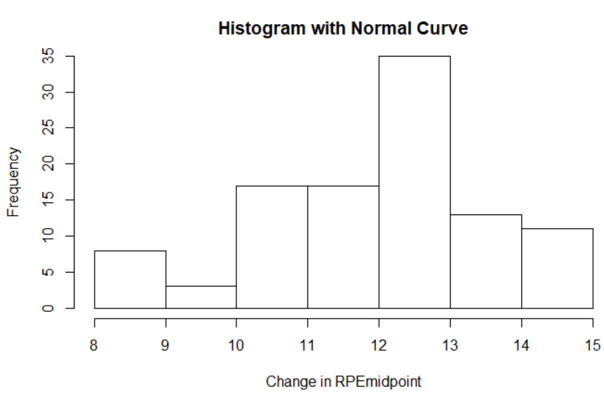
Hypothesis Six



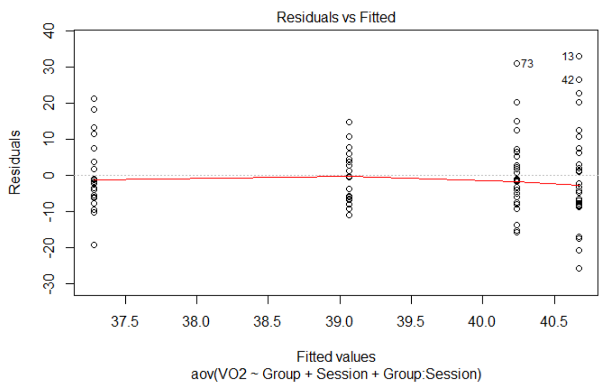


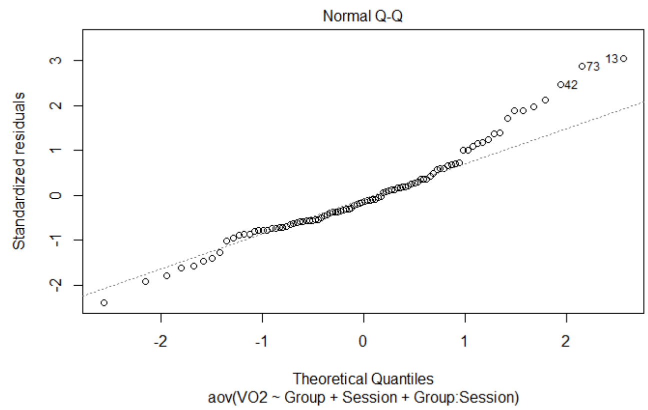


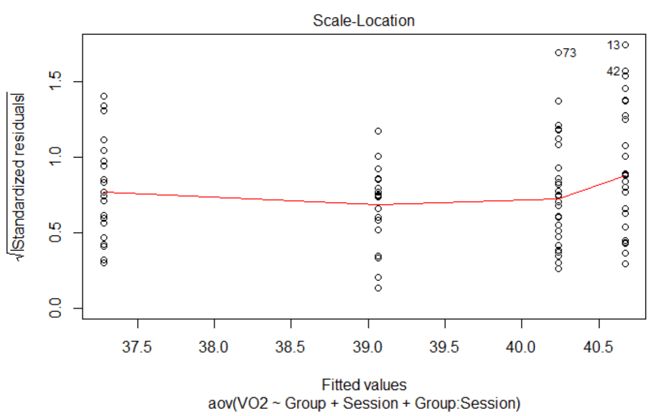


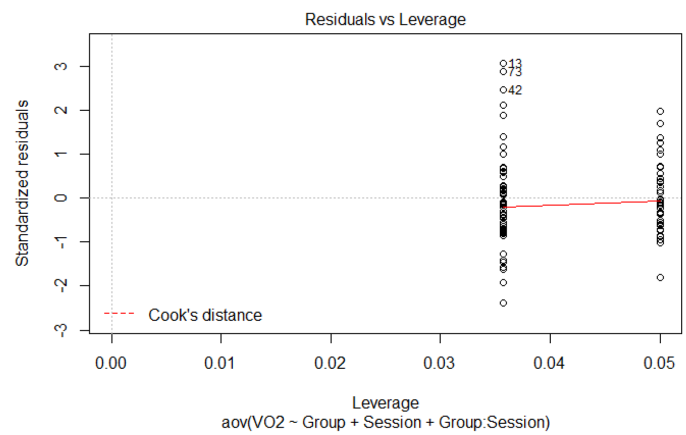


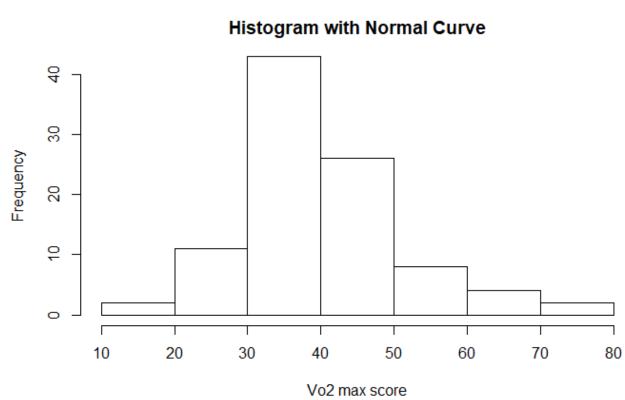
Hypothesis Seven





