PERSISTENCE OF OBJECT MEMORY PRESERVATION BY A BLUEBERRY-ENRICHED DIET IN AGED RATS

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ABSTRACT

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Previous studies found that four months of an antioxidant-rich diet supplemented with blueberries decreased memory impairment in aged rats, as indicated by performance on an object recognition task. The present study evaluated the aftereffects of shorter, one and two-month, versions of this diet on aged Fisher-344 rats. It determined whether any benefits would be maintained over a one-month period following termination of the diet. The performance of the rats maintained on the blueberry-enriched diet for one month declined markedly in two weeks. At four weeks, their performance was similar to that of aged rats that had been maintained on a control diet. In contrast, rats that had received the blueberry diet for two months largely maintained their superior performance. Shorter diets can preserve object memory in aged rats, and after several months their benefits can persist despite considerable interruption. The results are encouraging for dietary interventions in human aging.

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CHAPTER ONE: INTRODUCTION

Types of Memory

Memory is a broadly used term, which refers to the various operations of the mind that entail the encoding, retention, and retrieval of information and experiences (Birren & Schaie, 2006).

There are three types of memory that are commonly recognized by the majority of learning theorists. The first is sensory memory, which encompasses iconic and echoic memory. Next is primary memory, also called short-term memory, which consists primarily of working memory (memory which is needed only temporarily). Lastly, secondary memory, also called long-term memory, is classified according to three dichotomies: declarative and procedural memory, explicit and implicit memory, and episodic and semantic memory (Wilding & Valentine, 1997).

According to Wolfe (2001), everything pertaining to memory begins as sensory input from the environment. "Sensory memory is modality-specific, highly unstable and characterized by rapid decay" (Schneider & Rowe, 1996, p. 219). When a visual stimulus is presented, a sensory trace of that stimulus lasts for several hundred milliseconds after its offset. This is referred to as iconic memory. Echoic memory is analogous to iconic memory. However, it deals with auditory signals and it typically lasts much longer, usually for 1 or 2 seconds (Bjork & Bjork, 1996).

The two most studied types of memory are primary memory and secondary memory (Greene, 1992). Primary memory has a limited capacity and can store very few items. Even though primary memory has restricted storage capabilities, information is most often retrieved quite easily. Primary memory acts as a filter through which stimuli must pass to get to secondary memory (Tileston, 2004). In order for information to enter into secondary or long-term memory, rehearsal must take place. Rehearsal is used to maintain information in working memory, and it is the mechanism used to transfer information into secondary memory (Tileston, 2004).

Secondary memory consists of information that is stored for an indefinite period of time. Some researchers believe memories that are not utilized will be purged. Others believe memories are never lost, but the ability to recall them becomes increasingly difficult, especially with age (Sprenger, 1999).

The three dichotomies of secondary memory overlap in many ways, yet each dichotomy refers to pairs of distinct characteristics: 1) Explicit memory requires the deliberate recall or recognition of material previously experienced, such as repeating a list of words or drawing a visual pattern (Wilding & Valetine, 1997). Implicit memory, on the other hand, is shown indirectly by an effect of earlier experience on current behavior, such as completion of word stems with words which have recently been seen (Wilding & Valetine, 1997).

2) Declarative memory is the ability to store and recall information that an individual can speak or write. It requires conscious processing, unlike procedural memory. Procedural memory is the ability to store automatic processes for routine

actions. An individual performs these actions without the necessity for conscious thought (Wolfe, 2001).

3) Episodic memory requires remembering where and when information was acquired. On the other hand, semantic memory includes words, the symbols for words and the rules to abide by when using them. Semantic memory is generally quite accurate whereas episodic memory is sometimes inaccurate due to the brain reconstructing details of an experience when an individual cannot remember them (Wolfe, 2001).

