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The Effects of Berry Juice on Cognitive Decline in Older Adults.

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ABSTRACT

This study examined the effects of blackcurrant and boysenberry juices on cognitive processes in older adults. Current research suggests that fruits such as these may be able to reverse some of the effects of ageing on cognition.

The free radical theory of ageing proposes that individuals age because oxidative damage accumulates in cells and interferes with cell functions. The hardest working tissues such as the brain accumulate the most oxidative damage through respiration. Antioxidants can protect against free radical formation and damage. Anthocyanins can contribute to half of the antioxidant capacity of deeply coloured berry fruit. An increase in dietary antioxidants such as anthocyanins may help to alleviate free radical damage within the brain.

Research has shown that oxidative damage within the brain can impair cognitive functioning. Working memory shows age-related decline, along with visuospatial abilities, word retrieval and sustained attention. Some of this decline is thought to be related to oxidative damage of neurotransmitters such as acetylcholine and areas of the brain such as the hippocampus.

Past research with humans has shown that some antioxidants can affect cognitive functioning in an older population. Animal studies have also established that diets enriched with anthocyanins can improve memory, motor control and neurotransmitter functioning. The present study involved giving berry juice drinks to 52 older adults that had been assessed as having a mild impairment of cognitive function. The participants were divided into three groups and drank 200mL a day of either blackcurrant juice, boysenberry juice or a placebo for twelve weeks. The participants were assessed at three different times over the course of the experiment using the RBANS. The RBANS is sensitive to small changes in its tests of memory, visuospatial ability, language and attention.

The results of this study did not support previous research on antioxidants and cognitive functioning. There were no significant interactions between berry juices and any of the cognitive domains assessed by the RBANS over the course of the experiment. Some of the limitations of the study may be responsible for a lack of effect. The experiment was short with a low dose of antioxidants, and there was little control over the participants altering their own diet after being informed of the reasoning behind the study.

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INTRODUCTION

Our population is ageing. Through the increase in the adult lifespan the number of older adults is steadily growing. In the New Zealand 2001 Census (Statistics New Zealand, 2002) there were 450, 426 people aged 65 years or older. This made up twelve percent of our population. In 1951 older adults only accounted for nine percent of the population. This percentage is expected to double to 26 by the year 2051, which would mean approximately 1, 181, 000 older adults in New Zealand. Considering the ageing of the population, there is still relatively little known about the causes of ageing. There is also little known about methods to delay the ageing process.

Although there are many theories on ageing, there seems to have been no clear progression following a particular line of thought as there are in some other areas of psychology. There is no dominant theory and it seems likely that several systems work in conjunction with one another and different people may age from different combinations of factors (Balcombe and Sinclair, 2001).

While research is being done to advance these different theories of ageing, little is being done on trying to reverse age-related cognitive decline. This particular piece of research focuses on one particular theory of ageing, the free radical theory. This study addresses the effects of a particular group of antioxidants found in berry fruit on cognitive decline in a population over 65 years of age. The aim of this study is to determine the ability of antioxidants to reverse age-related cognitive decline.

The first chapter discusses several different theories of ageing in response to a set of criteria defining a plausible theory of ageing. The theories outlined in this chapter come from three different groups of theories based on programming events, random events and evolution. An integration of the basic ideas

stemming from these theories may be the direction of future research instead of focussing on them in isolation.

The second chapter is about the free radical theory of ageing. This theory states that damage caused by the oxidation of lipids, proteins and DNA is responsible for the ageing process. This damage occurs at the site of respiration, particularly in the brain where much of the body's oxygen is consumed. Because the brain is being damaged by free radicals, related cognitive processes are affected. Several of the previously discussed theories of ageing can be accounted for within the free radical theory.

Chapter three discusses antioxidants and their defence against free radical damage. Of particular interest are anthocyanins, a type of phenolic compound. Anthocyanins are found in high concentrations in fruit with dark pigmentation. Anthocyanins have a high antioxidant capacity and have been found to be absorbed unaltered in human plasma.

Age-related cognitive decline is the focal point of the fourth chapter. The conceptualisations and patterns of decline with reference to memory, visuospatial ability, language and attention are discussed. The biological decline underlying cognition is also detailed, particularly regarding free radical damage and its contribution to decline.

Chapters five and six are descriptions of past research on antioxidants and cognitive decline, and an outline of the present study. Chapter five discusses previous research on both humans and animals. Although not all of the studies with human participants supported the use of antioxidants to improve cognition, those that did not were often suffering from methodological flaws. Chapter six introduces the hypotheses underlying this study.

The method is outlined in the seventh chapter. The characteristics of the participants, the psychometric tests used, and the content of the berry juices are described in this chapter. The eighth chapter presents the results obtained from this research.

The final chapter is a discussion of the study and the results. The results of the experiment are discussed in relation to the three hypotheses of the study as well as each of the cognitive domains. The limitations of the study and directions for future research are also presented in this chapter.

THEORIES OF AGEING

Many different theories of ageing have been proposed over the years. They can generally be divided into three groups. One group is based on programming events and includes the neuroendocrine and immunological theories of ageing. There is also an evolutionary theory which is itself divided into the smaller theories of mutation accumulation and antagonistic pleiotropy. The final group of theories is based on random events and includes somatic mutation, caloric restriction, cross-linking and free radical theories of ageing.

In order to eliminate some confusion over what is a theory of ageing and what is a process associated with ageing, Bernard Strehler (as cited in Hayflick, 1994) developed four requirements that a theory of ageing must fulfil. First, it should be deleterious and explain why declines in physiological function and ability are observed as we age. Second, it should explain why such losses are progressive. Third, it should explain why losses are intrinsic and cannot be restored. Finally, the theory should explain why ageing is a universal phenomenon within a species.

Evolutionary Theories

In the overall evolutionary theory of ageing, senescence transpires because there is no longer the pressure of natural selection in the older members of a species (Hughes and Reynolds, 2005). Changes in genes that affect reproduction or survival at an early age improve that member's fitness much more than if the same changes were expressed later in life. Two specific evolutionary theories (mutation accumulation and antagonistic pleiotropy) provide explanations as to how changes in gene expression can lead to senescence.

The pressure of natural selection is reduced with age because the population is still at risk of extrinsic causes of mortality such as predation, disease and accidents (Patridge, 2001). Members of a population that died due to one of these causes may have already reproduced and passed on mutations with negative consequences in later life. These mutations can accumulate through many generations, leading to senescence in those members that survive long enough to express the mutations. This cause of senescence is the mutation accumulation theory (Patridge, 2001). Most of the mutations have small effects on an individual. The mutation in Huntington Disease is an extreme example of mutation accumulation (Hughes and Reynolds, 2005).

An alternative evolutionary theory to mutation accumulation is called antagonistic pleiotropy (Mangel, 2001). Pleiotropic genes are those that have more than one function. When a mutation occurs in such a gene, it can increase an organism's fitness by increasing reproductive ability, but may have negative effects later in life (Mangel, 2001). Selective pressure is higher during the reproductive years and so the mutation is selected into the species regardless of its effects on older members. Under this theory, mutations that are beneficial in early reproductive life will still be selected into the population even if they have detrimental effects for later life (Hughes, Alipaz, Drnevich, and Reynolds, 2002). A trade off is made between health and reproduction at an early age and health in old age. Bowles (2000) states that the evidence for this theory so far was conducted with methodological flaws. Bowles also believes that any genes that are beneficial in early life and detrimental in old age would have to be magical genes, and likens them to "Dr Jekyll and Mr. Hyde" (2000, pg.337).

Neuroendocrine Theory

The neuroendocrine theory of ageing proposes that hormones regulate senescence. Fabris (1991) states that this theory is based on three basic

assumptions. The first assumption is that the pituitary and its related glands regulate every bodily function in at least some way. The second assumption is that this complex system goes into decline as it ages. This decline is reflected in the decline of neuroendocrine cells and hormones in the ageing brain and body (Balcombe and Sinclair, 2001). The final assumption according to Fabris (1991) is that this decline comes from overexposure to potentially toxic hormones and neurotransmitters, or from other damage occurring at a cellular level. The degeneration of the neuroendocrine system would cause ageing through the gradual decline of the bodily functions it controls.

Other theorists propose that ageing stems from the degradation of the pineal gland. The pineal gland regulates many internal processes such as core body temperature and energy production (Pierpaoli and Lesnikov, 1994). Through its production of melatonin, the pineal gland acts as an internal time keeper for cells.

Melatonin is produced and is distributed to the cells as soon as it is made and can reach levels that are up to ten times higher at night than during the day (Touitou, 2001). Melatonin levels start to decline during adolescence and by the time old age is reached, the levels of melatonin may no longer be different during the night and day (Karasek, 2004). It is proposed that because the melatonin is no longer fulfilling its timekeeping role, the internal process lose their synchronisation and fall into disrepair (Reiter, 1995). Some of the evidence for the role of the pineal gland in ageing comes from transplantation studies. Pierpaoli and Lesnikov (1994) have conducted studies on mice in which they cross-transplanted pineal glands into old and young mice. The young mice who received an "old" pineal suffered from accelerated ageing while the old mice who received a "young" pineal were delayed in aging and death. The ageing of each of the mice was either accelerated or delayed by approximately six months (one quarter of their lifespan), depending on whether they received an old or young pineal.

Melatonin is also a powerful antioxidant that scavenges for the potent hydroxyl radical, and stimulates some of the brains own antioxidant enzymes (Reiter, 1995; Touitou, 2001). The impact of antioxidants and free radicals on ageing will be discussed throughout this research.

While some theorists state that the evidence points to the pineal gland as a source of senescence, why the pineal gland itself ages has not yet been established. The pineal gland suffers from calcium build-ups over the course of ageing but it is not known if these deposits disrupt the function of the gland (Touitou, 2001). Touitou also states that problems with the β-adrenergic receptors (such as decline in number or loss of responsiveness) within the pineal gland may be responsible for age-related changes in melatonin.

Immunological Theory.

The decline of the immune system is thought to be a possible cause of ageing. The immune system declines in both its ability to defend from the invasion of foreign bodies, and its ability to distinguish foreign bodies from the individuals own tissues (Wickens, 1998). The decreased efficiency of the immune system leaves aged individuals more open to disease, infection and auto-immune problems.

The thymus has been considered as the controlling mechanism of immunological decline. The thymus is important for maintaining healthy immune function but starts to suffer from atrophy after adolescence (Balcombe and Sinclair, 2001). The atrophy acts as a clock for the decline in immune function and ageing occurs. The thymus is located just above the heart and is where T-cells (immune system cells) mature (Steffens, Marchetti, Landay, and Al-Harthi, 1999). During ageing the thymus goes through the process of thymic

involution. As this is happening, the tissue of the thymus is being replaced with fatty tissue (Malaguarnera et al. 2001). Although the thymus does not undergo a visible change in size, the functional tissue left is greatly reduced by age. The reduced functionality of the thymus with age is thought to be a causal factor for the decrease in immunoresponsive hormones (Malaguarnera et al. 2001). The decrease in responsiveness of the immune system leaves the body open to disease and infection.

Recent research by Tian, Zhang, and Dai (2003) supports the notion that the pineal gland may be involved in thymic involution. In their study, old mice were supplemented with melatonin over a period of 60 days. There was evidence of tissue regeneration in the thymuses of the melatonin supplemented mice when compared to control mice. This research provides support for the thymus being the biological clock for immunosenescence but not for senescence as a whole. This suggests that the neuroendocrine theory is a more viable theory of ageing. Immunosenescence appears to be a symptom of neuroendocrine ageing.

Somatic Mutation

The somatic mutation theory of ageing posits that DNA mutations that occur in the cell accumulate and are responsible for ageing (Wickens, 1998). The mutations are acquired after the cell has been formed and may be caused by such events as background radiation or free radical damage (Ono, Uehara, Saito, and Ikehata, 2002). Any mutations acquired while an individual is young are of little consequence as there are many other fully functioning cells to take the burden. In early life, cells with mutations are a small drop in a large ocean. As we age, more cells are inhibited by mutations and there are fewer fully functioning cells to take the burden. Such mutations can be repaired but the repair mechanisms are not perfect and do not correct all mutations (Martus,

Dollé, Gossen, Boerrigter, and Vijg, 1995). Initially it was believed that mutations occurring within the nucleus caused the ageing process, and then mitochondrial mutations became a focus of research (Morley, 1995). As will be discussed later in this research, mitochondrial dysfunction can have a substantial effect on the cell as less energy is produced.

Somatic mutation theory relies on the premise that DNA mutations negatively affect cell functions. According to Vijg (2000), these mutations lead to one of three cellular events, cell death, transformation or senescence. This author suggests that cell death is a protective mechanism in which affected cells are eliminated and this loss leads to the cognitive and physiological changes of ageing. In this theory, the loss of brain cells (and the related cognitive function) that occurs with ageing would be attributed to cell death due to mutations. Cell transformation is the process that leads to such growths as cancer (Vijg, 2000). When mutations occur within the genes controlling the cell cycle, cancer can be the result. Finally Vijg (2000) noted that cell senescence was another cellular event caused by mutation. In this case, the cells have lost their ability to replicate, which may be a protective mechanism against passing on mutations.

Mutations occurring in the brain are much different to those in the rest of the body. It is thought that neurons may be able to sustain higher amounts of DNA damage as they do not replicate, yet they would need good repair mechanisms to maintain cellular functions (Evans, Burbach, and van Leeuwen, 1995). Somatic mutations occurring within the mitochondrial DNA of neurons and glia from the substantia nigra have been found (Cantuti-Castelvetri, In Press). These authors did not find a clear increase in mutations with age but indicated that this was likely because of their small sample size of six participants. Simon et al (2004) also studied mitochondrial DNA mutations in the brain and found that levels of accumulated mutations would at least partially account for age-related mitochondrial dysfunction.

The somatic mutation theory of ageing appears to have support in the research literature. However, the literature is sparse on the mechanisms of age-related change through somatic mutations. It may be that somatic mutation theory cannot account for age-related change on its own but may be vital as part of a more holistic theory.

Caloric Restriction

The caloric restriction theory of ageing is based upon research in which animals on a calorie controlled diet had longer life spans. When compared with controls on unrestricted diets, caloric restricted animals also show reduced free radical and mitochondrial damage, as well as delayed onset of age-related diseases (Timiras, Yaghmaie, Saeed, Thung, and Chinn, 2005).