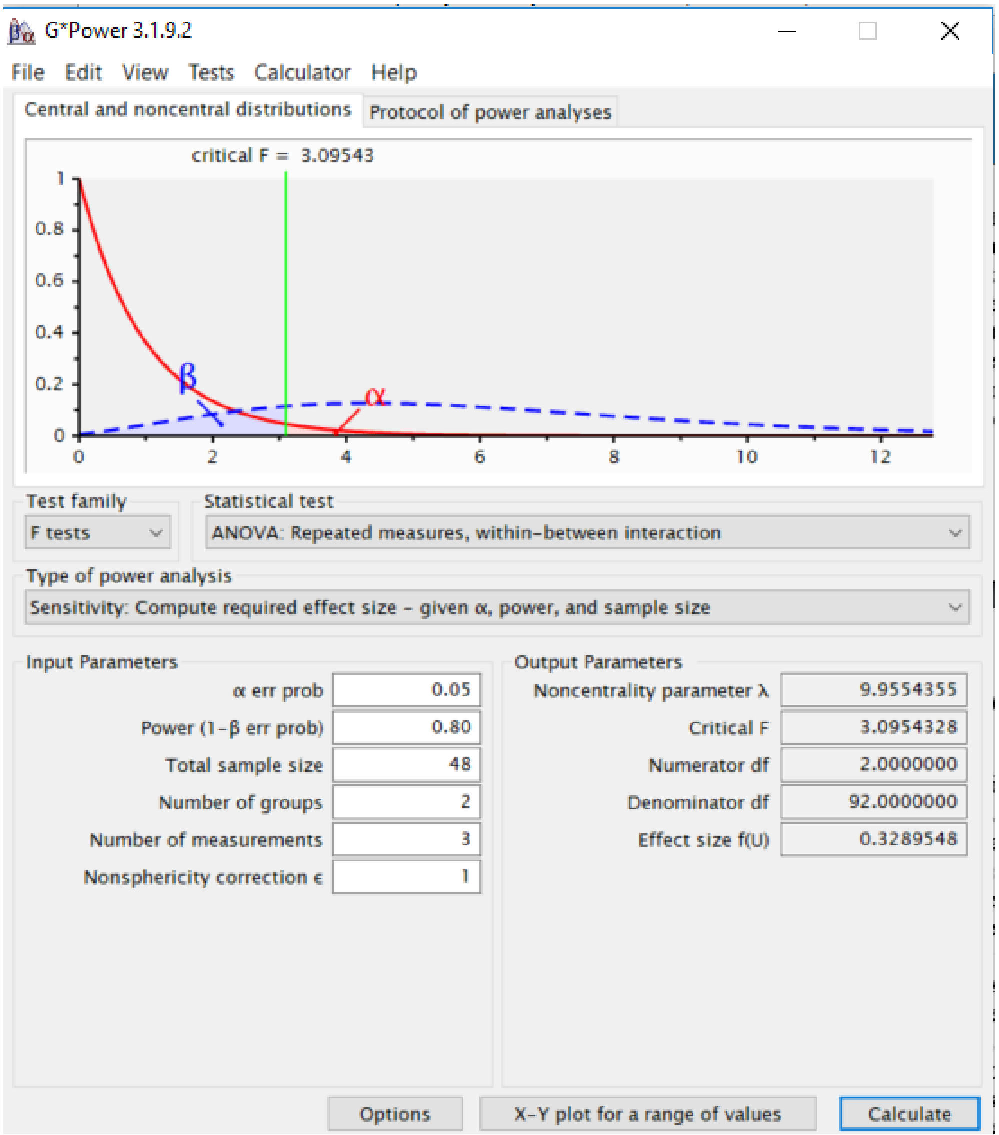




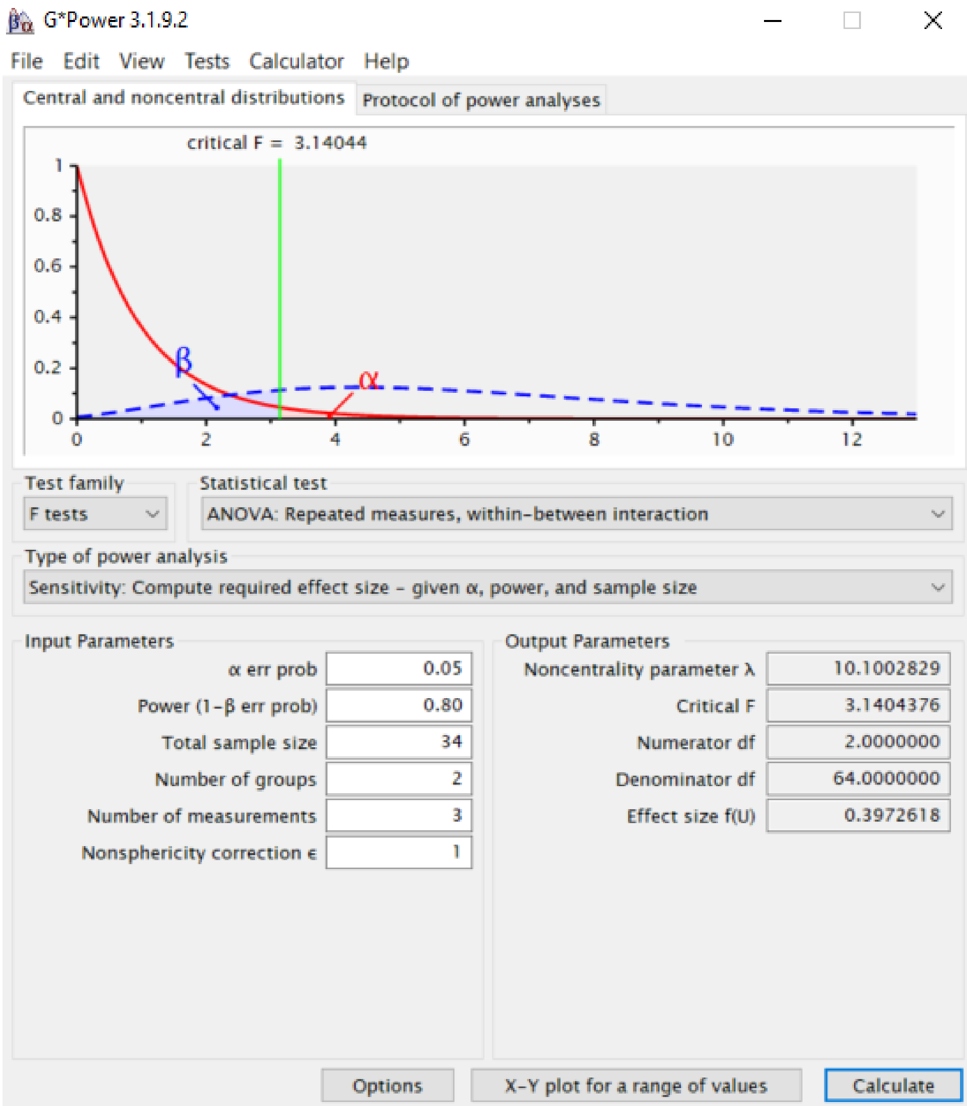


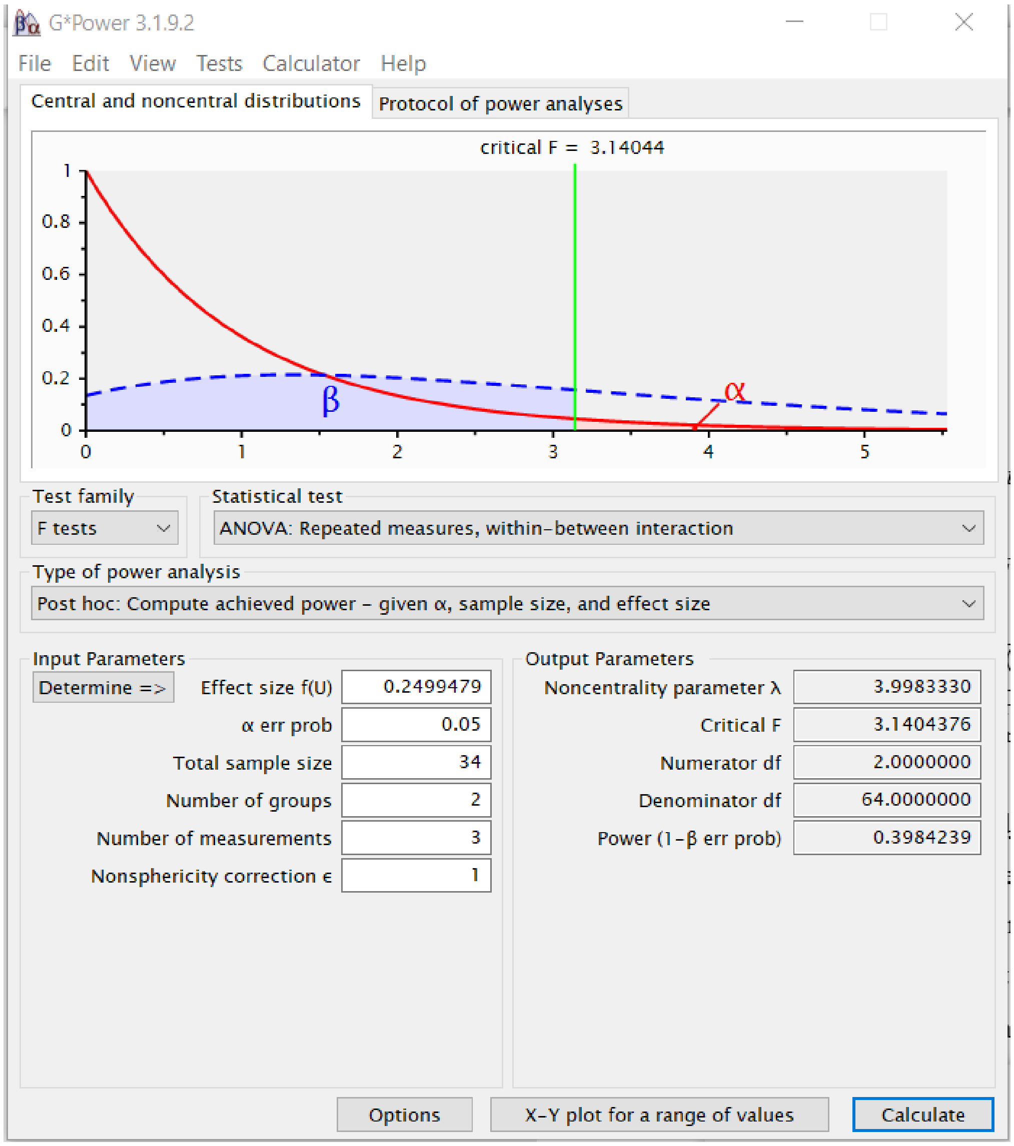
Appendix O – G*Power Post-Hoc Analysis

Hypothesis One

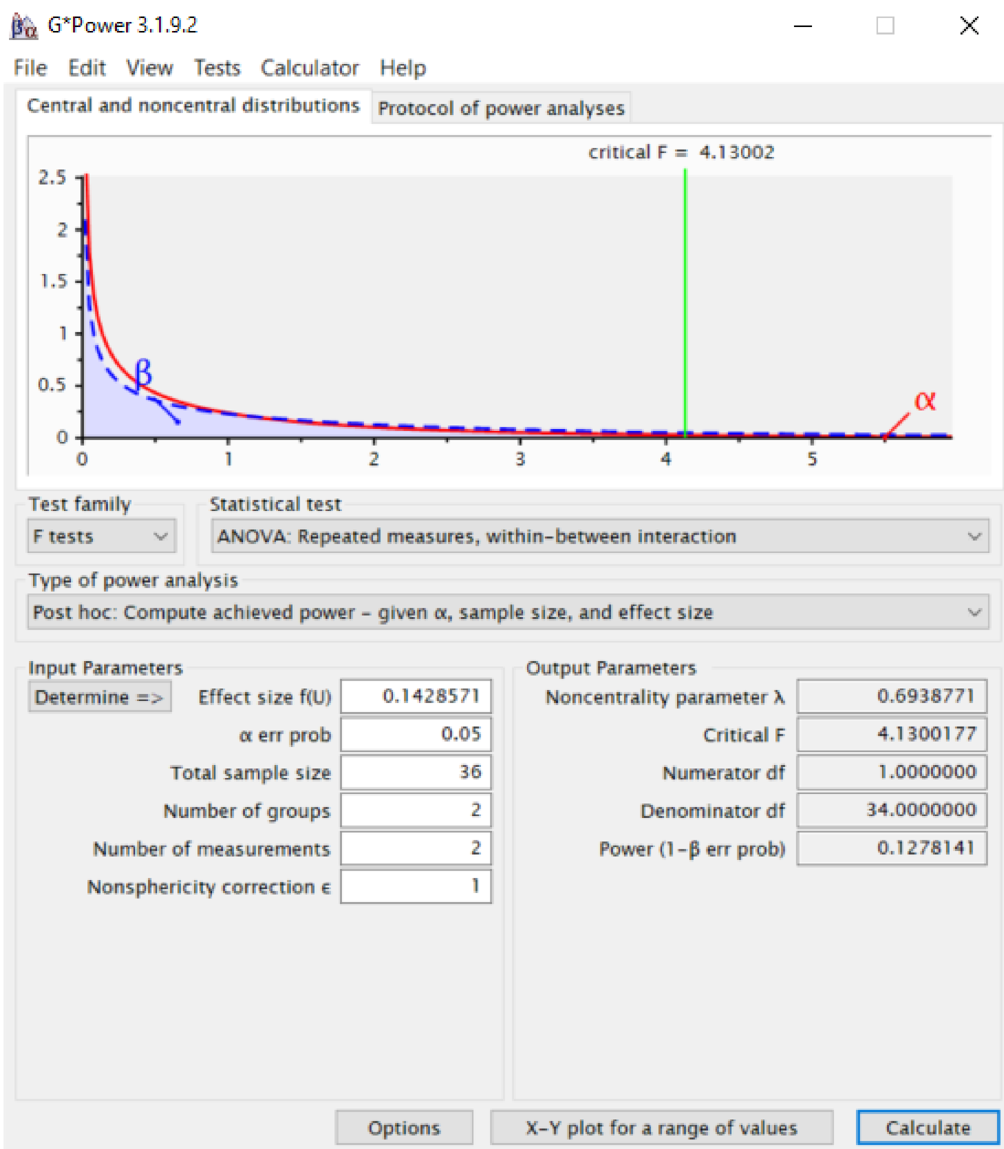


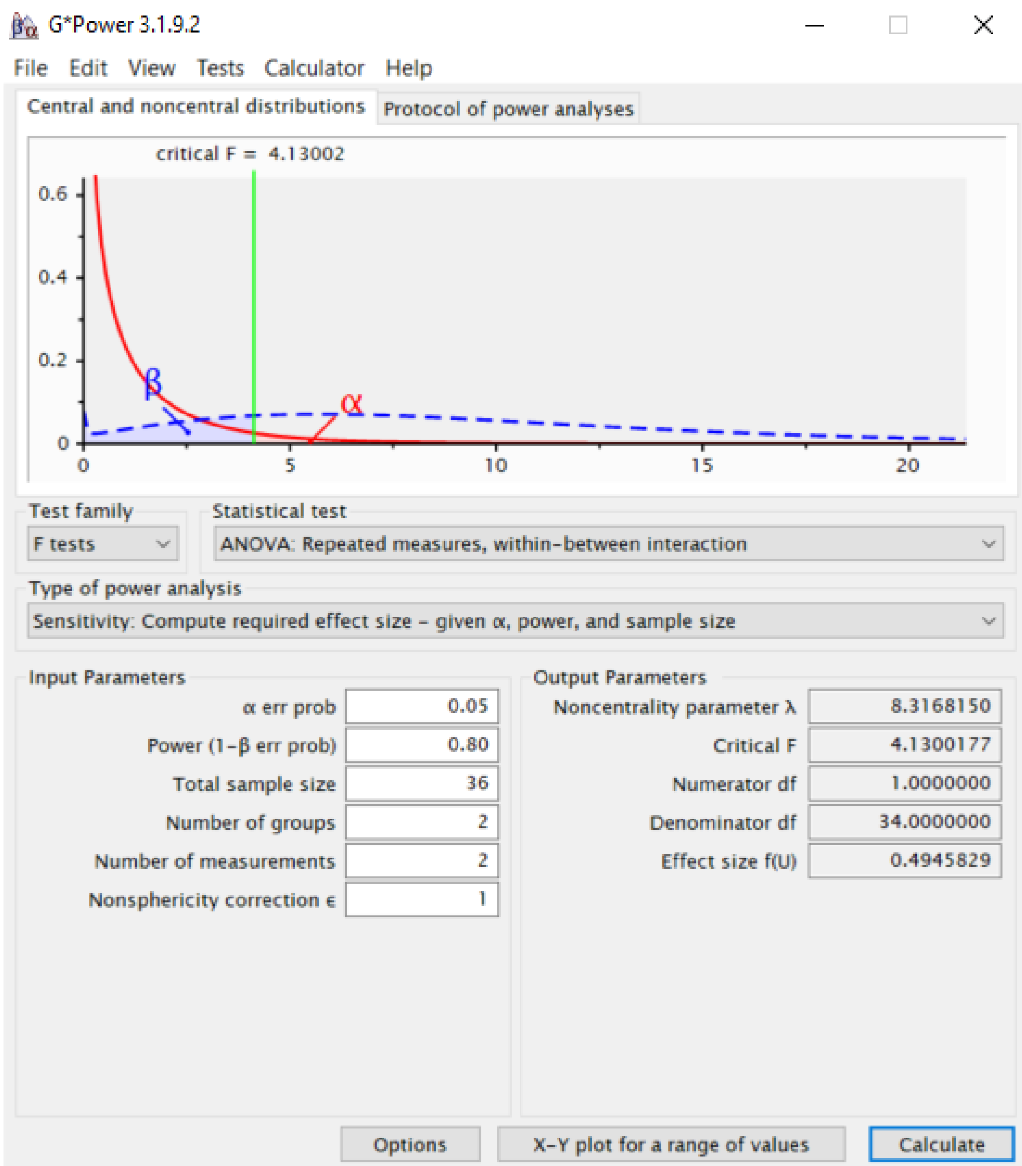
Hypothesis Two

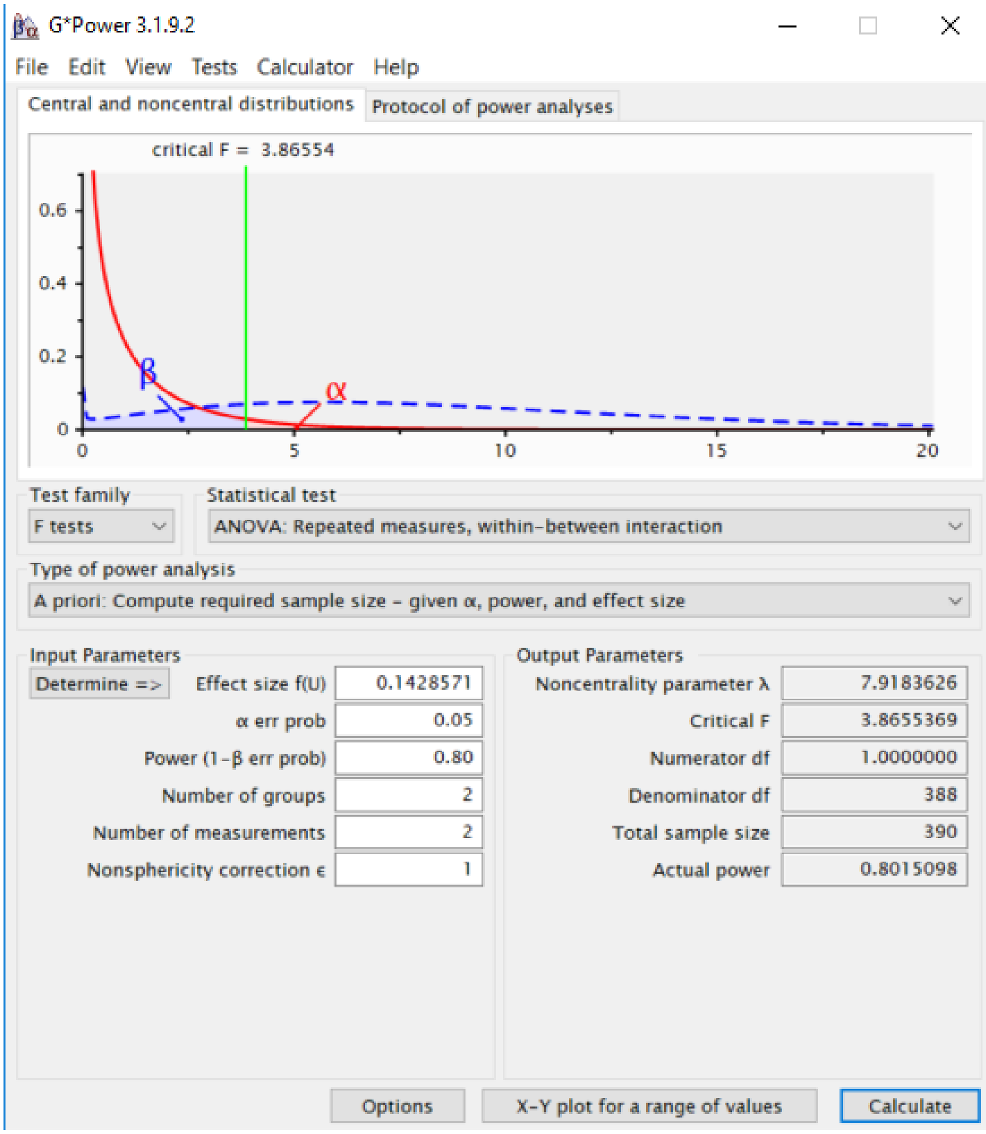



G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical F = 3.14044
β
α
Test family
F tests
Statistical test
ANOVA: Repeated measures, within-between interaction
Type of power analysis
Post hoc: Compute achieved power – given α, sample size, and effect size
Input Parameters
Determine =>
Effect size f(U) 0.2499479
α err prob 0.05
Total sample size 34
Number of groups 2
Number of measurements 3
Nonsphericity correction ε 1
Output Parameters
Noncentrality parameter λ 3.9983330
Critical F 3.1404376
Numerator df 2.0000000
Denominator df 64.0000000
Power (1–β err prob) 0.3984239
Options
X–Y plot for a range of values
Calculate