Memory Decline with Age

According to Birren and Schaie (2006), memory functioning in the aging adult can be affected by health, behavior, social and environmental factors. Memory generally declines with age, although the rate of decline varies dramatically between individuals (Albert, 1994). The differences in the rate of age-related changes can be attributed to numerous factors. Health, diet, motivation, social status and environmental issues are just a few components that may have an impact on the aging individual (Digiovanna, 1994).

According to Schneider and Rowe (1996), there are substantial differences between age groups when dealing with secondary memory. On the other hand, there are smaller differences between age groups in studies measuring sensory memory and primary memory.

Testing of primary memory most often involves having participants read a word list or listen to numbers being recited. Subjects are then asked to recall the information to the best of their ability. The findings of such studies usually differ depending on whether or not the subjects were asked to repeat the information, or whether they were asked to manipulate the information in some way, such as responding in a particular order. Older adults tend to have a more difficult time repeating words the order of which had to be manipulated. Therefore, it would appear that one factor in age differences when dealing with primary memory is the complexity of the task (Ferraro, 1997).

Zacks, Radvansky and Hasher (1996), found that older adults are less able than younger adults to suppress items that were designated to be forgotten. This was demonstrated in four separate experiments. In the first experiment, individuals were to read word lists. Each word was associated with a symbol that was representative of either "remembering" or "forgetting." After all of the word lists were shown, participants were to write down each of the words remembered and each of the words they were supposed to forget. This was done for six separate lists. After a 5 minute interval, participants were given 5 minutes to recall as many words as possible from both categories. The remaining three experiments were similar in nature to the one described above. All four experiments revealed that older adults are less able than younger adults to ignore information that is designated to be irrelevant (Zacks et al., 1996).

In a similar study (Andres, Linden, & Parmentier, 2004), a group of researchers found comparable results. They tested 72 young adults and 72 older adults. The participants were shown index cards containing trigrams for retention and then had to recall the trigram in its correct order. In the second scenario, participants were shown a

trigram for retention and immediately shown a second trigram, also for retention. Participants had to recall each trigram, in order and as separate lists. For the third scenario, individuals were shown two trigrams consecutively, and then a card which read, "to be forgotten." Later, participants then had to recall the first trigram. The results were that the older adults did not differ from the younger adults in their ability to remember one trigram. However, the older adults did show a marked decrease in performance compared to the younger adults when requested to recall both trigrams. In addition, the older adults were less likely to forget the second trigram when instructed to do so.

In a study conducted by Naveh-Benjamin (2005), it was found that a major factor underlying age-related memory problems appears to be the difficulty in binding items together and integrating events with their contexts. In this experiment, participants were to remember word lists for later recall. Overall, younger adults were reported to have higher accuracy and faster retrieval than the older adults. In this task, words that had been paired or were related were retrieved more often and faster than words which were not related or paired. Older adults performed particularly poorly when the word pairs were not related. Finally, older adults performed worse on cued recall tasks.

It requires more cognitive processing to transfer information into secondary memory than to simply encode information into primary memory. Most of the research pertaining to secondary memory involves subjects learning information at one time and recalling it at some later time. Older adults tend to perform better if they are told they will need to recall the information and if they are given strategies to aid in their learning. Overall, younger adults tend to perform much better than older adults on recalling information from secondary memory (Ferraro, 1997). A number of studies have

reported that recognition memory also declines in aging, although with great variability between individuals (Squire et al., 2003). This decline has also been found in aged non-human primates (Moss et al., 1997) and in aged rodents (Dellu et al., 1992).

Theories of Aging

The Telomere Hypothesis

Why do memory processes change with age, even in healthy older people? The brain, like the rest of the body, is subject to aging. There are several overarching hypotheses to explain the loss of bodily functions with age.

The telomere hypothesis offers an explanation for age-related deterioration based on a cell division counting mechanism. Telomeres are "the ends of eukaryotic chromosomes consisting of non-coding, repetitive sequences" (Weinstein & Ciszek, 2002, p. 616). Like the cap of a shoelace, the telomere protects the actual coding regions of DNA. According to the hypothesis, with each cell division, a telomere will gradually shorten in length.