Research into caloric restriction began in the 1930s and focused on its impact on the incidence of cancers in rats and mice (Weindruch, 2003). Now the focus of research is on the mechanisms by which caloric restriction extends life.

One such mechanism thought to underlie the effects of caloric restriction is reduced oxidative damage. As will be discussed later, oxidative damage is caused by free radicals and has been implicated in the ageing process. It is still unclear exactly how caloric restriction affects oxidative damage but it is thought to be either through reduced free radical production or increased antioxidant capacity (Wolf et al. In press). Barja (2002) reviewed several studies on caloric restriction and oxidative damage, and came to the conclusion that long term caloric restriction decreases free radical production at a particular site in the mitochondria. Fewer free radicals mean fewer mutations to mitochondrial DNA and a slower ageing rate.

With research starting to become available on the mitochondrial link with caloric restriction and mutations, there may be an opportunity to amalgamate the somatic mutation theory and the caloric restriction theory of ageing. Both theories appear complimentary with caloric intake and oxidative damage providing a cause of somatic mutations within the mitochondria.

Cross-linking

In the cross-linking theory, ageing is caused by the linking of proteins with one another. As more cross-links are created, they tangle together and can limit intracellular functions (Bjorksten and Tenhu, 1990).

Collagen is a tissue in which the molecules are replaced at a very slow rate. As the collagen ages it becomes less flexible due to the increasing numbers of links being made between the collagen fibres (Austad, 1997). These changes in collagen can affect the skin through the ageing process. While young skin stretches, older skin has lost much of its elasticity and is more prone to damage. Under the cross-linking theory, similar changes are occurring with the proteins inside the body.

According to Yin (2000) carbonyl toxification is one of the major causes of cross-linking. The author proposes that cross-linking occurs during the day due to this toxification but during sleep, melatonin reduces the level of carbonyls and repair mechanisms clear the damage caused by cross-links. Senescence begins when the damage caused becomes too much to repair and cross-linked proteins accumulate to a critical point.

Glycation is another process that can cause cross-links. Nonenzymatic glycation occurs when sugars such as glucose react with proteins (Suji and Sivakami, 2004). Glycation end products can cause protein cross-links that impede

intracellular transport (Bierhaus, Hofmann, Ziegler, and Nawroth, 1998). Glycation also creates free radicals as a by-product, but oxidative stress can also lead to an increase in glycation (Kikuchi et al. 2003) potentially creating a vicious cycle of free radicals and cross-linking production. The risk of cross-linking due to glycation increases with age. Glucose intolerance increases with age and older adults are exposed to low levels of hyperglycaemia more frequently than younger adults (Suji and Sivakami, 2004). The increased exposure of glucose in the blood stream means that glycation end products accumulate within the body, leading to cross-linked proteins.

Free Radical Theory.

The free radical theory is based on the idea that free radicals from both endogenous and exogenous sources can damage functions at a cellular level. The free radical theory of ageing will be looked at more closely in the next section as it is the theory that forms the basis for this study.

Fulfilling Strehler's Requirements.

The first of Strehler's requirements is that a theory of ageing should explain why deterioration occurs with age. The neuroendocrine theory does not explain the cause of deterioration by itself. Under this theory, ageing is caused by the decline of hormones and neuroendocrine cells within the body. No cause for the decline is attributed except to say that it comes from cellular level damage. The immunological theory cannot fully explain deterioration either. It appears that immunosenescence is regulated by the neuroendocrine system. Evolutionary theories and the somatic mutation theory attribute deterioration with age to mutations within the DNA. The cross-linking theory also attributes ageing to intracellular damage. All of these theories have cellular damage as their cause

of deterioration with age. A more advanced theory of ageing would be able to go one level deeper and establish why cellular damage occurs with ageing. The free radical theory of ageing does just that by attributing cellular damage to oxidation by free radicals and other reactive species.

Strehler's second requirement for an extensive theory of ageing is that it should explain why this deterioration is progressive. Again, the common theme within these theories of ageing is that cellular damage accumulates over time leading to a progressive deterioration in function. This is linked with Strehler's third requirement that a suitable theory must explain why this deterioration cannot be restored. Repair mechanisms are also the targets of cellular damage with age.

The final requirement for a valuable theory of ageing is that it needs to explain why ageing occurs to all members within a species. The evolutionary theories in particular, struggle to meet this requirement. In evolutionary theories, ageing is attributed to genes with negative consequences that accumulate or become active in later life. It is unlikely that every member of the human population has evolved and accumulated enough genetic mutations to cause ageing (Bowles, 2000).

In general, each of the previously discussed theories does not fulfil at least one of these requirements. Some theories such as the caloric restriction theory, fail to fulfil much more than one of these requirements. Instead, it seems that a more extensive theory is needed, one that looks into the mechanisms of cellular damage and repair. The free radical theory may make up for the shortfall of these other theories of ageing.

Summary

Much research has been done on different theories of ageing but there is no one conclusive theory as yet. The neuroendocrine and immunological theories

propose that different structures within the brain act as biological clocks to bring about senescence either through a decline in neuroendocrine or immune function. Evolutionary theories are based on the idea that there is not as much pressure from natural selection in older populations and so mutations with negative consequences in old age are not selected out of a species. Random event theories such as somatic mutation and cross-linking propose that errors accumulate over a life span and it is only a matter of time before such errors start interfering with vital functions and cause senescence. Another random event theory, the free radical theory, will be discussed more thoroughly in the following section. The free radical theory may be the link that helps the other theories to fulfil Strehler's requirements for a solid theory of ageing.

FREE RADICAL THEORY OF AGEING

The Free Radical Theory has become increasingly popular as a framework that accounts for ageing. It was first proposed by Denham Harman (1956) and maintains that damage caused by oxidation accumulates over time, contributing to ageing and its associated diseases.

Free radicals are made within the body, possibly causing damage over time. Older adults are at a greater risk because they have had more time to accrue damage from free radicals. Some of the more damaging free radicals are made from the oxygen used by cells to create energy. Oxygen can be a very reactive substance. It is relatively stable as molecular oxygen (O_2) and water (H_2O), but during the transformation from one to the other, reactive oxygen species can be generated. This transformation usually takes place in the mitochondria of the cell as they combine oxygen and hydrogen and then use this energy to make adenosine triphosphate (ATP) which is the cell's form of energy (Clark, 1999). The resultant free radicals cause damage in three main ways through attacking the cell's proteins, lipids and DNA. According to Digiovanna (2000) the damage to these three cellular components can have the following repercussions.

Damaging the proteins can reduce enzyme activity; make structural proteins more susceptible to destruction by enzymes, cause inflammation of tissues, and can interrupt the mechanisms controlling homeostasis (Digiovanna, 2000). Lipid damage can result in the cell membranes becoming less effective in their ability to allow substances to move through, and can decrease energy production by mitochondria. Damage to lipids can also lead to atherosclerosis by oxidising low density lipoprotein (Okada et al. 2004). The exact mechanism by which lipid peroxidation leads to atherosclerosis is not yet understood. Lipid peroxidation can lead to further oxygen radical production. DNA damage makes the replication process slower, which in turn means it takes longer to

produce new cells. It also slows down other cell processes and may lead to cancer (Klein and Ackerman, 2003).

It is estimated that around 1-2% of the oxygen consumed during metabolism is escaping the system as oxygen radicals (Finkel and Holbrook, 2000). These oxygen radicals are then free to attack the cell from the inside. Most of the damage occurs in the mitochondria themselves because they are the point at which approximately 90% of the cell's oxygen is consumed (Hughes and Reynolds, 2005). Because of the evolution of mitochondria their own DNA is not wrapped up in protective histone proteins, making it much more prone to oxidation. Damaging the mitochondria affects the amount of energy produced by the cell which limits its functions and causes even more leakage within the mitochondria (Clark, 1999). It is thought that older adults have accumulated more damage to their mitochondria than younger adults and so have a more limited supply of energy.

Free radicals could at least partially explain the caloric restriction, the somatic mutation, and the crosslinking theories of ageing. Caloric restriction may increase the lifespan and decrease the effects of ageing by producing fewer free radicals (Clark, 1999). Metabolic capacity (the ability of mitochondria to process oxygen) is decreased on a calorie restricted diet. Free radicals may also be responsible for at least some somatic mutations because they damage DNA and cause chromosomal abnormalities. Because of the exponential nature of free radical production, the damage by mutations may be extensive as we age. Hayflick (1994) notes that free radicals can also produce crosslinks in some proteins, contributing to the inevitable slowing of functions that are postulated under the crosslinking theory. It thus seems that free radicals may be responsible for a fair proportion of the decline that occurs as we age.

Table 1 (Lister, 2003; Matsuo and Kaneko, 2000; Halliwell and Gutteridge, 1999) lists the major free radicals, where they are formed, and the damage that they

do to the body. As can be seen from this table there are both reactive oxygen species and reactive nitrogen species. The most reactive free radical is the hydroxyl radical. It is a by-product of breaking down hydrogen peroxide and causes damage to DNA, lipids and proteins. Fortunately it has a short half-life and so is usually contained to a relatively small area once it has been produced. Reactive nitrogen species are used in different mechanisms by design but they become dangerous when their levels become excessive.

Table 1

Damaging free radicals within the body

Reactive Species	Source	Effects
Oxygen Species		
• Superoxide	Respiration (mitochondria); pollutants	Damage to DNA, proteins and lipids is negligible. Can initiate free radical chain reactions. Interferes with energy metabolism and antioxidant defence mechanisms.
• Hydroxyl radical	Hydrogen peroxide break down	The most reactive of the free radicals. Damages DNA, proteins and lipids. Damage limited to the production site due to a very short life.
Nitrogen Species		
• Nitric oxide	Immune cells, artery cells and nerve tissue; pollutants	Damaging when in excessive amounts. Reacts with superoxide to form peroxynitrate.
• Nitrogen dioxide	Formed in the reaction of nitric oxide and oxygen; pollutants	Damages membrane lipids. Is more reactive than nitric oxide.

Free radicals have unpaired electrons and like to acquire extra electrons. Non radicals do not have unpaired electrons but they still react in similar ways to free radicals, doing the same sorts of damage (Lister, 2003). Taking electrons from other molecules changes the structure of those molecules and they become oxidised. Changing the structure of the oxidised molecules is responsible for the damage done to them. Table 2 (Lister, 2003; Matsuo and Kaneko, 2000; Halliwell and Gutteridge, 1999) describes the non-radicals that occur within the body and also lists their sources and the damage they can do. As can be seen from this table, hydrogen peroxide itself is not very dangerous but it can react to form the hydroxyl radical. Because hydrogen peroxide can cross cell membranes easily, the hydroxyl radical may be able to get into more places than it would on its own. Hypochlorous acid can also damage by itself or react to form the hydroxyl radical.

Table 2

Damaging non-radicals within the body

Reactive Species	Source	Effects
Oxygen Species		
• Hydrogen peroxide	Phagocytes; respiration	Easily crosses cell membranes. Does negligible damage to DNA and proteins itself but is easily converted to the very reactive hydroxyl radicals.
• Singlet Oxygen	Absorption of energy	Can be involved in DNA mutations and causes rapid lipid peroxidation. Can contribute to crosslinks in proteins.
Chlorine Species		
• Hypochlorous Acid	Oxidation of chloride	Potentially damages some proteins and chlorinates some DNA bases. Can react with superoxide to form hydroxyl radicals.

The reactive species described above can be formed through many different processes within the body. According to Shigenaga, Hagen and Ames (1994), the mitochondria are the biggest producers of oxidants. Other endogenous sources of free radicals and reactive species are through the reduction of toxins and fatty acids (Beckman and Ames, 1998; Elliot and Elliot, 2001). The immune system also uses reactive species as a protective factor within white blood cells. Foreign bodies absorbed by these cells are destroyed by exposure to high levels of oxygen radicals (Austad, 1997).

These sources (except for the mitochondria) are ways in which the body has evolved to use reactive species in a beneficial manner. Unfortunately these reactive species are not always contained and can cause damage to the surrounding tissue. This damage does not affect younger adults as they have more undamaged tissue to carry the burden. As older adults accumulate more damage there is less healthy tissue to support physiological functions. This damage is particularly noticeable in the brain and in cognitive functioning in older adults (Timiras, 2003).

The tissues that show the most oxidative damage are the hardest working such as the brain (Clark, 1997). There are many different potential sources of oxidative stress for the brain, the most obvious being the high rate of oxygen consumption within this key organ. When we are resting, the brain, liver and heart are responsible for approximately half of our metabolic rate (Kriketos, Peters and Hill, 2000). The rate of superoxide produced is proportional to the amount of oxygen used. The brain is a very small area of localised high oxygen use, making it vulnerable. As noted earlier, superoxide can react with other molecules to become the very dangerous hydroxyl radical. Furthermore metabolic processes such as the oxidation of dopamine by monoamine oxidases within the brain generate hydrogen peroxide (Halliwell and Gutteridge, 1999).

Another source of oxidative stress in the brain results from neurotransmitters reacting with oxygen to form reactive species. A result being iron and copper ions are released from damaged brain tissue. These ions can contribute to lipid peroxidation, the oxidation of these neurotransmitters, forming a vicious cycle of free radical reactions (Halliwell and Gutteridge, 1999). The presence of excitotoxins can induce oxidative stress and create yet another vicious cycle of damage and free radical reactions.

In general, the antioxidant mechanisms within the brain cannot keep up with the production of free radicals or reactive species from the sources just discussed (Halliwell and Gutteridge, 1999). Each of these sources produces free radicals within the brain over time, damaging the tissue. Again, this damage accumulates and can impact on cognitive functioning in older adults. As will be discussed further in this research, free radical damage can affect cognitive functions such as memory, visuospatial skills, language, and attention in older adults.

The Eva Study

The EVA (Etude du Vieillissement Artériel) study was a large longitudinal study on ageing conducted in France (Berr, Balansard, Arnaud, Roussel, and Alpérovitch, 2000). According to these authors, part of the study involved assessing the relationship between cognitive decline and oxidative stress in 1166 high functioning older participants. Cognitive functioning was tested with the Mini-Mental Status Examination (MMSE). Biological markers of oxidative stress and antioxidant activity were tested by plasma levels of thiobarbituric acid reactive substances (marking for lipid peroxidation), selenium, carotenoids, and the level of Vitamin E in red blood cells. One of the major findings from this study was that there was a significant relationship between lipid peroxidation and cognitive decline at the four year follow up. It was also

found that lower levels of selenium and carotenoids were a risk factor for cognitive decline.

