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.