In a study conducted in the 1960's by Hayflick and Moorehead, it was discovered that fibroblasts grown in vitro had a finite life span. These cells failed to replicate and divide after reaching their limit of 50 ± 10 divisions. Hence, the term "Hayflick limit" was coined after this major discovery (Ferraro, 1997). "At the Hayflick limit, telomeres trigger a permanent growth arrest known as replicative senescence or mortality stage one" (Cong, Wright & Shay, 2002, p. 408).

The replicative potential depends on the cell type, species and age of the subject (Schneider & Rowe, 1996). Cell samples taken from older individuals can divide even fewer times. According to Schneider and Rowe (1996), there are only a few types of dividing cells that fail to senesce; they are as follows: the germ cells, cancerous cells and some primitive somatic stem cells. Nerve cells do not divide. However, many glial cells that support neuron function do divide, so the telomere theory might still apply to brain aging.

Additional Theories

It should be noted that possible mechanisms of aging are not limited to the telomere hypothesis and the free radical hypothesis which forms the basis of this thesis. There are numerous other changes that take place in an aging cell. One theory is that as a cell ages, incorrectly constituted proteins become fatal to the cell or the cell will become filled with useless enzymes and proteins and eventually die (Ferraro, 1997).

The "Wear and Tear" theory suggests that aging is the byproduct of abuse, use, disease, toxins and an accumulation of other factors that have a negative effect on the body. This particular theory was very popular at one time. However, due to lack of evidence, the theory has lost credibility (Digiovanna, 1994).

Another theory that has lost attention over the years is the "Error Catastrophe Theory." According to this theory, the actual genes are not damaged, but the RNA and protein molecules that read them are. Therefore, erroneous messages are being carried throughout the body and to the cells. This, in turn, leads to biological age changes. This particular theory is faulty in that known mutagens do not produce error messages that

lead to senescence. In addition, when abnormal amino acids (building blocks of proteins) are added to an animal diet, there is no evidence than an "error catastrophe" has occurred (Strehler, 1977).

There are numerous other theories of aging that have been proposed throughout the years. These other theories will not be discussed. However, researchers are still investigating them to identify their significance in the aging process. The theories are often based on hormone levels, collagen molecules, toxins in the body and endocrine functioning. The most compelling theory that has recently been given a great deal of attention by researchers is the free radical theory.

Free Radical Theory

"The free radical theory states that aging is caused from an accumulated injury produced by free radicals" (Digiovanna, 1994, p. 27). Basically, a free radical is a highly reactive molecule. These contain atoms (often oxygen) that are highly reactive because they contain an odd number of electrons in their outer orbital. Since electrons "seek" to pair up, the free radicals constantly tend to pair up with other molecules to obtain an electron pair. The body is equipped to eliminate free radicals via endogenous antioxidants such as glutathione, which help to neutralize free radicals. In addition, exogenous antioxidant compounds may be supplied though dietary intake. Nevertheless, the cumulative amount of free radicals can eventually overcome the body's ability to neutralize them. Free radicals damage normal, healthy molecules such as DNA, lipids, proteins molecules and enzymes (Digiovanna, 1994).

Reactive oxygen species (ROS) are commonly free radicals because they have unpaired electrons (Kregel & Zhang, 2006) "ROS is a general term for molecular oxygen-derived molecules that are reactive species or that are converted easily to reactive species" (Matsuo & Kaneko, 2000, p. 1). ROS can induce oxidative damage to cells. This leads one to the hypothesis that an accumulation in reactive oxygen species will lead to an increase in the aging process (Harman, 1956).

Organisms are constantly exposed to oxidative stress. Oxidative stress occurs when the natural balance of oxidants and antioxidants is disturbed. "When the oxidant capacity outweighs the antioxidant capacity, a small amount of ROS may escape from the antioxidant defense in cells" (Matsuo & Kaneko, 2000, p. 25). Due to a decrease in food intake, an increase in waste elimination and meager food absorption, the older population is at risk for antioxidant deficiencies. Therefore, it is reasonable to conclude the aged would benefit substantially from an increased antioxidant regimen (Carmeli, Lavian, & Reznick, 2000, p. 95).