This study shows that free radicals causing oxidative stress have affected the cognitive functioning of the participants. As previously discussed, lipid peroxidation is a type of damage that is caused by free radicals and other reactive species. As the levels of free radical induced lipid damage increase, the cognitive functioning of the participants has declined. This study reveals that free radicals may be responsible for age-related cognitive decline.

Summary

The free radical theory of ageing proposes that free radicals damage DNA, lipids and proteins within the body causing losses of functions associated with ageing. Free radicals from both endogenous and exogenous sources produce damage that accumulates in the body with age, particularly in the areas with high metabolic rates such as the brain. Mitochondrial dysfunction from free radical damage causes a loss of energy to cells. This loss of energy interferes with many cellular functions, possibly leading to the decline seen in older adults. The free radical theory may also account for the caloric restriction, somatic mutation and cross-linking theories of ageing. Each of these theories can be linked with free radical damage.

The free radical theory fulfils Strehler's requirements. It explains why declines are observed with age (Strehler's first requirement), by postulating that free radicals which are produced as part of our metabolic processes cause damage to our DNA, lipids and proteins. It explains how losses are progressive (the second requirement) because the damage accumulates with age. This theory states that free radicals produce damage to surrounding tissue, creating the production of more free radicals in the nearby cells. The losses from free radical

damage eventually cannot be restored. Free radicals assault the body throughout the lifespan, doing more damage each time. After a while the body's defence mechanisms can not restore all the damage (the third requirement). Strehler's last requirement is that the theory be universal to all members of a species. Every person suffers from free radicals to different degrees and a good case could be made for the free radical theory to be a universal phenomenon in humans. This theory of ageing is worth more attention in terms of its history, its effects on older adults and protective factors that have been established.

Fortunately there are some mechanisms that defend against this free radical damage. Antioxidants protect against this damage by reacting with the free radicals before they react with any cellular structures. Antioxidants and their impact on the ageing process will be discussed in the next chapter.

ANTIOXIDANTS

Antioxidants are substances that can provide protection from the damage caused by oxygen radicals. Some antioxidants may also reverse oxidative damage by assisting the bodies repair mechanisms (Ramirez-Tortosa et al. 2001). These substances occur naturally and when they are present in low concentrations can prevent oxidative stress (Basu, 1999).

Antioxidants defend against oxidation in ways that can be divided into primary and secondary defence mechanisms. The primary defence mechanism consists of antioxidants that prevent reactive species from developing whereas the secondary mechanism consists of antioxidants that scavenge reactive species (Lister, 2003). Scavenging antioxidants are those that either react with oxidants before they get a chance to do damage, or those that prevent or terminate oxidant chain reactions (Melhorn, 2003). Unfortunately, this defence system is not perfect and so reactive species are still produced and can do damage before they are neutralised.

The various antioxidants protect against different types of oxidants. They also differ in the biological systems they protect (Halliwell and Gutteridge, 1999). The primary defence antioxidants are all endogenous. The secondary defence antioxidants include the enzymes glutathione peroxidase, which scavenges for hydrogen peroxide, and superoxide dismutase which scavenges the superoxide anion (Ji and Hollander, 2000). It has been shown that people obtain many scavenging antioxidants from their diet including vitamin C, Vitamin E (tocopherols and tocotrienols), carotenoids and flavanoids (Lister, 2003).

Ageing involves the decline of metabolic, neurological and immunological function. All of these declines can be at least partially attributed to free radical damage. Metabolic function declines with accumulated damage to mitochondrial DNA and lipids. This decreases the amount of energy that cells

can produce and limits metabolic function. Hagen, Wehr, and Ames (1998) studied the effects of nutrient supplementation on the energy production of ageing mitochondria. They found that the antioxidant N-tert-butyl-α-phenyl-nitrone (PBN) significantly decreased age-related free radical production in rats.

As will be discussed later, neurological function is inhibited by free radical damage to neurotransmitter systems and structures within the brain. Immune cells are also under threat from free radicals because of their membrane lipids are more vulnerable to oxidative damage (De la Fuente, 2002). The effects of antioxidants on ageing are reflected in their effects on these three systems.

Phenolic compounds are antioxidants that are obtained from food, particularly fruits and vegetables. There are many phenolic compounds including flavanoids, isoflavones, phenolic acids, chalcones, tocopherols and tocotrienols (Morello, Shahidi and Ho, 2002). In vitro, many phenolic compounds act by "inhibiting lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers" (Halliwell and Gutteridge, 1999, p.225). Peroxyl radicals are formed during lipid peroxidation and have been shown to induce DNA damage (Lim et al., 2004). Phenolic compounds can stop the chain of lipid peroxidation and peroxyl radical formation by reacting with the peroxyl radicals. Free radicals can attack membrane lipids producing a lipid peroxidation chain reaction which means that one free radical can cause a massive amount of damage (Croft, 1999).

An intriguing group of the flavanoids called the anthocyanins are responsible for the blue, purple and deep red colours found in fruit. Anthocyanins and other flavanoids have high levels of antioxidant activity (Joseph, Denisova, Bielinski, Fisher and Shukitt-Hale, 2000). While the antioxidant activity of phenolic compounds is higher in combination, Zheng and Wang (2003) still found anthocyanins to be a significant factor for antioxidant activity. They used oxygen radical absorbance capacity (ORAC) assays to study the antioxidant

capacity of anthocyanins and phenolic compounds in blueberries, cranberries, chokeberries and lingonberries. Anthocyanins accounted for more than half the antioxidant capacity of blueberries. Even though it has been found that some berries are low in antioxidant vitamins C and E, they can still have high antioxidant capacities which have been attributed to flavanoids and anthocyanins in particular (Mazza, Kay, Cottrell and Holub, 2002).

Absorption of anthocyanins

For anthocyanins to be effective as antioxidants they need to be absorbed into the body. A few studies have focused on the absorption of anthocyanins by humans in particular. Cao, Muccitelli, Sánchez-Moreno, and Prior (2001) investigated the absorption of elderberry anthocyanins on a small sample of elderly female participants. This study revealed that anthocyanins could be absorbed in their original forms. Anthocyanins were detected in both the plasma and urine of these participants and had mostly left the body after four hours.

A study by Mazza et al. (2002) focused on the human absorption of anthocyanins. Participants were fed freeze dried blueberries (containing 1.20g of anthocyanins) dissolved in water, and then measured serum levels of anthocyanin. There were no detectable anthocyanins present in the serum before the treatment commenced but the levels of anthocyanins did become significant within the serum. The maximum levels were reached four hours after administration. One of the important things to note with this research is that most of the anthocyanins were in their original form and had not been broken down into ineffective components.

Youdim, Shukitt-Hale and Joseph (2004) conducted a study to determine whether flavanoids were able to cross the blood-brain barrier. They found that

some flavanoids including several anthocyanins did pass through their in vitro blood-brain barrier model in measurable quantities. Anthocyanins are reported to remain in endothelial cells and help to protect them against reactive species. Youdim et al. (2004, pg. 1688) also report that flavanoids "were localized in regions such as the cortex, hippocampus, cerebellum, and striatum" within the brain.

Anthocyanins have a high antioxidant capacity, are absorbed in their original form, and can cross the blood-brain barrier. Because of this they may be able to alleviate the cognitive damage caused by free radicals.

Antioxidant capacity of anthocyanins

Anthocyanins are antioxidants because they are able to donate an electron to stabilise free radicals while still remaining a stable molecule themselves (Ramirez-Tortosa et al. 2001). In vivo antioxidant effects of anthocyanins have been well established in animal studies (Prior, 2004). The effects of anthocyanins in humans are less conclusive.

Ramirez-Tortosa et al. (2001) conducted a study to assess the antioxidant capacity of anthocyanins in rats. The rats were kept on a diet that was deficient in vitamin E for 12 weeks. The vitamin E deficiency was designed to increase the rats' susceptibility to oxidative damage. Ten weeks into the study one group of the rats were fed an anthocyanin rich diet. At the end of the experiment, rats that were fed the anthocyanin rich diet had lower scores on indices that reflected lipid peroxidation and DNA damage in their livers. The authors were unsure if this was due to antioxidant scavenging or other mechanisms such as increasing DNA repair mechanisms or antioxidant enzymes.

Lazzé et al. (2003) demonstrated that antioxidant properties of anthocyanins can exert protective effects against the damage of free radicals. In this study, *tert*-butyl-hydroperoxide (t-BHP) was used in vitro to cause cell death and DNA damage by inducing single strand breakages within the DNA and by oxidising DNA bases. The t-BHP also leads to lipid peroxidation. Rat smooth muscle and hepatoma cells were incubated in an anthocyanin solution for two hours before being exposed to t-BHP for one hour. At the end of the incubation it was found that anthocyanins protected against cell death, DNA strand breakages and lipid peroxidation. These protective effects are illustrative of the antioxidant effects of anthocyanins in vitro. Wang et al. (2000) found similar effects in vivo. They found that treating rats with hibiscus anthocyanins for five days before injecting them with t-BHP reduced oxidative damage to the liver.

Summary

Research has shown that antioxidants can protect against free radical damage. The primary defence antioxidants stop reactive species from forming while the secondary defence antioxidants scavenge the reactive species. Anthocyanins are a specific antioxidant found in many deep coloured fruits and account for much of the antioxidant capacity of berries. Anthocyanins have been found to be absorbed in their original forms and reach maximum concentrations in the body within approximately four hours. Anthocyanins have also been shown to cross the blood brain barrier. Studies with animals have revealed that anthocyanins can act as antioxidants *in vivo*.

COGNITIVE DECLINE

As has been previously discussed, free radical damage may lead to a decline in cognitive functioning. There is an extensive literature on the decline of cognition as part of the ageing process. Studies looking at the different aspects of cognition such as the Seattle Longitudinal Study have documented an overall pattern of decline with age. Other studies have focused on particular aspects of cognition and its decline in late adulthood and have also come to similar conclusions. This chapter focuses on aspects of cognition that undergo age-related decline, and how free radicals may be affecting this process.

The aspects of cognition under study in this research are memory, language and attention. This section will begin with a brief outline of the Seattle Longitudinal Study to provide a general overview of decline in adulthood. It will then explore the models of understanding for each of these aspects of cognition. This section will also discuss research into the patterns of decline for each cognitive process. The biological changes underlying the decline of these cognitive processes will be discussed in the next section.

The Seattle Longitudinal Study

The Seattle Longitudinal Study was a sequential study designed by K. Warner Schaie (1994) to study intellectual function over adulthood. It started in 1956 and the original sample contained approximately 5, 000 participants from Washington. The participants were tested every seven years and at each wave of testing, new participants were recruited (Schaie and Hofer, 2001). The top 75% of socioeconomic levels are represented within the Seattle Longitudinal Study (Maitland, Intrieri, Schaie and Willis, 2000).

The Seattle Longitudinal study was designed to assess the path of intellectual abilities in ageing adults (Schaie, 1994). The study demonstrates the ages at which aspects of cognitive decline become significantly noticeable. The only ability that showed significant age-related decline before the age of 60 years was word fluency (Schaie, 1994).

At the testing stage in 1991, the results indicated that inductive reasoning, perceptual speed, verbal memory and spatial orientation all declined in a linear fashion from early adulthood into old adulthood (Schaie, 1994). In this cross-sectional sample, inductive reasoning and spatial orientation showed the steepest degree of decline. Numeric ability and verbal ability peaked in middle adulthood before declining with age, although numeric ability showed the least decline of all the abilities tested.

While a general decline in cognitive function was seen in this study, it is important to note that there were generational and cohort differences within the samples. The disparity between some studies on age-related cognitive decline may be a reflection of the differences between cohorts used. There were also non-random dropout effects experienced in this study. Participants who continued with the study consistently obtained higher scores on the ability tests than those participants who chose not to return for the next wave.

Memory

As can be seen from the Seattle Longitudinal Study, memory is an aspect of cognition that undergoes age-related decline. Because older adults are a vastly heterogenous population, the degree and rate of cognitive decline among the elderly vary considerably. All memory functions seem to show some degree of decline (Backman, Small and Wahlin 2001) although some are more affected than others. Our memory represents our ability to process and recall past

information. As we age our memory function declines which results not only in the obvious problem of forgetfulness, but also other areas of cognition because many cognitive functions are dependent on having a fully functional memory (Timiras, 2003). The two main types of memory are working memory and long-term memory. Working memory is where the information currently being processed is stored. From there, the information may go into long-term memory where it will be kept for future reference.

Working memory is an intermediate state where information obtained from sensory memory is processed. The working memory can usually deal with seven (plus or minus two) pieces of information. Anything beyond that material starts to get lost. Baddeley (1981) proposed a model of working memory based around a central executive. In this model the central executive directs what is to happen with the material currently in working memory. It directs how information is to be encoded for long-term memory and also orders material to be retrieved from long-term memory. Older adults working memory and central executive may not be able to deal with as much material at one time. Having studied older adult's digit span abilities, Craik and Jennings (1992) found that there was a slight effect of age on the number of digits that could be repeated. This difference was magnified for repeating digits backwards. Age differences show up more strongly on tasks that are higher in complexity, but attention and mnemonic strategies may underlie such differences. It may not solely be due to memory decline.

After being in working memory, the material may make its way into long-term memory. Declarative memory is a specific type of long-term memory for the kind of material that can be explained. It is divided into the two sections of episodic and semantic memory. Episodic memory is memory for experiences. Episodic memory "makes possible mental time travel through subjective time" (Tulving, 2002 pg.5). These memories involve looking within and re-experiencing the past. Semantic memory stores information on facts and

general knowledge that are acquired across time and context (Baddeley, 2001). Knowing the capital of ones own country, the meaning of the word purple, or that chocolate melts when it gets hot are all examples of semantic memory. Episodic memory is the long-term memory system that shows the biggest decline and age related differences, especially when the participant is trying to remember the source in which the experience occurred (Smith and Earles, 1996). As can be seen from the results of the Seattle Longitudinal Study, some tests of semantic memory such as word fluency show early age-related decline.