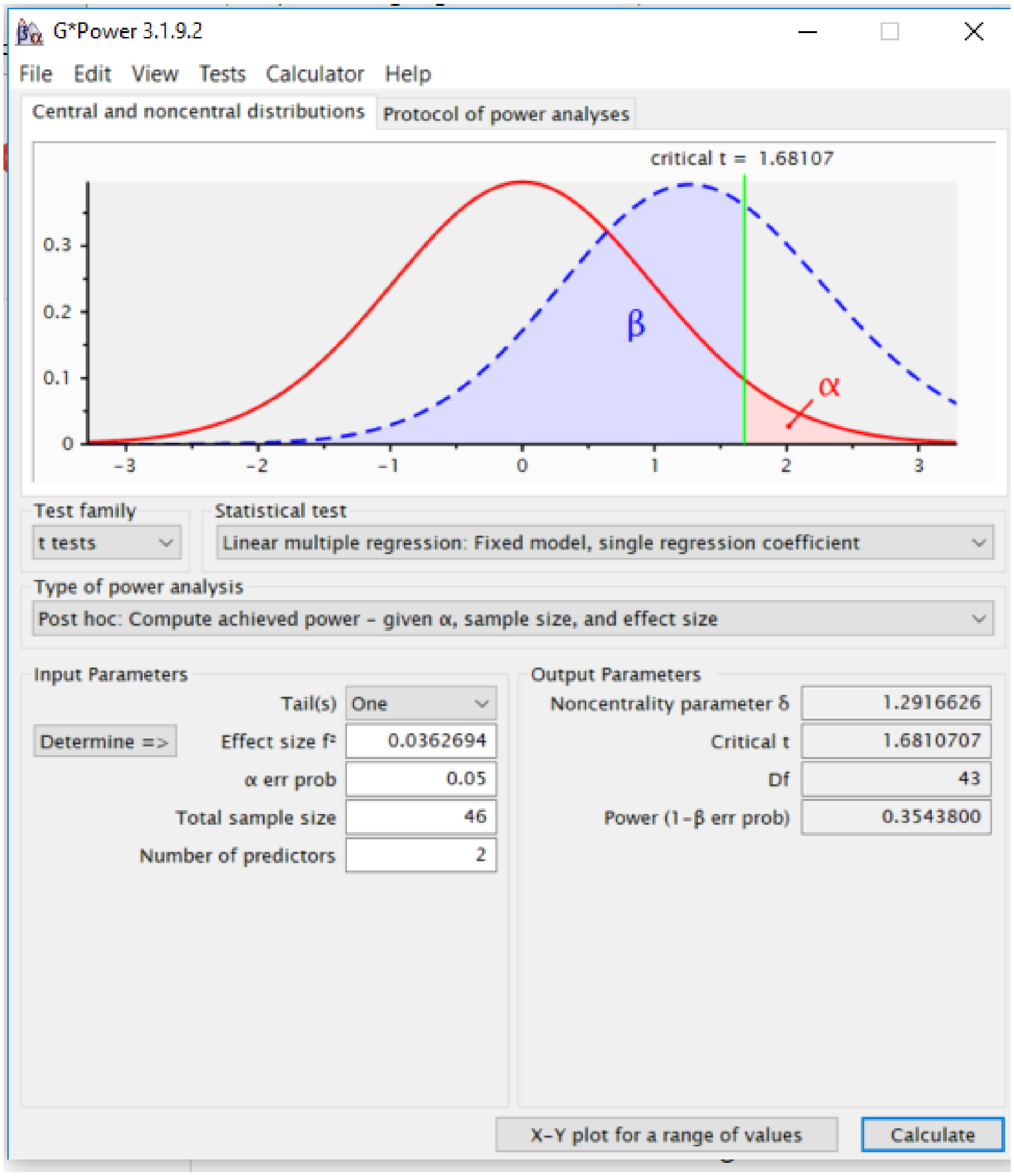
Hypothesis Three

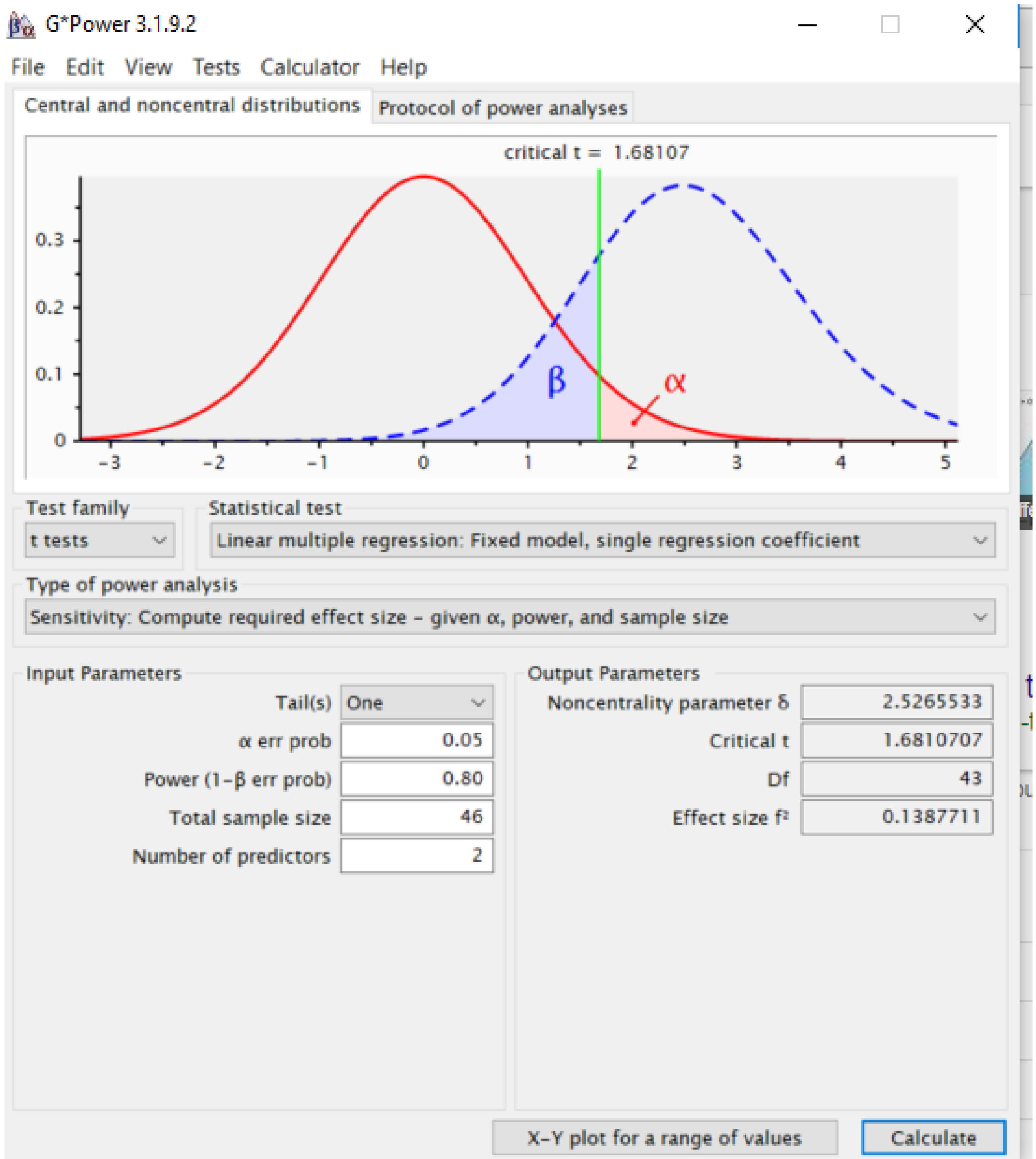


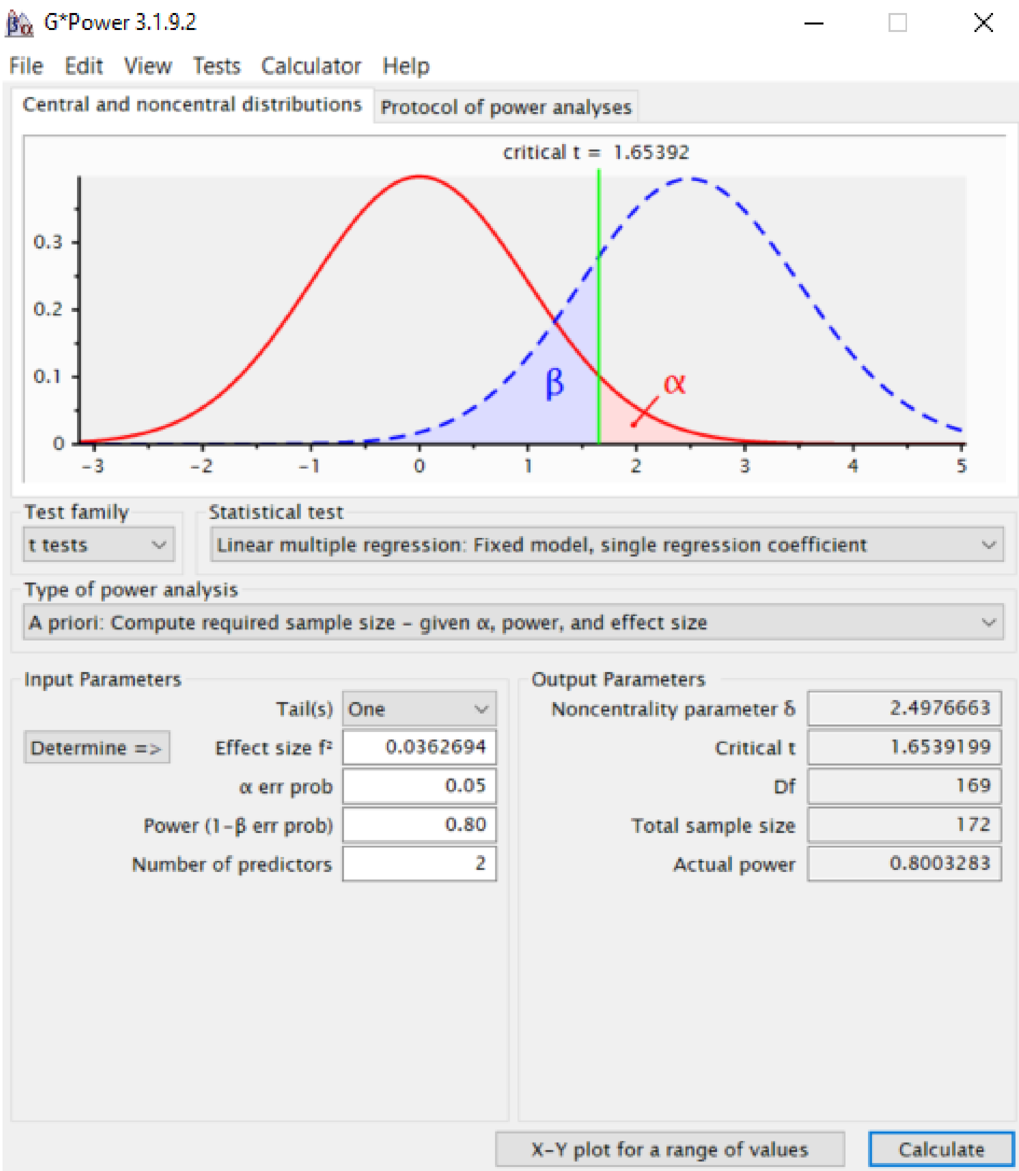

G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical F = 4.13002
0.6
0.4
0.2
0
0
5
10
15
20
β
α
Test family
F tests
Statistical test
ANOVA: Repeated measures, within-between interaction
Type of power analysis
Sensitivity: Compute required effect size – given α, power, and sample size
Input Parameters
α err prob 0.05
Power (1-β err prob) 0.80
Total sample size 36
Number of groups 2
Number of measurements 2
Nonsphericity correction ϵ 1
Output Parameters
Noncentrality parameter λ 8.3168150
Critical F 4.1300177
Numerator df 1.0000000
Denominator df 34.0000000
Effect size f(U) 0.4945829
Options
X-Y plot for a range of values
Calculate


G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical F = 3.86554
Test family
F tests
Statistical test
ANOVA: Repeated measures, within-between interaction
Type of power analysis
A priori: Compute required sample size – given α, power, and effect size
Input Parameters
Determine =>
Effect size f(U) 0.1428571
α err prob 0.05
Power (1–β err prob) 0.80
Number of groups 2
Number of measurements 2
Nonsphericity correction ϵ 1
Output Parameters
Noncentrality parameter λ 7.9183626
Critical F 3.8655369
Numerator df 1.0000000
Denominator df 388
Total sample size 390
Actual power 0.8015098
Options
X–Y plot for a range of values
Calculate