There is a probable association between aging and mitochondrial dysfunction. Mitochondria are one of several organelles (intracellular formed bodies) with a special function found in eukaryotic cells. This particular organelle serves as the site of cellular respiration and production of cellular energy in the form of adenosine triphosphate (ATP). Mitochondria are thought to be a source of oxidants that damage cellular proteins and nucleic acids (Morrison et al., 2005). "Damage to mitochondrial DNA blocks the rejuvenation of the mitochondrial population and leads to bioenergetic decline and cellular death" (Carmeli et al., 2000, p. 95).

Liu et al. (2002), conducted a study which suggests that feeding ALCAR (acetyl-L-carnitine) and LA (R-α-lipoic acid) to old rats improve performance on memory tasks by lowering oxidative damage and improving mitochondrial functions. LA is a coenzyme that is necessary for the production of ATP in mitochondria. LA is also reduced in mitochondria to an antioxidant. L-carnitine is required to transport long chain fatty acids into the mitochondria for oxidation and ATP production. ALCAR, the acetylated form, is used more often that L-carnitine because it crosses the blood-brain barrier more efficiently.

In this experiment, Fisher 344 rats were used, since they grow old without growing large. At the start of the experiment, the young rats were 4.5 months of age and the old rats were 24.5 months of age. The experimental rats were fed ALCAR, LA or both. The control rats were given a standard AIN93M rodent diet. The rats were tested on the Morris Water Maze to test spatial memory. This particular test requires rats to find a platform that is submerged in a pool of water. Each rat was tested for four consecutive days with four trials per day. On the fifth day, the platform was removed from the pool and rats were measured on how long they spent at the site where the platform used to be. On the sixth day, a visible platform was introduced into the pool. Rats were measured on how long it took them to reach the platform. This portion of the experiment was to determine visual function and motor ability in the absence of a demand on memory (Liu et al., 2002).

Overall, the young rats took significantly less time than the older rats to find the hidden platform. The rats given the ALCAR or LA spent a shorter time than the control rats but the results were not significant. However, the group receiving both supplements

did have significantly shorter latencies than the control group. In addition, young rats reached the visible platform faster than the older rats. However, all three of the older experimental groups improved. The loss of memory function with age seems to be caused partially by oxidative mitochondrial deterioration in neurons (Liu et al., 2002).

In a study conducted by Navarro et al. (2005), mice were given vitamin E supplements to determine the effects on mitochondria functioning. "Vitamin E is the most important fat-soluble chain-breaking antioxidant in the body" (Ji & Hollander, 2000, p. 42). Vitamin E can be found in almost all cell membranes, with a portion in the inner mitochondrial membrane. For this experiment, the control group was given standard laboratory animal food. The experimental group was given the same food, supplemented with vitamin E, from the age of 28 weeks until death. The diet was started when the mice were young adults to avoid any adverse effects that vitamin E might have caused during normal growth and development.

The results showed that mice that were given the supplemented diet did show an increase in survival. Males showed a 40% increase in median life span and a 17% increase in maximal life span. Females, on the other hand, showed a 14% increase in median life span and there was no effect for maximal life span. The lesser effect on the female life span is most likely due to the lower mitochondrial production of oxidants in females compared to that in males (Navarro et al., 2005).

Antioxidant Foods

As mentioned previously, the body is capable of neutralizing free radicals via antioxidants. Antioxidant compounds are found in the intracellular system and in extracellular fluids of the body. The intracellular antioxidants can be divided into those that are lipid soluble and membrane-located and those water-soluble compounds that are present in the fluid portion of the cytoplasm (Scalbert, Johnson, & Saltmarsh, 2005).