Long-term memories are thought to be created in a specific neurological process called long-term potentiation, which makes a cell more receptive to similar information (Gazzaniga, Ivry, and Mangun (2002). The neurotransmitter glutamate is implicated in creating and maintaining long-term potentiation through its NMDA and nonNMDA receptors in the hippocampus. According to Lu et al. (2004) DNA damage occurring as a result of oxidative stress may be responsible for the reduced expression of the NMDA receptor. Lu et al. (2004) conducted a study on ageing and gene regulation. They found that there are a group of genes that show decline in expression with age and another group that show increased expression with age. Some of the most seriously affected genes are those involved in synaptic function, especially those concerning learning and memory. They discovered that the genes involved in synaptic plasticity for NMDA receptors were among the most significantly affected by age. This affects the systems involved in long-term potentiation and memory storage. This means that as we age, oxidative stress and free radical damage can lead to a decline in memory function because we can no longer produce long-term potentiation in the hippocampus as well as we used to.

Visuospatial ability

Visuospatial abilities involve the processing and manipulation of visual information. This term refers to a vast spectrum of tasks from those requiring a low level of processing such as line orientation tasks, to high level processing tasks such as the mental rotation of objects (Ogden, 1990).

Visuospatial abilities also decline in the course of normal ageing. There are greater age-related differences for visuospatial tasks than there are for verbal tasks (Kaszniak and Newman, 2000). Two different explanations for this age-related difference have been discussed by Jenkins, Myerson, Joerding, and Hale (2000). The first explanation offered in their discussion is that verbal abilities remain intact in later life due to being constantly practised. Visuospatial tasks are proposed to be a much less frequent occurrence, especially the types of tasks used for experimental purposes. The alternative explanation offered by Jenkins et al (2000) is that the age-related difference is due to differences in the ability to process these types of information. Their own research and that of Schaie and Willis (1993) supports this idea by showing consistent differences in visuospatial and verbal information for cognitive domains such as memory. Jenkins et al (2000) found a larger age-related difference for visuospatial working memory than for verbal working memory.

Language

On the whole, language abilities remain intact as we age, with only some smaller areas of skills such as word retrieval showing decline. Why such deteriorations occur is still debated and evidence points to a number of different system failures. One such system failure pertains to the quality of the data coming in. Because older adults start to lose auditory and visual acuity, language functions may be hindered by the low quality data that is being

perceived (Wingfield and Stine-Morrow, 2000). Another system failure that is partially responsible for some aspects of language decline is the lack of resources available to language functions (Wingfield and Stine-Morrow, 2000).

Declining resources available to language abilities include diminishing working memory capacity and problems in inhibiting other information. As was previously discussed, working memory decline in older adults shows up more for tasks that are high in complexity. Because many different levels of processing are required when language (either written or spoken) is used, it would qualify as a complex task. The working memory has to process several different tasks simultaneously but its capacity for information is limited in the elderly. Older adults may not be able to hold as much of the information they need in working memory, or they may not be able to process it as fast. Kemper and Mitzner (2001) suggest that working memory is the source of language difficulties in ageing but they also note that this research is mostly correlational.

Hasher and Zacks (1988) proposed a different source of language decline. Instead of problems with working memory being the main focus, they proposed that inhibition is weakened with age and that older adults are not as good as younger adults at stopping information (be it internal thoughts or external stimuli) from interfering with the task of reading. Again Kemper and Mitzner (2001) outline numerous studies that show support for this inhibition deficit hypothesis. Van der Linden et al. (1999) came to the conclusion that age related differences in language abilities exist alongside a poorer working memory which they attributed to slower processing and increased interference. It would seem that both a reduction in working memory and an inhibition deficit are responsible for some decline in language skills in older adults.

Word retrieval is another of the language skills that shows age-related decline. While it does not seem to be significant, it has many repercussions in the lives of the older adults it affects as it makes conversation less fluent, can contribute

to a negative self-image and can result in reduced social activities (Burke and Shafto, 2004). The inability to remember names and an increase in tip-of-the-tongue experiences are the first things that older adults usually identify in their perception of their own cognitive decline.

While it is established that such word retrieval problems occur with increasing age, there is less research on why this is. Word retrieval problems in people with dementia of the Alzheimer's type can be put down to problems with semantic memory but because semantic memory shows little age-related decline, word retrieval problems in normal ageing cannot necessarily be attributed to deficits in semantic memory.

Two hypotheses that may explain word retrieval problems in ageing are impaired lexical access and semantic degradation. Impaired lexical access occurs when access to the word is impaired and may be interfered with by blocking or interloping words (Evrard, 2002). The semantic degradation hypothesis refers to a down grading of connections in semantic memory between the meaning of the word and the representation of that word (Barresi, Nicholas, Connor, Obler and Albert, 2000). Barresi et al (2000) looked at impaired lexical access and semantic degradation in approximately 40 adults aged 50 years or older. They found that name retrieval problems were attributed to impaired lexical access rather than semantic degradation but that semantic degradation had more influence in those aged 70 years or more. Word retrieval problems in adulthood are "attributed to an impairment in access to the phonological form of the word." (Barresi et al. 2000, pg 170).

Attention

Attention is like memory in that it is a term used to describe a vast number of different cognitive activities. Parasuraman (1998) believes that there is little

value in chasing an overarching theory of attention and that we should be focusing on researching the different aspects of attention in their own right.

Based on the ideas of Posner and Boies, Parasuraman (1998) came up with a three part model of attention. The components were selection, vigilance and control. According to Parasuraman (1998) selective attention is the most widely studied area of attention. It is our ability to parallel process incoming goal-directed information. Vigilance refers to our ability to sustain attention over periods of time. The age-related differences in vigilance are task dependent but in the case of attention tests that require a decision to be made, age-related differences are apparent (Deaton and Parasuraman, 1993). Attentional control is similar to the central executive of working memory (McDowd and Shaw, 2000). Attentional control is responsible for organising and directing where our attention should focus and how much attention we pay to each activity (Rogers and Fisk, 2001). Again age-related differences vary with the nature of the tasks and their complexity.

Declines in aspects of attention affect other cognitive abilities such as memory and language. Attention declines have been blamed for memory problems in older adults because attention is used in three different ways to create memories. First it needs to be decided what should be remembered, and then attention needs to be focused on this information. Finally attentional capacity to parallel process demanding tasks may be required (McDowd and Shaw, 2000). Attention also takes some of the blame for memory decline due to decreasing inhibition in older adults. This decreased inhibition is also thought to be responsible for language difficulties in the elderly, especially in the tip-of-the-tongue experience.

The above decline of attention as an aspect of cognitive functioning has been reflected in the deterioration of some neurotransmitter systems such as acetylcholine and glutamate. Declines in attentional abilities of older adults

may be a product of a decrease in acetylcholine. Acetylcholine has been implicated in attention and increases for tasks where attentional requirements are high (Muir, 1996). Overall cognitive decline has also been linked to decreasing levels of acetylcholine (Blandina, Efoudebe, Cenni, Mannaioni, and Passani, 2004). This may be due to attention being required for many other cognitive abilities. It is thought that age-related decline in certain cognitive functions such as attention, memory and learning may be because of the decline in acetylcholine and its associated mechanisms. There are two main types of cholinergic receptors, the nicotinic and muscarinic receptors. Muscarinic receptors have been shown to decrease with age in both animals and humans (Kelly and Roth, 1997). Also, the receptors can be vulnerable to oxidative stress (Joseph, Fisher, Carey and Szprengiel, 2004).

One of the main groups of cholinergic projection neurons leads into the hippocampus (Thiel, 2003). As will be discussed in the next section, the hippocampus is crucial to creating new memories. Acetylcholine affects long-term potentiation in the hippocampus by increasing its likelihood (Ye, Qi, and Qiao, 2001). One of the affects of acetylcholine on long-term potentiation may be that it reduces the threshold for potentiation to occur. Yamazaki, Hamaue, and Sumikawa (2002) found that lesions to the cholinergic pathway in the hippocampus of rats resulted in a higher threshold for long term potentiation to be reached. They found that administering nicotine (instead of acetylcholine) to bind with the nicotinic receptors reversed the impairment of long term potentiation induction by increasing the release of glutamate. Segal and Auerbach (1997) describe a type of long term potentiation that is limited to muscarinic receptors. In their research they have found muscarinic long term potentiation to be severely lacking in the brains of aged rats. They propose that this may be the cause of the inability of aged rats to learn cognitive tasks.

Summary

Many aspects of cognition decline with the ageing process. Some studies have focused on cognition and ageing in general. Others have broken cognition down into different domains and researched one particular aspect of cognition. This study focuses on the domains of memory, language and attention.

There are several different ways to conceptualise memory. Here memory has been divided into working memory and long-term memory. Working memory is subject to age-related decline, particularly with complex tasks. Long-term memory, in particular, declarative memory is thought to show age-related decline. Episodic declarative memory is reported to have the largest degree of decline in ageing, while semantic declarative memory undergoes smaller changes. Many systems are thought to be responsible for the subsequent age-related memory decline. The glutaminergic system affects memory via the decreased expression of genes controlling NMDA receptors. This decreased expression may be due to free radical damage to DNA.

Visuospatial abilities also decline with age. Visuospatial tasks show a higher degree of age-related decline than do verbal tasks.

Language remains mostly intact throughout the ageing process with some problems occurring with word retrieval. Research provides two main reasons for the minor changes in language due to ageing. Firstly it is believed that a diminished working memory capacity means there are fewer resources available to older adults when producing language. Secondly, it has been proposed that older adults have problems with inhibition and information interrupts their thoughts and their speech.

Attention can be divided into selection, vigilance and control. There are age-related declines in complex tasks of attention. Such declines in attention are

thought to be partly responsible for memory and language problems in older adults. The cholinergic system is vital for learning, memory and attention. Age-related declines in levels of acetylcholine have been found. Acetylcholine receptors needed to create memories are also vulnerable to oxidative damage.

STUDIES LINKING ANTIOXIDANTS TO COGNITIVE FUNCTION

Research linking antioxidants and cognition has been growing over the past ten years. While research with humans started with vitamin antioxidants, the initial research on flavanoids began with animals. Although this research has showed that diets high in flavanoids can reverse some age-related decline, there has been little research on flavanoids among human participants.

Human Studies

Perrig, Perrig and Stähelin (1997) investigated the relationship between antioxidants and memory in 442 people aged from 65-94 years in Switzerland. They hypothesised that memory decline in ageing is due to oxidative stress. To test this hypothesis they studied the plasma levels of Vitamins E, C and β-carotene and tested for implicit memory, working memory, explicit memory and semantic memory. The plasma levels of the antioxidants had been measured in 1971 and again in 1993, with the memory testing being carried out at the same time. They found that the semantic memory test (WAIS-R vocabulary) had the highest correlations with the plasma antioxidant levels, while free recall scores were correlated with β-carotene and ascorbic acid. There was also a significant main effect in ANOVAs with semantic memory and an explicit memory task (recognition) when the participants were divided into low and high vitamin groups. Perrig et al. (1997) believe that these findings demonstrate a link between memory function and antioxidants providing evidence for oxidative stress as a cause of memory decline. While this study was designed to provide support for oxidative stress and the free radical theory it provides no information about the value of supplementing diets to improve memory function. There was one hint about the main effect for some types of memory when the group was divided by vitamin level. The previously discussed research on gene regulation as we age and synaptic receptors in the

hippocampus would have us believe that a higher antioxidant intake would improve memory in older adults but there is little evidence to support this in humans.

Another study looking at dietary antioxidants in the United States of America found no relationship between antioxidant intake and cognitive performance (Peacock, Folsom, Knopman, Mosley, Goff, and Szklo, 2000). These authors researched antioxidant intake as part of a larger study on atherosclerotic disease in a sample of 12 187 people aged between 48 and 67 years. The participant's antioxidant intake was calculated by establishing their diet through a food frequency questionnaire. The participants were divided into four levels of antioxidant intake. Cognitive function was not related to any of these levels. The authors also found that antioxidant supplement intake was not related to cognitive function in their study. However, the authors note that there are reliability problems inherent within dietary questionnaires and that this may interfere with any relationships presented. No information on the frequency or strength of the supplements was used in assessing the relationship between antioxidant supplements and cognitive function.

Manders et al. (2004) conducted a review of 21 controlled and randomised studies of nutritional supplementation and cognitive function. Four of these studies specifically addressed multivitamin supplementation. Significant positive effects were found in those studies where cognitive function was broken down into domains. The domains looked at were short and long term memory, executive function, verbal ability, abstract thinking, attention and problem solving ability. The studies showing no significant effect used a broad measure of cognitive function which may not have been specific or sensitive enough to detect any changes. The studies showing significant positive effects used healthy community dwelling participants. The studies that showed no effects used participants that were either identified as frail community dwelling elderly or hospital in-patients.

The authors described four factors that influenced the outcomes of these nutrition studies. This involved the level of cognitive decline, level of nutritional deficiency, the dose of the vitamin or nutrient, and the duration of the experiment.

There appear to be more studies showing support for nutritional interventions affecting cognitive function than there are studies that fail to support this (Manders et al, 2004). The studies that show no significant effects appear to be suffering from design flaws that could influence the results. Unfortunately there is a dearth of research on antioxidant vitamins, particularly phenolic compounds such as anthocyanins with human participants. This gap in the literature exists even though Lampe (1999) believes them to be more powerful as antioxidants than are vitamins C and E.

One study conducted by McAnulty et al. (2004) addressed blueberry polyphenols and their ability to reduce oxidative stress from exercise. These authors found that participants supplemented with 2/3 of a cup of blueberries for seven days had significantly lower levels of lipid hydroperoxides which result from the oxidation of omega fatty acids. While this research did not focus on cognitive function, it is important to note that phenolic compounds can play an antioxidant role within the human body.

Animal Studies

Socci, Crandall and Arendash (1995) developed an experiment involving rats that provides support for the free radical theory. They gave three antioxidants (Phynyl-α-tert-butylinitrone, vitamin C and vitamin E) to aged rats for 5 months. After two months the rats were tested in the Morris water maze where they have to swim around a pool and remember where the platform is from

visual cues around the edges of the maze. After the rats had been taught where the platform was, it was removed and they were timed to see how long they spent in the correct quadrant looking for it. This time was taken as the measure of retention. After four months of supplementation, these same rats were placed in a passive avoidance chamber. The chamber was divided into a light chamber and a dark chamber, with the rats being placed initially in the light chamber. When they entered the dark chamber the rats were given a small electric shock. This time retention was measured by the amount of time it took them to go back into the dark chamber the next time they were in the same situation. Socci et al (1995) found that the antioxidant treatment improved the rats' memory for the water maze.