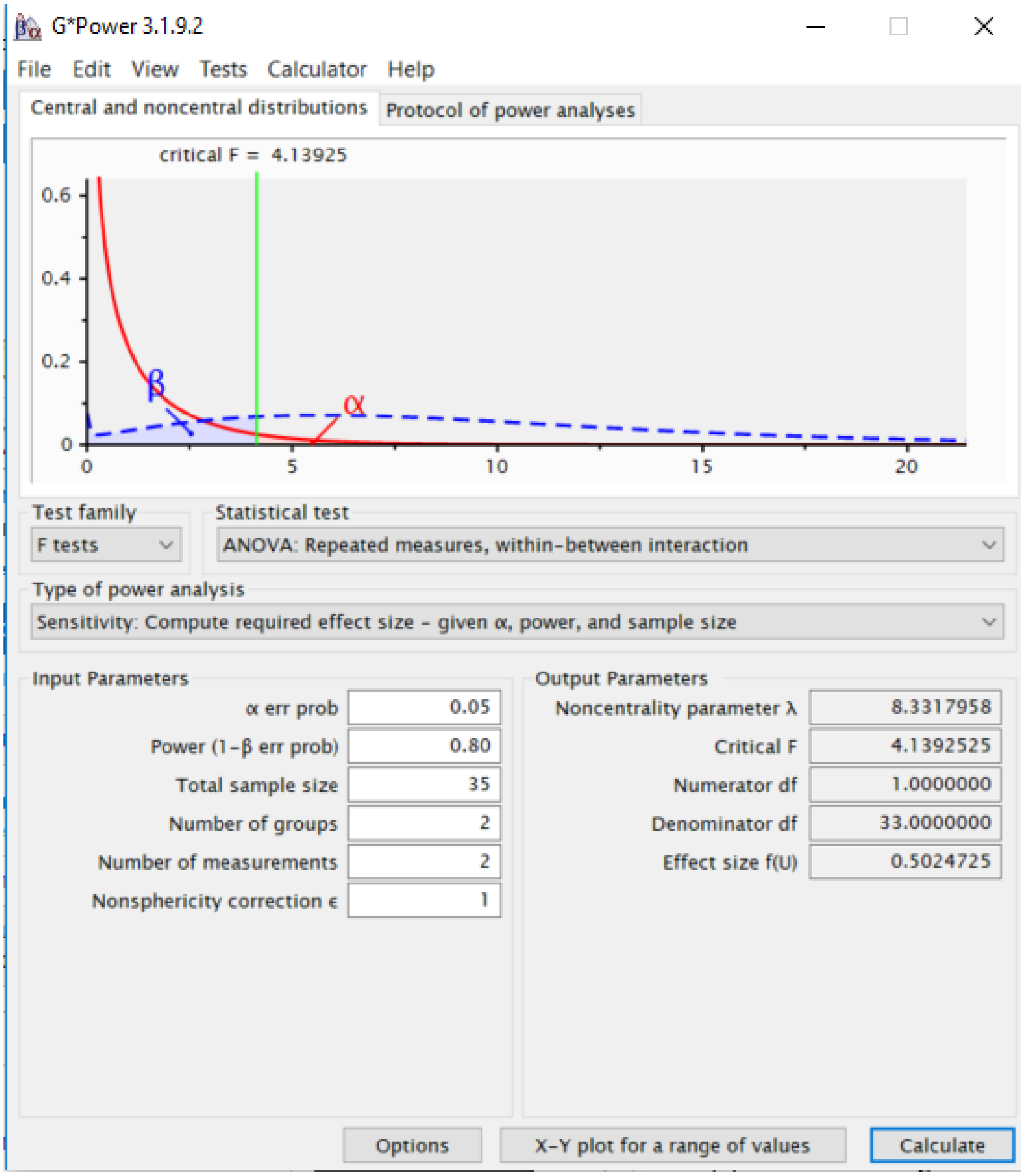
Hypothesis Four



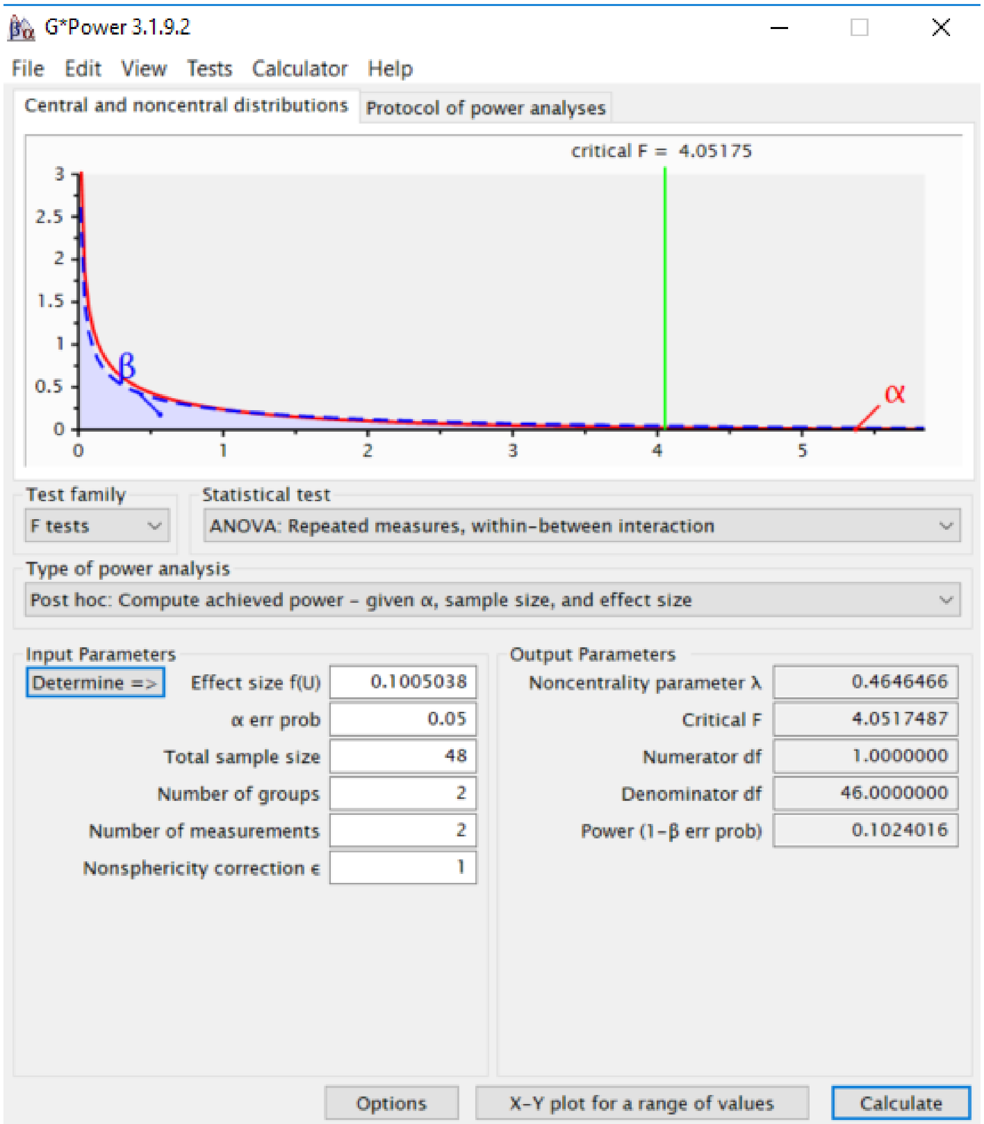

G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical t = 1.68107
β
α
Test family
t tests
Statistical test
Linear multiple regression: Fixed model, single regression coefficient
Type of power analysis
Sensitivity: Compute required effect size – given α, power, and sample size
Input Parameters
Tail(s) One
α err prob 0.05
Power (1-β err prob) 0.80
Total sample size 46
Number of predictors 2
Output Parameters
Noncentrality parameter δ 2.5265533
Critical t 1.6810707
Df 43
Effect size f² 0.1387711
X-Y plot for a range of values
Calculate