Much of the original research on supplementing polyphenols in animals has been conducted at the Human Nutrition Research Centre on Aging at Tufts University in Boston by James Joseph and his colleagues. The aim of one of the first studies was to see if feeding a diet high in antioxidants to rats would decrease the oxidative stress induced decline in receptor sensitivity and cognitive performance (Joseph et al. 1998). The 80 rats used for the study were fed either a control diet or a diet enriched with strawberry, spinach, or vitamin E for eight months until the rats were 15 months of age. They found that the rats fed on either spinach or vitamin E diets showed an improvement in memory using the Morris water maze. These two supplement groups were also found to increase the endogenous antioxidant activity in the Striatum. Endogenous antioxidant activity was also increased in the cerebellum, with the Vitamin E fed rats showing the greatest increase and the spinach fed rats showing the smallest increase among the supplemented groups. The authors also found that each of the supplemented groups slowed the age-related decline seen in muscarinic receptor sensitivity. This research prompted Joseph and colleagues to look more closely into which flavanoids have a high antioxidant capacity.

A later study by Joseph et al. (1999) focused on whether age-related decline could be reversed by antioxidant intake in aged animals. In the first study above, the rats were 6 months old and fed a supplemented diet for over half their lives. In this study, the rats were 19 months old and were only fed a supplemented diet for 8 weeks. Using aged animals allowed the authors to see if a diet high in antioxidants could reverse age-related decline or prevent it. In this case, the diets were supplemented with spinach, strawberry or blueberry. The rats fed the blueberry diet showed the greatest reversal of age-related neuronal function as measured by increased muscarinic receptor sensitivity and improvements in calcium homeostasis. The only group to reverse age-related motor problems were the blueberry fed rats. Psychomotor skills were assessed by tasks such as rats walking on a rotating rod or walking across planks of various sizes to check balance and coordination. The blueberry fed rats also took less time to complete the Morris water maze, a task of memory. This study showed that a diet high in flavanoids, such as that of the blueberry fed rats, can reverse some age-related declines seen in neuronal and motor functioning.

Goyarzu et al. (2004) found that aged rats (19 months old) fed on a blueberry supplemented diet for four months differed significantly to the control rats on an object recognition test. As well as a control group of aged rats, there was also a group of young rats aged 8 months. The aged blueberry fed rats performed just as well on the one hour delayed memory recognition task as the young control rats. This finding is complementary to the finding by Joseph et al. (1999) that blueberry fed rats showed greater spatial memory on the Morris water maze.

Joseph et al. (2003) studied the effects of polyphenolic compounds from blueberries in mice. The mice used for this study were genetically predisposed to develop build ups of amyloid β protein similar to those seen in Alzheimer's disease. The mice in the blueberry condition were fed a blueberry supplemented diet from the age of four months. Testing in a maze was done at

twelve months of age. They found that the blueberry supplementation for the transgenic mice increased the level of performance on the maze to a level comparable with the non-transgenic mice. Upon dissection of the mice brains it was found that improvements in maze performance were parallel with increased neuronal signalling, particularly in the hippocampus, cortex and striatum. Joseph et al. (2003) attribute the improved neuronal communication to the antioxidant effects of polyphenols.

Martin, Prior, Shukitt-Hale, Cao and Joseph (2000) also researched the effects of fruits and vegetables and vitamin E on different tissues within the rat. They were particularly interested in vitamin distribution in brain tissue, and fed rats diets supplemented with spinach, strawberry or vitamin E. A significantly higher concentration of vitamin E was found in the hippocampus and cortex of the spinach fed rats and that all of the special diets were in some way able to reduce age-related decline. This experiment highlighted the importance of studying antioxidant compounds found in fruit and vegetables, and also came with a warning about the potentially toxic effects of excessive doses of vitamin E.

In a different study by Bickford et al. (2000) rats were fed diets supplemented with spinach, strawberry or blueberry. Glutathione levels of the cerebellum were significantly higher in the blueberry and strawberry fed rats in comparison with the control group. This suggests that the berry diets may have helped to increase the levels of the endogenous antioxidant glutathione. It was also found that each of the diet supplemented groups performed better on the motor learning task than did the control group, with the spinach group being the most effective. The authors did not attempt to identify which nutrients were responsible for the effects.

Summary

The previously mentioned studies do support the use of antioxidants to increase cognitive performance in both animal and human populations. The human studies have investigated the effects of β-carotene, ascorbic acid and multivitamin supplements on cognitive performance in older adults. There is little research on phenolic compounds such as anthocyanins in humans. However, one study found that phenolic compounds are active within the human body in an experiment designed to assess their effects on exercise induced oxidative stress. Phenolic compounds and anthocyanins have been studied more fully in animal populations. These studies have found that diets supplemented with blueberry and spinach can prevent and even reverse declines in neuronal functioning which is thought to affect cognition and motor functioning. One of the findings from these studies was that a berry fortified diet appeared to increase levels of some endogenous antioxidants as well as to improve cognitive performance in rats.

So far, research has shown that there is a relationship between antioxidant vitamin levels and cognitive functioning in humans. Research has also shown that anthocyanins are potent antioxidants. Diets that are high in these substances have been found to have an effect memory performance (in the form of maze negotiation) in aged rats. Up until now, little research has been done on the effects of anthocyanins in a human population, particularly in regard to their effects on cognition.

THE PRESENT STUDY

The studies reviewed suggest that antioxidants can have a limited effect on cognitive processes in both animals and humans. The free radical theory of ageing posits that reactive oxygen species cause dysfunction within cells and that this damage is responsible for the ageing process. If this damage was to be ameliorated it would follow that cognition would improve.

The present study used the antioxidants found in dark coloured berries as the method by which to ameliorate the damage done by reactive oxygen species. Of particular interest are the cognitive processes of memory, visuospatial ability, language and attention, all of which have been reported to show some decline with normal ageing. The participants were all older adults (65 years of age and older) suffering from mild memory impairment. Participants were chosen with mild memory impairment because it was thought that this would allow for more improvement to be seen in cognitive testing scores. Because the participants were expected to obtain lower scores, an improvement in cognition would be easier to see before being affected by ceiling effects.

The data for the present study were collected as part of a larger study run in conjunction with Massey University's Institute of Food, Nutrition and Human Health (IFNHH). As part of the larger study, data was obtained on immune functioning, dietary history, and levels of oxidative damage in the blood for the participants in this study. The information on dietary history and oxidative damage although potentially beneficial to the present study was restricted and so does not contribute to the findings presented here.

The participants were given berry juice to drink every day. There were three different berry juices used, blackcurrant, boysenberry and a placebo. The blackcurrant juice had an anthocyanin level that was approximately twice that of the boysenberry juice. There were no anthocyanins detectable in the placebo

juice. At three times over the course of the experiment (12 weeks) the participants were assessed using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Randolph, 1998), which includes tests for memory, attention, language skills and visuospatial skills. The first session of testing provided a baseline with which to compare the other two sessions.

The aim of this study was to explore the effects of boysenberry and blackcurrant juices as a means to enhance cognitive functioning in older adults. Because these particular berries are both high in anthocyanins and other antioxidants, it was expected that their antioxidant capacity would help to alleviate the effects of reactive oxygen species within the brain and so improve cognitive functioning.

The first hypothesis of this study was that the berry juice groups would show an improvement in cognitive function that was significantly larger than any change seen in the placebo group. It was expected that any improvement shown would be significantly different from any improvement found in the control participants. As previously discussed, antioxidants have the potential to increase brain efficiency by enhancing neuronal receptor sensitivity.

The second hypothesis was that the participants drinking the blackcurrant juice will obtain significantly higher scores on the RBANS indices than the participants drinking the boysenberry juice. The blackcurrant juice had a much higher concentration of anthocyanins and antioxidant capacity than the boysenberry juice. The higher antioxidant capacity could thus exert greater protective effects for cognitive function in the blackcurrant drinking participants.

METHOD

Participants

Advertisements were run in the local newspapers in Palmerston North to recruit participants into the study. The advertisements included a brief description of the study and specified that anyone 65 years of age or older who felt they were more forgetful now than in their youth would be welcome to express their interest. Those people that registered their interest were sent an information pack and were assessed on medical grounds for inclusion using the inclusion questionnaire (see appendices A and D). After interested people had read their information packs and passed the inclusion criteria, they were accepted into the study.

Potential participants were excluded if they had suffered from any of the following; serious coronary trouble, stroke, diabetes, thyroid disease, Parkinson's disease or tremors, kidney problems, blood disorders, problems with a full visual field, liver problems, impaired immunity, or fruit allergies. Participants were also required to bring with them to the screening session a full list of the medications they were currently prescribed. The medications were examined by the Institute of Food, Nutrition and Human Health's Medical Officer to check that the participants would not be taking any medications that could interfere with their cognitive performance or immune system functioning.

The participants were also screened for mild memory impairment using the Rey Auditory Verbal Learning Test (RAVLT). Participants who obtained a score at or below the mean score for their age were included in the study. Those potential participants that scored above the mean were excluded from further involvement in the study. Only those participants exhibiting mild memory impairment were included in the study. The participants all scored close to the

mean on the RAVLT and so none were thought to be suffering from severe memory impairment.

There were 52 participants in total, including 23 females. The number of participants was low because of the very restrictive entry criteria. Over 150 potential participants who met the medical criteria for entry with the RAVLT were screened. Two thirds of those people obtained scores on the RAVLT that were above the norm and so only 52 participants were eligible and able to complete the study. The participants were aged between 65 and 91 years. The average age was 72.06 years with a standard deviation of 5.90 years. One male participant withdrew after the first testing session due to illness and so was not included in the significance tests. The participants were divided into three groups consisting of 17 members each.

Materials

The Rey Auditory Verbal Learning Test

The RAVLT is a simple pencil and paper test designed to assess verbal memory (Spreen and Strauss, 1998), taking approximately 15 minutes to administer. A list of 15 words is read out at the rate of one word per second and after listening to the full list, the examinee repeats as many words as they can remember. The same list is read aloud a further four times and each time the examinee repeats as many words as they can remember (trials A1 to A5). Although the words are always read out in the same order, the examinee does not have to repeat them in order. Once the list has been read out five times, an interference list of 15 words is read aloud and again as many as are remembered are repeated (trial B1). After the interference list has been repeated, the examinees are asked to say any of the words that they can remember from the first list (trial A6). The final trial (A7) involves repeating the initial list after a 20 minute delay.

The RAVLT only has a moderate test-retest reliability of .55 (Snow, Tierney, Zorzitto, Fisher, and Reid, 1988) over a one year testing interval. The construct validity of the RAVLT was assessed by MaCartney-Filgate and Vriezen (1988). These authors found that there were some significant correlations between the Buschke Selective Reminding Test and the RAVLT ranging from r= .58 to r= .69 for different trials of the RAVLT. In a factor analytic study by Vakil and Blachstein (1993), the RAVLT was found to have three factors which they labelled, acquisition, storage and retrieval. These three factors are thought to represent the three stages of memory processing (Vakil and Blachstein, 1993).

The RAVLT was used as the screening test to identify potential participants. The number of words that the examinees remembered in trial A6 were compared to standardised norms and those who scored at or below the mean were asked to be part of the study.

The Repeatable Battery for the Assessment of Neuropsychological Status.

The RBANS was originally designed because there was proposed to be a lack of appropriate tests to assess for possible dementia. This Battery was designed to be sensitive to mild dementia and to detect small changes in cognitive decline, while also being broad enough to use with a vast range of cognitive levels (Randolph, Tierney, Mohr, and Chase, 1998). It takes approximately 30 minutes to administer and includes twelve subtests. Each of the subtests contribute to the five domains of immediate memory, delayed memory, attention, language, and visuospatial/constructional. There are two equivalent forms of the test (Form A and Form B) for retesting purposes.

Each of the five domains relates to an Index within the test. The Immediate Memory domain tests the participant's abilities to recall information at the end

of its presentation. It is used as a measure of short-term or working memory. Subtests used to calculate the Immediate Memory Index are List Learning and Story Memory. List Learning requires the participants to recall a list of the same ten words over four trials. The Story Memory subtest measures the participant's ability to remember a two sentence long story, verbatim, over two trials.

The Visuospatial Constructional domain tests the participant's ability to work with spatial information. In the RBANS, this is assessed by the Figure Copy and Line Orientation subtests. The Figure Copy test involves the copying of an abstract figure from the Stimulus Book that is shown to the participant, and is on display while they draw the figure. The Line Orientation test involves getting the participant to match the placement of two lines on a page with those lines on the key above them. There are 10 different trials of the Line Orientation test.

The Language Index uses Picture Naming and Semantic Fluency to test the participant's basic language skills. Picture Naming involves giving the correct name for ten simple drawings presented from the Stimulus Book. This subtest can be sensitive to tip-of-the-tongue experiences. Semantic Fluency requires the participants to think of as many examples of a given category as they can within one minute.

The attention domain measures sustained attention or vigilance across short periods of time. The Attention Index uses the two subtests of Digit Span and Coding to test for vigilance. The Digit Span test requires the participants to try to remember a string of numbers that increases in length each time. The strings start off two digits long and increase by one digit each time, stopping at a string nine digits long. Once the participant fails two strings in a row the subtest is stopped. Coding requires the participant to convert symbols to numbers by the use of a key detailing the symbols and their corresponding numbers. The participants have 90 seconds to code as many symbols as they can.

Delayed memory is the fifth domain that the RBANS tests. The Delayed Memory Index consists of tests that ask the participant to remember information from the beginning of the testing session. It is used for testing working memory and long-term memory. List Recall involves trying to remember as many of the ten words from the List Learning trials, and List Recognition involves deciding whether or not each word from a list of 20 was from that original list. Story Recall requires the participants to remember as many details from the short story used in Story Memory as they can. Figure Recall requires the participants to draw the abstract figure they copied at the beginning of the test without the figure in front of them.

All of these Index Scale Scores are totalled and converted to the Total Scale Index Score. This Total Scale Index Score is an approximation of the participant's level of cognitive performance.

The norms for the RBANS have been standardised on a sample of 540 adults reflecting the United States of America census data. As well as norm information on age and gender, there is also information on level of education. Randolph (1998) reports the test-retest reliability of Form A to Form A is r = .88 and the test-retest reliability of Form A to Form B is r= .82.

Randolph (1998) also presents comparison studies between the RBANS and other measures of cognitive function to provide evidence for construct validity. The Immediate Memory Index is reported to have a modest correlation of r= .61 with the Verbal Memory Index from the Wechsler Memory Scale (revised edition). The Delayed Memory Index from the RBANS has a correlation of r=.49 with the Delayed Recall Index of the WMS-R. The Visuospatial/Constructional Index of the RBANS is also reported to correlate well with other measures visuospatial ability. For the Judgement of Line Orientation test r= .62, and for the Rey Complex Figure Copy test r= .79. The Attention Index of the RBANS has a high correlation of r= .82 with the Attention/Concentration Index of the

WMS-R. The Boston Naming Test was also highly correlated with the Language Index of the RBANS with r=. 75.

Berry Juice

There were three different types of berry juice, black currant juice, boysenberry juice and a control cordial made specially to taste like the other juices. The placebo contained sugar, citric acid, and synthetic colours and flavours. The black currant juice was adjusted so that the anthocyanin concentration was 500mg per serving. New Zealand boysenberry concentrates have 6954mg of anthocyanins per litre (Hay, 2000), so the boysenberry juice would contain approximately 250-300mg of anthocyanins per serving. The two treatment drinks were also expected to have other antioxidants such as vitamin C and other phenolic compounds.

Each of the drinks were packaged in 100mL lots and participants were instructed to take two together and have a 200mL serving. They were asked to drink their juice at the same time every day.

Procedure

Potential participants were recruited and underwent a screening process to determine their suitability for the study. After their medical history was established over the phone using the inclusion questionnaire, an appointment was made to come to the Institute of Food, Nutrition and Human Health at Massey University. The RAVLT was administered by one of two interviewers and if the score was at the mean or below, the potential participant was able to be part of the study. It was made clear at this point that coming to their

screening visit did not bind them to being in the study and they were free to change their minds. The screening took place over four weeks.

As each participant was selected into the study, they were randomly assigned to one of three groups that would be drinking different types of juice. Only one member of married couples was randomly assigned and the other was put into the same group. This was done to avoid confusion and to stop couples from accidentally consuming the wrong drink, and to stop couples from trying each others drinks to see if they could tell which group (i.e. the control) they were in. The experiment was run as a double blind study and only the people making and packaging the juices at HortResearch knew which groups applied to which drinks.

After the participants had been selected into the study, they came back for a baseline testing session. They were administered Form A of the RBANS and were then given a supply of their juice to take home. Fresh juice was delivered to the participants homes either every week or fortnight.

Testing intervals were six weeks long and testing was staggered over three week periods. Six weeks after participants came for their baseline testing, they came back for a test in the middle of the experimental period. This time they were administered Form B of the RBANS. Twelve weeks from the start of the experiment the final testing session took place. The participants were assessed again with Form A of the RBANS.

All appointments took place in the mornings in either the human laboratory or at the meeting room in the Institute of Food, Nutrition and Human Health. The same two interviewers were used for all the screening and testing.

RESULTS

This chapter analyses the data from the present study in six sections. The first section deals with the descriptive data from each Index Score of the RBANS (immediate memory, visuospatial constructional, language, attention, delayed memory, and the Total Scale score) for the three juice conditions. The second section presents the ANOVA results for each of the indices.

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 11 software. A repeated measures ANOVA was conducted for each of the indices. The berry juice type of blackcurrant, boysenberry and placebo were the between groups factors. The time of testing (baseline, six weeks, and twelve weeks) were the within group factors.

The assumptions of a repeated measures ANOVA are that the participants are randomly assigned, the data are normally distributed, and that there is homogeneity of variance and covariance (Cohen, 2001). The assumptions for the ANOVA were met in this study. Participants were randomly assigned to the experimental conditions, although married couples were treated as one unit for the purposes of random assignment. According to Cohen (2001) it is not as important for data to be normally distributed and have a homogenous variance in a repeated measures ANOVA as it is with an unrelated ANOVA. The homogeneity of covariance is an important assumption to be met for a repeated measures ANOVA as a lack of homogeneity can increase the chances of committing a type I error by inflating the *F* ratio. The following analysis was done using the Greenhouse-Geisser *F* test to correct any significant lack of homogeneity of covariance. This more conservative statistic adjusts for the increased likelihood of committing a type I error (Cohen, 2001).

Descriptive Data

Table 3 presents the means and standard deviations for each of RBANS scales, divided by juice group. The differences in test scores over time in the placebo group showed a consistent trend towards improvement, along with the boysenberry and blackcurrants juices.

The Immediate Memory Index Score of the RBANS tests short-term memory. As can be seen from Table 3, there was a trend towards improvement in each of the groups. The biggest difference between testing sessions was seen in the placebo group. Next to the placebo group, the blackcurrant juice group showed the largest mean difference over time. The boysenberry juice group had a mean difference in immediate memory that was lower than both the placebo and blackcurrant groups.

Table 3.

Mean and standard deviations for each Scale Index Score of the RBANS by juice type.

		Boysenberry		Blackcurrant		Placebo	
		M	SD	M	SD	M	SD
Immediate Memory	Baseline	83.41	17.53	88.18	15.65	85.82	16.70
	Six weeks	92.94	21.27	95.41	13.87	95.00	12.10
	Twelve weeks	89.88	18.10	95.00	19.07	93.41	11.86
Visuospatial Constructional	Baseline	85.88	15.24	93.35	15.48	88.06	15.11
	Six weeks	87.82	13.39	96.53	13.23	92.35	21.14
	Twelve weeks	93.53	17.74	95.47	11.16	90.82	15.15

		Boysenberry		Blackcurrant		Placebo	
		M	SD	M	SD	M	SD
Language	Baseline	98.35	16.63	101.65	10.95	98.24	8.67
	Six weeks	101.71	9.68	95.29	6.63	98.18	7.88
	Twelve weeks	104.18	15.18	102.35	10.65	101.29	7.18
Attention	Baseline	90.53	15.72	102.00	13.00	97.65	15.13
	Six weeks	98.35	14.71	103.76	12.62	100.18	12.75
	Twelve weeks	101.35	17.83	102.65	14.91	104.24	14.09
Delayed memory	Baseline	82.35	16.83	83.35	16.68	85.35	14.53
	Six weeks	86.76	18.87	89.35	17.45	91.24	12.86
	Twelve weeks	89.29	17.13	93.24	16.22	95.88	7.19
Total scale index	Baseline	84.35	13.99	91.24	12.28	87.71	11.35
	Six weeks	91.12	15.13	94.12	11.61	93.24	10.75
	Twelve weeks	94.35	17.04	96.71	13.14	95.41	8.83

The Visuospatial Constructional Index measures the participant's spatial abilities. The boysenberry juice group was the only group to show a consistent increase in the Visuospatial Constructional Index Score, with the other two groups showing little change. The placebo group showed a marginally larger mean difference than the blackcurrant juice group.

The Language Index measures participant's basic language skills, particularly in naming items. The Language Index Scores for the boysenberry group increased somewhat, as did the placebo group to a more limited extent but the scores of the blackcurrant group had improved little by the final testing session and the mean score was lower at the six week stage than it was at the beginning.

The Attention Index Score measures sustained attention over short periods of time. Both the placebo group and the boysenberry group showed a tendency towards improvement. The boysenberry group showed the biggest change in sustained attention with a difference of 11.29 points. The blackcurrant group showed little improvement.

The Delayed Memory Index Scale corresponds more with long-term memory than with short-term. It measures the participant's abilities to remember information presented at the beginning of the test after being distracted by other subtests for approximately 20 minutes. All of the groups showed a general improvement in their Delayed Memory Index Scores, with the placebo group showing the greatest change and the boysenberry group showing the least.

The Total Scale Index Score is the final total for the RBANS. The Index scores are added together and a conversion table is used to calculate the total score. As can be seen from Table 3, all of the groups showed a consistent improvement over time. The boysenberry group showed the most improvement on this index, followed by the placebo group. The blackcurrant group showed the least improvement in mean scores over time.

Analysis of Variance Results

A repeated measures analysis of variance was used to determine the significance of results for each index in this study. Differences between groups and differences across time were tested for significance. ANOVA was also used to assess the significance of any interactions between group and time for the indices. Any interactions seen would indicate that at least one of the groups had shown an improvement that was significantly different from the other two. Had there been any significant interactions, post hoc testing would have been conducted to determine which group was different. For the berry juices to have had an effect in this experiment, the blackcurrant and boysenberry juices would have shown an improvement across time that was not reflected in the placebo group.

Table 4 shows that for the Immediate Memory Index there was a significant effect for the within subjects factor over time F (2,47) = 14.05, $p < .001$. The between subjects factor of juice group was not significant. This means that none of the juice groups were significantly different from each other on the Immediate Memory Index over the course of the experiment. There was also no within subjects interaction between group and time. An interaction between these two factors would have indicated that at least one of the groups improved significantly more than the others. These results do not support this finding and so there is no noticeable effect of berry juice on immediate memory in this case.

Table 4

Analysis of variance for the Immediate Memory Index.

Source	*df*	*F*	η	*p*
	Between Subjects			
Juice Group	2	.33	.01	.718
Juice Group within group error	48	(665.57)		
	Within Subjects			
Time	2	14.05*	.23	<.001
Group X Time	4	.13	.01	.97
Group X Time within group error	95	(77.28)		

Note. Values within parentheses are mean square errors.

* Values significant for at least $p < .05$

There were no significant effects or interactions found for scores on the Visuospatial Constructional Index. The between subjects factor indicates that none of the groups were significantly different from the others at any stage of the experiment (Table 5). Also, there was no significant improvement over time seen in the Visuospatial Constructional Index. Because there is no significant interaction between group and time in table 5, it can be inferred that visuospatial abilities did not improve more across the experiment in any particular sample group.

Table 5

Analysis of variance for the Visuospatial Constructional Index.

Source	*df*	*F*	η	*p*
	Between Subjects			
Juice Group	2	1.18	.05	.32
Juice Group within group error	48	(436.93)		
	Within Subjects			
Time	2	1.68	.03	.19
Group X Time	4	.53	.02	.70
Group X Time within group error	91	(151.34)		

Note. Values within parentheses are mean square errors.

As with the previous two Indices, there were no significant differences in Language Index scores found between the groups at any stage during the experiment (table 6). There was a significant main effect for the within subjects factor of time in the Language Index scores, $F(2,47) = 5.09$, $p < .05$. This means that the participants did improve on this index across the trials. Because there was no significant interaction between group and time, none of the groups showed an improvement that was different from the rest. The Language Index scores were the closest to having a significant interaction between group and time, with $p=.08$.

Table 6

Analysis of variance for the Language Index.

Source	*df*	*F*	η	*p*
	Between Subjects			
Juice Group	2	.25	.01	.78
Juice Group within group error	48	(258.67)		
	Within Subjects			
Time	2	5.09*	.10	.009
Group X Time	4	2.16	.08	.08
Group X Time within group error	91	(51.08)		

Note. Values within parentheses are mean square errors.

* Values significant for at least $p < .05$

Table 7 shows that there were no significant differences between the juice groups at any stage for the Attention Index. There was a significant effect for the improvement of sustained attention across time, $F(2,47) = 6.82$, $p < .05$, so at least one of the trials had scores that were significantly different from the others. Because there was no interaction between juice group and time, the improvement in trials was not limited to only one or two of the groups. Each group improved their scores on the Attention Index.

Table 7

Analysis of variance for the Attention Index.

Source	*df*	*F*	η	*p*
	Between Subjects			
Juice Group	2	.97	.04	.39
Juice Group within group error	48	(499.71)		
	Within Subjects			
Time	2	6.82*	.12	.002
Group X Time	4	1.79	.07	.14
Group X Time within group error	95	(71.39)		

Note. Values within parentheses are mean square errors.

* Values significant for at least $p < .05$

Delayed Memory scores were not significantly different between the groups in this sample (table 8). There was a significant improvement over time, F (2,47) = 13.49, $p < .001$. There was no interaction between group and time. This means that no single juice group showed an improvement over time that was different from the others.

Table 8

Analysis of variance for the Delayed Memory Index.

Source	*df*	*F*	η	*p*
	Between Subjects			
Juice Group	2	.47	.02	.62
Juice Group within group error	48	(576.59)		
	Within Subjects			
Time	2	13.49*	.22	<.001
Group X Time	4	.20	.01	.93
Group X Time within group error	91	(84.14)		

Note. Values within parentheses are mean square errors.

* Values significant for at least $p < .05$

The trends seen in the above mentioned Index scores were reflected in the Total Scale Score (table 9). There were no differences between groups at any stage of the experiment and all groups improved over time, $F(2,47) = 18.99$, $p < .001$. There was no interaction between juice group and time. The degree of improvement in cognitive function did not differ between the three groups.

Table 9

Analysis of variance for the Total Score Scale.

Source	*df*	*F*	η	*p*
	Between Subjects			
Juice Group	2	.51	.02	.60
Juice Group within group error	48	(415.77)		
	Within Subjects			
Time	2	18.99*	.28	<.001
Group X Time	4	.64	.03	.63
Group X Time within group error	96	(41.51)		

Note. Values within parentheses are mean square errors.

* Values significant for at least $p < .05$

Summary

Each of the three groups showed significant improvements on a number of the Index scores over the duration of the experiment. The only Index score that did not show an improvement over time was the Visuospatial Constructional Index. No group was significantly different from the others on any of the Index scores or the Total Scale Score. There were no significant interactions between berry juice group and time for any of the indices. The Language Index had an interaction that was close to significance.

DISCUSSION

The results of this study suggest neither of the hypotheses were supported in this sample. If there is any effect to be found, it may be masked behind placebo and practice effects. The results are discussed in relation to each of the cognitive domains assessed in this study and also in relation to the free radical theory of ageing. Limitations of the study are presented towards the end of this chapter.

Hypothesis One: That the berry juice groups would show an improvement in cognitive function that was significantly larger than any change seen in the placebo group.