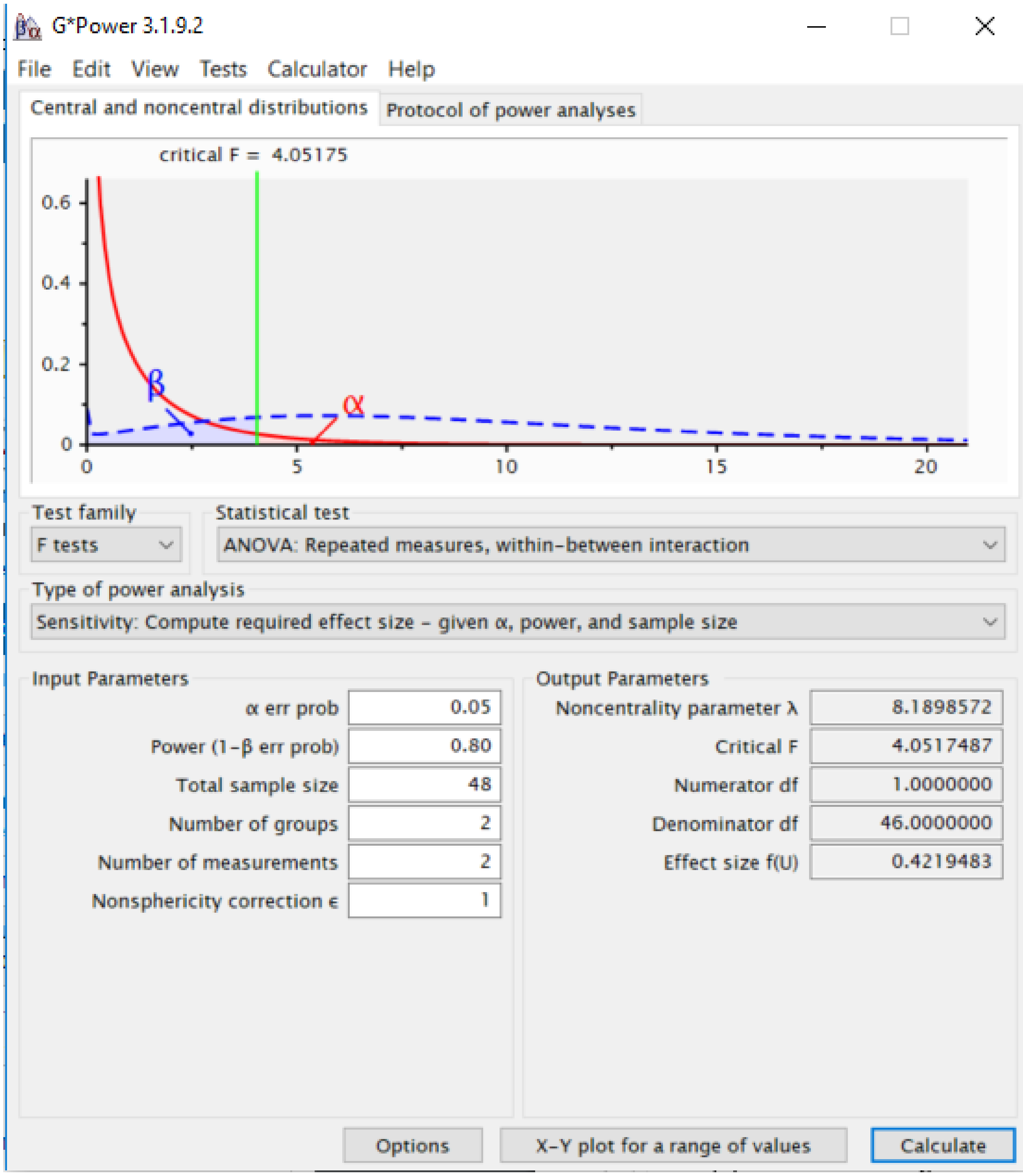

G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical t = 1.65392
0.3
0.2
0.1
0
-3 -2 -1 0 1 2 3 4 5
β
α
Test family
t tests
Statistical test
Linear multiple regression: Fixed model, single regression coefficient
Type of power analysis
A priori: Compute required sample size – given α, power, and effect size
Input Parameters
Tail(s) One
Determine =>
Effect size f² 0.0362694
α err prob 0.05
Power (1-β err prob) 0.80
Number of predictors 2
Output Parameters
Noncentrality parameter δ 2.4976663
Critical t 1.6539199
Df 169
Total sample size 172
Actual power 0.8003283
X-Y plot for a range of values
Calculate

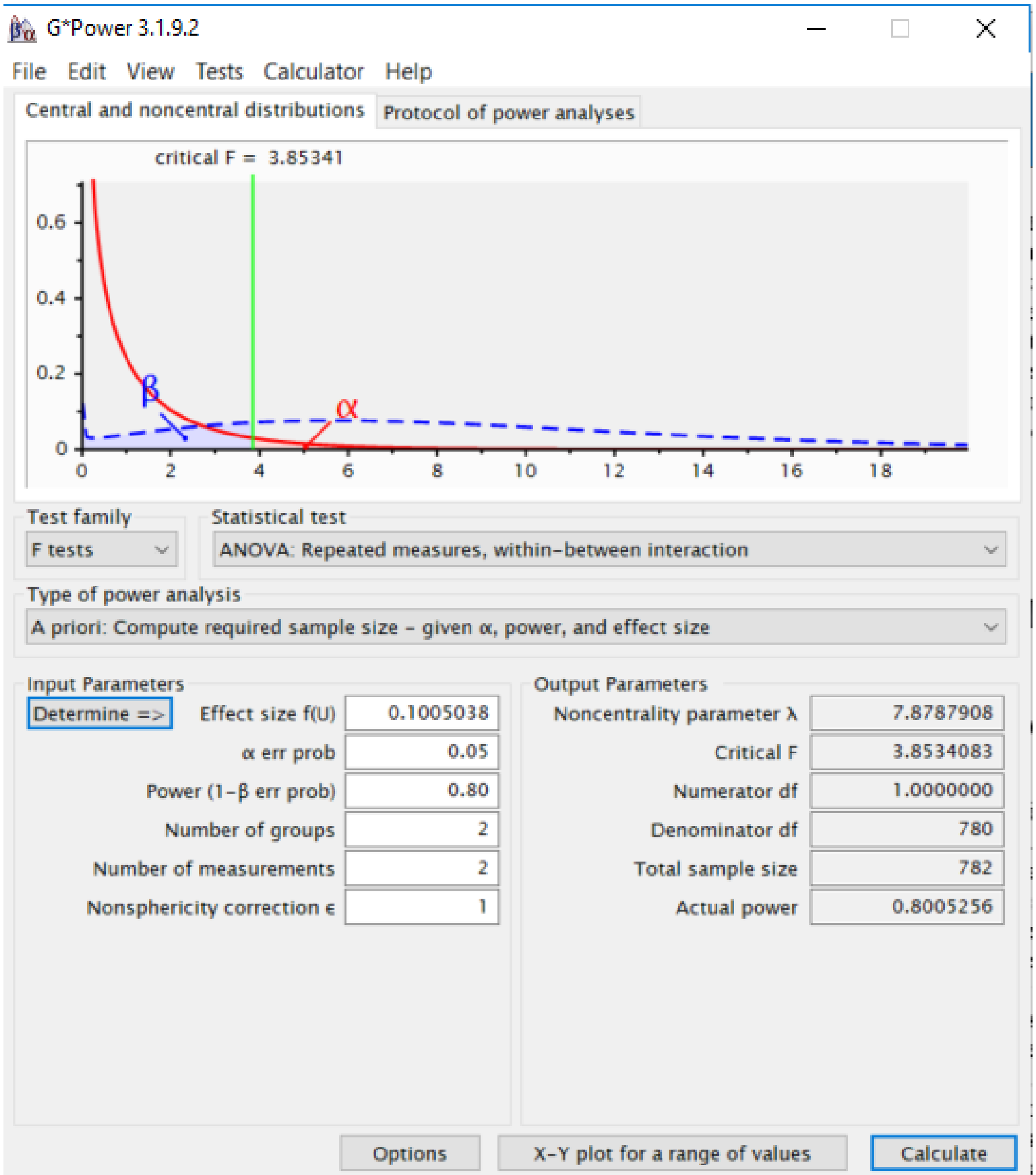
Hypothesis Five



Hypothesis Six




G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical F = 4.05175
Test family
F tests
Statistical test
ANOVA: Repeated measures, within-between interaction
Type of power analysis
Sensitivity: Compute required effect size – given α, power, and sample size
Input Parameters
α err prob 0.05
Power (1–β err prob) 0.80
Total sample size 48
Number of groups 2
Number of measurements 2
Nonsphericity correction ϵ 1
Output Parameters
Noncentrality parameter λ 8.1898572
Critical F 4.0517487
Numerator df 1.0000000
Denominator df 46.0000000
Effect size f(U) 0.4219483
Options
X–Y plot for a range of values
Calculate


G*Power 3.1.9.2
File Edit View Tests Calculator Help
Central and noncentral distributions
Protocol of power analyses
critical F = 3.85341
Test family
F tests
Statistical test
ANOVA: Repeated measures, within-between interaction
Type of power analysis
A priori: Compute required sample size – given α, power, and effect size
Input Parameters
Determine =>
Effect size f(U) 0.1005038
α err prob 0.05
Power (1-β err prob) 0.80
Number of groups 2
Number of measurements 2
Nonsphericity correction ϵ 1
Output Parameters
Noncentrality parameter λ 7.8787908
Critical F 3.8534083
Numerator df 1.0000000
Denominator df 780
Total sample size 782
Actual power 0.8005256
Options
X-Y plot for a range of values
Calculate

Hypothesis Seven