The berry juice groups did show a significant improvement in cognitive function over time. The only exception was that there was no significant improvement noted in visuospatial ability. The first hypothesis was not supported by the data in this study however, as there was no significant difference between the berry juice groups and the placebo group.

While it was thought that placebo and practice effects would influence all three groups, it was still expected that the berry juice groups would show a significant improvement over and above any improvement seen in the placebo group. As can be seen from the results, the participants in the placebo group improved significantly over the twelve week experimental period. This group demonstrated higher scores for each index except for the index testing visuospatial abilities. There were no effects of berry juice on cognition that could be detected in this sample.

Longitudinal studies have repeatedly shown that age-related cognitive decline does occur. These studies measured cognitive decline over long periods of time. In the case of the Seattle Longitudinal Study, testing sessions were seven years apart. In the present study, the testing sessions were only six weeks apart. The

same form of the RBANS was used for the baseline testing and the final testing sessions. Because of this, practice effects may have been partially responsible for the improvement seen in the control group.

Hypothesis Two: That the participants drinking the blackcurrant juice will obtain significantly higher scores on the RBANS indices than the participants drinking the boysenberry juice.

The results of this study do not support this hypothesis because there was no interaction between juice group and time. This indicates that no single group was significantly different from the others at any testing period. It was expected that the difference would be the result of the higher anthocyanin levels in the blackcurrant juice.

The participants drinking the boysenberry juice had the highest difference in means between the baseline and final testing sessions. This large difference in means existed for every RBANS index except for the Immediate Memory and Delayed Memory indices. On these two indices, the placebo group had the highest mean difference. As has been discussed, this difference was not significant.

Placebo Effect

Because the participants were aware of the purpose of the study, placebo effects may have been responsible for the improvements in cognition. According to Stewart-Williams and Podd (2004, pg. 326) a placebo effect is defined as "... a genuine psychological or physiological effect, in a human or another animal, which is attributable to receiving a substance or undergoing a procedure, but is

not due to the inherent powers of that substance or procedure." In this case, there was an improvement over time but as this was not dependent on the contents of the berry juice, the improvement may have been attributable to placebo effects.

The expectancy theory is one particular theory that accounts for the placebo effect. The expectancy theory maintains that simply thinking a particular substance has an effect can induce the said effect (Brody and Brody, 2000). Participants in this study were informed that animal research had suggested a relationship between the reversal of age-related decline in cognition and berry fruit (see appendix A). In this situation the participants were led to expect an improvement in cognition as a result of drinking the berry juice.

The experiment was run in a double blind fashion, and so the participants did not know if they were in the placebo condition. Because the placebo juice was made to be as similar in taste and appearance to the berry juices as possible, most of the participants admitted that they thought they were drinking either the blackcurrant or boysenberry juice and not the placebo. Placebo effects could be responsible for the improvement seen across all of the berry juice groups as most of the participants expected to improve. The berry juice may have had a small effect that was masked by placebo effects. If the placebo effects were stronger than any possible effect of the berry juice, the berry juice effects would not have shown up in the statistical analysis.

Memory

As reported in the results chapter, all of the groups improved significantly on the Immediate Memory score over the experimental period. No single group was significantly different from the other two at any stage during the experiment. This suggests that the improvement may be more from practice

effects on the RBANS rather than the berry juices increasing short term memory.

Because the Immediate Memory subtests involved repeating back information just after it had been received, the participants could get a sense of how well they had done. Many of the participants gave the impression that they had failed their own internal standards when they were retrieving items. They would apologise for what they thought was their poor performance. This may have lead to the participants focusing and concentrating more in the follow up testing sessions.

The participants who drank the blackcurrant juice had a greater change in mean score than did the participants in the boysenberry group in both the Immediate Memory and Delayed Memory indices. This difference was not significant and the participants in the control group showed a change in mean scores on both these indices that was greater than the participants drinking either of the juices.

Visuospatial/Constructional Ability

The Visuospatial Constructional subtests did not show any significant improvement for any of the groups over the trial. The berry juice had no effect on visuospatial abilities in this study and there did not seem to be any practice effects over the three testing sessions.

The Visuospatial Constructional subtests were the only tests that did not show any improvement over time. This may have been due to the fact that participants could not get an understanding of how well they were doing, and so any practice effects were less noticeable. The Visuospatial Constructional subtests were hard to practice in between testing sessions as some of the participants reported doing for some of the other subtests.

There was no precedent for the improvement of visuospatial abilities as a consequence of antioxidants in previous research. It was thought however, that visuospatial abilities would improve in this study with an increase in attentional ability. The visuospatial subtests in the RBANS required a high level of attention. As will be discussed shortly, it was thought that attention would improve as the anthocyanins protected the cholinergic system from oxidative damage.

Language

As with the indices of memory in this study, improvements in scores on the Language index occurred across all groups including the control group. This indicates that the differences in scores across time were again due to placebo and practice effects rather than the antioxidant effects of the berry juices. Although there was no significant interaction between juice group and testing period, the different juice groups accounted for 8% of the variance for Language Index scores. This was the largest effect size in the study. With a larger sample size, blackcurrant and boysenberry juices might have elicited a significant effect from Language Index scores.

As outlined earlier, age-related declines in language are thought to be due to a decreased ability to inhibit information and to a poorer working memory. Therefore in this study a significant interaction between Language Index score and berry juice could have been related to improvements on Immediate Memory Index and Attention Index scores.

Attention

Scores on the Attention Index of the RBANS also showed a significant increase over time. As with the other indices there was no interaction between juice group and test scores indicating that this increase was again due to practice or placebo effects.

Under the Free Radical Theory of Ageing, attention should have increased due to the protection of the cholinergic system from oxidative damage. The cholinergic system, it's muscarinic receptors in particular, are susceptible to oxidative damage. As well as being beneficial to memory, acetylcholine is also important for tasks that require a high level of attention. Theoretically, the anthocyanins in berry juices would have reduced the damage caused by oxidative stress. This being the case, attention should have improved as repair mechanisms fixed previous damage. Any improvement in the attention domain would potentially have contributed to an increase on the other cognitive domains as each of the RBANS tasks required sustained attention. This study does not rule out the potential for such changes to occur. The juice groups accounted for 7% of the variance for Attention Index scores. This indicates that there may be a small interaction between juice group test score but that too many other variables were interfering with the interaction in this case.

Free Radical Theory of Ageing

Free radicals are held responsible for the ageing process by causing oxidative damage that accumulates over a life time. The damage to proteins, lipids and DNA accrues in cells, compromising vital cell functions such as oxygen metabolism. Antioxidants from food sources can help to scavenge for, and neutralise such free radicals. The blackcurrant and boysenberry juice

anthocyanins used in this study have been shown to have a high antioxidant capacity *in vivo.*

The results show that the experimental conditions of this study did not have an effect on cognition. Those participants drinking either of the two berry juices did not show significant improvements in cognition over those participants drinking the placebo juice.

The differences between the mean scores at the beginning and end of the experiment were higher for the boysenberry group than the blackcurrant group on every test except for the memory indices. This was despite the boysenberry juice having a lower concentration of anthocyanins. On both of the memory indices, the blackcurrant group showed a mean improvement that was larger than the boysenberry group. The placebo group, however, showed the largest improvement on these indices. The placebo group always showed a larger mean improvement over time than the black currant group on every index. The boysenberry group had the largest mean improvement for every index except for the memory indices.

These results do not support the hypothesis that antioxidant activity of the berry juices is responsible for the test score improvements. New Zealand grown blackcurrants have an antioxidant capacity that is twice that of boysenberries (Just the Berries, n.d.). The berry juice antioxidants in question have also been assessed for their *in vivo* absorbance and antioxidant activity (Mazza et al., 2002; Youdim, Shukitt-Hale and Joseph, 2004). Any improvements in cognition may have been attributable to the high antioxidant capacities of blackcurrants and boysenberries. However, these results suggest that placebo and practice effects were responsible for the increased scores on the RBANS indices and that the cognitive improvement was not a result of increased antioxidant intake.

Limitations of the Present Study and Suggested Further Research.

There were several major limitations in this study. Firstly, any existing effect may not have been detected due to other variables such as placebo and practice effects masking any differences between groups. Secondly, the duration of the study may have been too short to detect a reversal of age-related cognitive decline. Thirdly, the concentration of antioxidants being consumed each day may have been too low. Fourth, the participants may have altered their own diet due to increased awareness of the effects of antioxidants on memory, motor control and immune function. Finally, the sample size was quite small with only 17 participants in each condition.

While it needs to be acknowledged that berry juice and anthocyanins do not have a significant effect on cognition in this sample of older adults, it also needs to be acknowledged that any effect may have been masked by limitations of this particular study. Although the RBANS is sensitive to small changes in cognition and is a useful tool in detecting problems such dementia and mild cognitive impairment (Randolph, 1998), any change in cognition due to the berry juices may have been lost amongst background noise. Placebo effects, practice effects, personal concerns and emotions of the participants, and two different test administrators may all have contributed to drowning out any possible effect.

This study would have benefited from a natural history group that only came in for testing and did not consume any type of juice. This type of group would have allowed for placebo effects to be measured. Because a natural history group would not have the expectation of cognitive improvement due to berry juice, they would have provided a good benchmark to compare the placebo group to.

One limitation of this study was that the trial was run over a relatively short period. In the review of nutritional supplements on cognition by Manders et al. (2004), the studies that administered multivitamins (including antioxidant

vitamins) daily for periods of 4-17 weeks found no significant effect. The studies of a longer duration, up to one year, found significant improvements in cognitive testing. Also, the studies on antioxidant diets and animals discussed previously used an experimental period ranging between eight weeks and eight months. Even eight weeks is a much longer proportion of a rat's life. As rats only live for approximately two years, having a rat on an antioxidant enriched diet for eight months is the equivalent of having people on an enriched diet for one third of their life.

The relatively small amount of anthocyanins and antioxidants consumed each day as part of the study may be another limitation. The participants of this study were only consuming 250-500mg of anthocyanins once a day. In the animal studies conducted on antioxidant supplemented diets, the rats and mice were only eating a supplemented diet and nothing else. The administration of the antioxidants and anthocyanins were prolonged throughout the day as most of the animals were fed *ad lib.* In one such study by Goyarzu et al. (2004), the rats were eating the equivalent of 4.4g of fresh blueberries each day. This many blueberries would represent a much larger percentage of the rats' daily food intake than would 200mL of blackcurrant or boysenberry juice in the participants used for this study. While this may not have been much of a limitation on its own, it combines with the other limitations to make any effect that much harder to find.

It is also a possibility that the participants altered their diet to accommodate a larger volume of fruits and vegetables. The participants were aware that research on animals has provided evidence for berry fruit contributing to improved memory and motor control from the information sheet sent out to them. The participants were concerned about their own personal memory loss, which was one of the advertised requirements. The participants were also aware that the impact of berry fruit on immune function would be under investigation. As the study coincided with the beginning of winter, and the

participants were looking for something to improve their cognitive functioning, it is possible that they increased their intake of fruits and vegetables. While they had been asked not to do this, they may not have been aware that they were altering their diet in any way that would affect the study.

Another limitation was the small sample size of only 17 participants in each of the three groups. By screening participants for average or below memory functioning using the RAVLT, the population that the participants could be drawn from was reduced. That, in combination with other strict screening criteria meant that there were very few participants eligible to continue on the study. The small sample size meant that the power to detect significant effects within the study was very low. According to the SPSS statistical analysis, the power to detect interaction between juice group and test scores ranged from an 8% to a 62% chance of finding a significant effect. The sample size would have had to have been much larger to obtain a satisfactory chance of finding a significant effect.

Research to correct all such limitations would be both expensive and time consuming. Having participants drink several glasses of berry fruit juice or eat several cups of fresh berry fruit each day for 5-10 years is more than likely not feasible. That being said, some of the research on vitamin intake in humans has successfully provided evidence supporting positive antioxidant effects on cognition. It may be that correcting only one of these limitations may reveal an effect that was not apparent in this study.

Summary

The present study investigated the effects of blackcurrant and boysenberry juice anthocyanins on cognitive decline in an elderly population. In particular, tests of memory, visuospatial ability, language and attention were used to assess

cognition. This study was unable to detect any interactions between berry juice type and domains of cognitive function. The present study does not therefore provide additional evidence to support the hypothesis that free radical damage may be responsible for age-related cognitive decline. It is acknowledged however, that failure to demonstrate an improvement in cognitive function over and above that of the control group may have been influenced by several limitations. These limitations include placebo and practice effects, a short duration and low anthocyanin intake, small sample size and the possibility that participants may have altered their diet.

APPENDICES

Appendix A

Information Sheet

Massey University

The effect of berry juice on memory and immune function

INFORMATION SHEET

Who are we?

The Institute of Food, Nutrition and Human Health is part of Massey University. Our role is to carry out research into the links between food and human health. Assoc. Prof. Marlena Kruger is one of the Institute's Senior Research Scientists, and is interested in the effect of food in helping to provide protection from various diseases.

Why are we doing this trial?

The evidence that eating fruit and vegetables may promote good health by providing protection against various diseases, including cancers and coronary heart disease, continues to increase. Fruit and vegetables, especially those of colours red, orange, purple, yellow etc., are known to have high concentrations of antioxidants which have been shown to be important in reducing cell damage. Research undertaken at the Human Nutrition Research Centre on Ageing (HNRCA) on elderly animals showed that addition of a berryfruit extract could enhance memory, balance and co-ordination. Previous collaborative research between HortResearch and HNRCA, showed that the beneficial effects may also be produced by both blackcurrant and boysenberry.

Our immune systems need to be healthy if we are to have optimal well being. Several studies in the elderly have shown that anti-oxidants (vitamin E, carotenoids, Se) are able to enhance immune function. However, little is known about the effect of the antioxidants from fruit on immune function in the elderly. For this reason we will be looking at the effect of the berry drinks on several measurements of immune status.

In this study we will have three groups of 20 people.

1) Group 1 will take the control (dummy) drink, a synthetic berry fruit drink (200 mL) containing synthethic colours and flavours, sugar, and citric acid.
2) Group 2 will take a freshly manufactured commercial blackcurrant drink (200 mL) such as Barker's Blackcurrant syrup with supplementation of natural pigment concentration. This will adjusted to 500 mg/serve.
3) Group 3 will take a freshly manufactured commercial Boysenberry drink (200 mL) with the Boysenberry concentration adjusted to single strength expressed juice.

As the blackcurrant drink is fortified, the group 2 and 3 will be compared to the control and not to each other.

Would you like to take part?

We would like to invite 60 people, aged from 65 years to join our study.

To fit in to our study you should :

- ***be feeling that you are more forgetful or not thinking as well as you used to***
- ***be a non smoker***
- ***not be taking drugs that impair memory***
- ***have not been diagnosed with cancer, vascular disease, diabetes or mental illness***
- ***not have a neurological disorder***
- ***not have renal impairment***
- ***not mind undergoing a simple screening questionaire for memory decline over the telephone***
- ***not be taking any vitamin or mineral supplements***

If you are interested in taking part please contact Marlena Kruger or Chris Booth who will be happy to discuss the project and answer your questions.

Contact details:

Assoc. Prof. Marlena Kruger

Institute of Food, Nutrition and Human Health

Massey University

Private Bag 11222

Palmerston North

Telephone: 06-350-5905 (or 06-350-5966)

Fax: 06-350-5446

e-mail: *m.c.kruger@massey.ac.nz*

Mrs Chris Booth

Human Nutrition Studies Laboratory

Institute of Food, Nutrition and Human Health

Massey University

Private Bag 11222

Palmerston North

Telephone: 06-350-5901 (or 06-350-5966)

Fax: 06-350-5446

e-mail: *c.l.booth@massey.ac.nz*

What is involved?

This is a 12-week food study.

You will be randomly allocated to one of our 3 groups, (you won't know which group you are in).

If you are in group 1 you will be asked to drink 200 ml of the (dummy) control beverage daily for 12 weeks.

If you are in group 2 you will be asked to drink 200 ml of the blackcurrant beverage daily for 12 weeks.

If you are in group 3 you will be asked to drink 200 ml of the boysenberry beverage daily for 12 weeks

We will ask you to go to Medlab Central (Victoria Ave) 3 times over the 12 week period for blood samples to measure any effects of the drinks on your immune function and the antioxidant levels in your blood. We will provide you with transport to and from Medlab if required.

We will ask you to provide a small urine sample on the same 3 occasions to measure your antioxidant levels.

We will also ask you to take part in a memory assessment procedure on 3 different occasions so that we can assess any changes (note these are not intelligence tests). These tests will be done in your home or a suitable place of

your choice, and will take 60 minutes each time. The total amount of time that the whole trial will take is approximately 8 hours.

What are we going to measure?

Body composition/Nutritional status:
The following measurements will be done:

- *Body weight* will be measured using ordinary weighing scales. You will be asked to remove your shoes and jumper or jacket.
- *Standing height* will be measured using a height meter
- *Waist to hip ratio* will be derived from measurements of waist and hip circumference made using a measuring tape.

The food you eat (dietary history)

We would like you to answer some questions that help us to find out about the amount and kinds of food you normally eat. This will take about 15 minutes. In the questionnaire we will ask about your routine dietary intakes and the frequency you eat certain foods. We want to try to assess your habitual antioxidant intake as well as the levels of vitamins and other nutrients in your diet in order to assess the impact of the supplemented drinks on your antioxidant status.

Medical assessment:

We will ask you to fill in a form about your health and current medications. We will also ask you to sign a consent form to allow us to send a general questionnaire about your health to your GP to ensure that you are suitable to take part in the study. We will give you a copy of this so you can see the sort of questions that will be asked.

Blood

We will ask you to visit Medlab Central (Victoria Ave) before we commence the trial, to have 30 ml (6 teaspoons) of blood taken, to assess your heart, liver and kidney function, which must be normal for you to be included in the trial. If required, we can provide transport to and from Medlab.

When you have been accepted as part of the study we need another blood sample, to assess your baseline values. You will then start taking the drink for 12 weeks.

We will need another blood sample after 6 weeks, and the final blood samples after 12 weeks.

We would like you to have all the blood and urine samples done in the morning between 7.30 am and 9.30am. This is because the levels of the chemicals that we measure change during the day so we have to take them at the same of the day. We shall use the blood to measure levels of damaged protein, fat and DNA (oxidized protein, lipid peroxidation, and oxidative DNA damage), as well as the levels of antioxidant (antioxidant capacity). We will also measure the ability of your immune cells to multiply and attack germs and the ability for them to send chemical messages to each other (lymphocyte proliferation, cytokine secretion, phagocyte monokine production and phagocyte phagocytosis). These measurements will help us monitor the impact that the blackcurrants juice is having on your body.

Not all the blood will be used for the tests above. We will freeze a small amount in case we need to repeat any tests but we will contact you for your permission to do this.

Urine samples:

We will ask you for a urine sample (20 ml) as you start the study, after 6 weeks and again at 12 weeks which we will measure for oxidized DNA.

Cognitive performance

As part of our screening process we will ask you to undergo a test called the Rey Auditory Verbal Learning Test (RAVLT). This is primarily a test for memory functioning. The RAVLT takes approximately 10 to 15 minutes and is a test where you are asked to recall a list of words a few times.

During the trial your mental performance will be tested using a computer based method using playing cards, which specifically tests changes in memory (cognitive) performance in a 15-20 minute assessment procedure, during which a number of your mental functions will be assessed:

1) the time you take to react to a stimulus.

2) the time you take to make a decision.

3) your ability to remember things.

We will also use a second test, the Repeatable Battery for the Assessment of Psychological Status (RBANS). It involves recall of a story that is read to you, repeating back of a list of words, and recalling the previously read story once again, to see how well you can remember.

Your memory function varies according to food intake, and the time of day, so the assessments will be done at a similar time of day. You will be given dietary suggestions so that the food you eat before the test does not interfere with the tests, in particular coffee.

If significant differences in your mental capacity and blood tests are not observed after the 12 weeks we would to extend the study. If you are happy with the extension we will provide blackcurrant or Boysenberry drink for a further 6 months. We will assess your mental capacity at the end of this period.

To carry out the memory assessment we would like to visit you at your home or a suitable place of your choice. Assoc. Prof. Marlena Kruger, who has a PhD in Human Physiology from the Medical University of Southern Africa, South Africa, supervises our nutrition laboratory. Marlena's Technical Officer is Chris Booth, who has a degree in Biochemistry, and expertise in human nutrition. Chris is trained in the procedures and it will be Chris who will be interviewing you.

A schedule of measurements is given below:

Time	Measurements
Before the intervention	1. Measurements of health, food intake and memory at your home 2. Blood sample to screen for health status at Medlab
At the start of the trial **Week 0**	3. Blood and urine samples for baseline measurements at Medlab 4. Memory assessment at home
Intervention (12 weeks) **Weeks 6** **Week 12**	5. Blood and urine samples and memory assessment at 6 weeks 6. Blood and urine samples and memory assessment at 12 weeks

Are any of the procedures harmful or painful?

This study involves routine clinical and laboratory testing procedures, which are widely used around the world. We appreciate that some people are anxious about having blood taken, and that this procedure sometimes causes local bruising. For this reason all samples will be taken by a trained technician. Volunteers also have access to the City Doctors if they develop problems from the testing or have side effects from the berry drink. The cost for such a visit will be covered by the researchers.

Who will see the information about me?

When you join the trial you will be given a number and thereafter all information will be filed with the code number, and stored in a locked filing cabinet accessed by the research team only. When information from all the volunteers has been pooled, and made anonymous, it will be used in presentations to academic societies, scientific publications and reports to the funders, Blackcurrant NZ Ltd and HortResearch. No names will be used, just the designated numbers. We will give you a summary of these findings of our research if you would like one.

All personal data will be destroyed of at the end of the trial. Scientific data, filed on paper, will be shredded and electronic data will be deleted from our computer records and databases after 5 years, which is the requirement for Massey University.

Who is funding this research?

This research is funded by Blackcurrant NZ Ltd and the Horticultural Institute of New Zealand, which is a Crown Research Institute involved in research on horticultural crops, the environment, plant breeding and the beneficial components of fruits and vegetables.

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 Injury Prevention, Rehabilitation and 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 from Massey University.

Will I get any financial compensation?

You may have to drive to Medlab with your own car and therefore we will reimburse all your travel costs at a rate of 50c/km. We will also give you a retail voucher to compensate you for your inconvenience and time. The researchers will cover any costs, associated with Doctors visits, resulting from the trial. You will not have any further costs to participate in the trial.

What are my rights?

We respect your rights to:

- *refuse to answer any particular question, and to withdraw from the study at any time*
- *ask further questions about the study that occur to you during your participation*
- *provide information on the understanding that it is completely confidential to the researchers. All information is collected confidentially, and it will not be possible to identify you in any reports that are prepared from the study*
- *be given access to a summary of the findings from the study when it is concluded.*

If you would like to participate in this study please call Chris Booth on 350 5901.
You will be visited in your home by Chris Booth or Marlena Kruger to explain the study and to do some body measurements.

This study has received ethical approval from the Massey University Human Ethics Committee, PN Application 04/30 and the Manawatu Human Ethics Committee 04/05/ 014. If you have any concerns about the conduct of this research, please contact Professor Sylvia V Rumball, Chair, Massey University Regional Human Ethics Committee: Palmerston North, telephone 06 350 5249,

email human ethicspn@massey.ac.nz

Mail to: humanethicspn@massey.ac.nz

Appendix B
Consent Form

Massey University

The effect of berry juice on memory and immune function

CONSENT FORM

Request for an interpreter			
English	*I wish to have an interpreter*	*es*	*o*
Maori	*E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero*	*e*	*ao*
Samoan	*Oute mana'o ia iai se fa'amatala upu*	*oe*	*eai*
Tongan	*Oku ou fiema'u ha fakatonulea*	*o*	*kai*
Cook Island	*Ka inangaro au I tetai tangata uri reo*	*e*	*are*
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu	E	Nakai

- *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 understand that I have chosen to take part in this study and that I have the right to withdraw from the study, or any test, at any time.*
- *I agree to provide information to the researchers on the understanding that my name will not be used without my permission.*
- *I understand that the study will be stopped if it appears harmful to me.*
- *I understand the compensation provisions for this study*
- *I have had time to consider whether to take part*
- *I know I can contact Assoc. Prof. Marlena Kruger or Mrs Chris Booth at any time during the study*
- *I agree to participate in this study under the conditions set out in the Information Sheet.*
- *I understand that the Massey University Human Ethics Committee have approved this project.*
- *I consent to the researchers storing specimens for later determination of antioxidant status on the understanding that I will be contacted for consent.*
- *I consent to the researchers using data collected in this trial for publication in academic/medical publications.*
- *I would/would not like one of the researchers to discuss the outcomes of the study with me*
- *I understand that, in the event of any findings that are relevant to my medical welfare being incidentally discovered during the course of this experiment, I will be informed of this, and I may give permission for these findings to be relayed to my regular medical practitioner, or other person whom I may depute, on a confidential basis.*

I ………………………………………………………………………….

hereby consent to take part in this study

Signature of volunteer………………………………………………

Name of witness……………………………………………………….

Signature of witness………………………………………………

Project explained by ………………………………………………..

Role in project…………………………………………………….

Signature…………………………………………………………

Date…………………………………………………………….

This study has received ethical approval from the Massey University Human Ethics Committee, PN Application 04/30 and the Manawatu Human Ethics Committee 04/05/ 014. *If you have any concerns about the conduct of this*

Appendix C
Request of Information from General Practitioner

Massey University

Dear Doctor

The following patient of yours, has volunteered to participate in a study investigating the effect of antioxidants in a food substance, berry juice, in restoring age related deterioration in mental capacity.

In order to obtain maximum benefit from this study it is important to exclude subjects who have disorders that may influence response.

Accordingly, we request a moment of your time to scan this patient's file in order to ascertain whether they have any of these conditions. A fee of $20.00 is payable to you on receipt of your report.

Please find enclosed a permission slip signed by your patient allowing us access to this information and a summary of the study.

Yours sincerely

Chris Booth
Institute of Food, Nutrition and Human Health
Massey University
Palmerston North
Phone 06 350 5901
Email C.L.Booth@massey.ac.nz

The effect of antioxidants in a food substance, (berry juice), in restoring age related deterioration in mental capacity.

Please tick the appropriate box if there is any history of the condition and return the completed form in the enclosed envelope.

Name:
DOB:
Address:

	Tick if yes
cerebrovascular disease	
diabetes mellitus	
mental incapacity	
cancer	
vascular disease	
myxodema	
amyloidosis	
alcohol abuse	
anaemia	
malabsorption	
neurological disorders including debilitating cognitive degeneration	
multiple sclerosis	
parkinsons disease	
alzheimers	
epilepsy	
renal impairment	
compromised immune status	
visual impairment, intact visual fields	
current consumption of night sedation	
current consumption of tranquillisers	
current consumption of antipsychotic	
current consumption of antidepressants	
iron supplements	
vitamins and antioxidants	

Appendix D
Inclusion Questionnaire

Inclusion Questionnaire

Name_____
DOB_____
Telephone Number_____
Cellphone Number_____
Address_____
Email Address_____

Have you ever had any of the following: ✓ if yes X if no

Coronary artery trouble, angina or heart attacks
Stroke, mini stroke or CVA
Diabetes or persistent sugar in the urine
Mental disorder such as depression, schizophrenia or eating disorder
Thyroid disease
Parkinsons or persistent tremors
Kidney problems

Blood disorders such as anaemia, leukaemia or multiple myeloma

gaps with your vision

Been told that you are badly over or under weight

Disorders of the liver

Impaired immunity

Do you smoke?

How many drinks containing alcohol do you have a day?

Have you ever been told you have a high alcohol consumption rate?

Have you ever been allergic to any sort of fruit or fruit juice eg blackcurrant ?

Have you ever been allergic to any sort of food colouring?

Do you take any of the following √ if yes or X if no

Vitamin supplements	
Mineral supplements	
Iron supplement	
Other health foods	
Extra calcium	
Pills for anaemia	
Pills for gastric ulcer	
Pills for diabetes	
Pills to lower the blood fat levels	
Antidepressant medication	
Blood thinning pills eg warfarin or aspirin	
Nerve pills (tranquillisers)	
Pills for epilepsy	
Sleeping pills	
Water pills (diuretics)	

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