Vitamin E, A and beta-carotene are the major antioxidants that are found in membranes; they are considered lipophilic in nature. Vitamin E is considered the most important lipid-soluble antioxidant. This is because the quantity of vitamin E is the highest of all membrane antioxidants (Scalbert, Johnson, & Saltmarsh, 2005).

Most exogenous antioxidants are derived from fruits and vegetables. Vitamin E is abundantly found in palm oil and sunflower seeds. Beta-carotene and other carotenoids are found in carrots, parsley and other vegetables. The water-soluble antioxidant flavonoids and other polyphenols are found in green tea, red wine, and some fruits and vegetables. A good source of antioxidant vitamin C is citrus fruit (Scalbert, Johnson, & Saltmarsh, 2005).

ORAC Index

There are numerous methods used in research studies to calculate antioxidant capacity in foods. According to Wu et al. (2004), there are flaws in many of these research methods.

Oxygen radical absorbance capacity (ORAC) is a scalar method of measuring the antioxidant capacity in foods. According to Wu et al. (2004), in order to obtain an accurate ORAC value, the antioxidant capacity of both lipophilic and hydrophilic antioxidant components must be measured. In addition, the researchers measured the total phenolic content of certain foods to obtain the total antioxidant activity (TAC), since phenolic compounds account for the major portion of antioxidants in many plants.

The primary concern is that these methods do not take into account humanly or environmentally introduced radicals (from fertilizers, pesticides, etc.) that do not occur naturally in the biological system. Therefore, there are numerous contradictory reports on the rank order of antioxidant capacity in many foods. In addition, it is important to remember that metabolism and absorption of nutrients plays a crucial role when evaluating the physiological effects of antioxidants (Wu et al., 2004).

Wu et al. (2004), collected produce in two different seasons from twelve cities around the United States. Other foods were purchased from a local supermarket. Some of the foods were analyzed in the raw state, and others were cooked to compare the differences. Various food items had to undergo extraction and centrifugation in order to create samples for testing. The total antioxidant capacity was calculated by summing the totals of the lipophilic (L-ORAC) and hydrophilic (H-ORAC) values. In some instances, there was not a lipophilic value because 90% of the TAC was hydrophilic. In this situation, only the hydrophilic value was used towards the total amount (Wu et al., 2004).

The most surprising result from this particular study is that the researchers found in the dried spices that L-ORAC values were much higher than the H-ORAC values. This leads one to believe the essential oils in the spices contribute considerable quantities

of antioxidants. The major hydrophilic antioxidants are derived from phenolic and cinnamonic acid. The two spices with the highest TAC were ground cloves at 3,144.46 and ground cinnamon at 2,675.36. The spice with the lowest TAC was poppy seed with a value of 4.80. One downside with regard to dried spices is that it is difficult to measure the amount consumed since most spices are used very sparingly. Most of the foods in the bread and cereal group had a value around 20.00. Another interesting finding was that baking chocolate had a very high TAC value of 1,039.71, while milk chocolate had a much smaller value of 81.70. Overall, the ORAC values for the foods in the nut group were in the median range. The highest values in this food group were hazelnuts (96.45) and pecans (179.4). Pine nuts (7.19) were the lowest value, although, several points higher than the melons in the fruit group. Prunes had the highest value for the dried fruits at 85.78 (Wu et al., 2004).

When the fruits and vegetables were analyzed, it is important to mention that all of the beans were evaluated in the highly concentrated dry form. The foods in the fruit and vegetable group with the lowest TAC were cucumbers with a 1.15 value, honeydew melons at 2.41, watermelons with a 1.42 rating, lima beans at 2.43 and snap beans at 2.67. Wu et al. (2004), found that fresh fruits had a much higher H-ORAC than vegetables. The foods with the highest TAC were small red beans with a value of 149.21, red kidney beans at 144.13, pinto beans with a 123.59 rating, black beans at 80.40, cooked artichoke at 94.09, cranberry at 94.56 and lowbush blueberries at 92.60 (Wu et al., 2004).

Polyphenols

Blueberries contain many polyphenols, chemical substances found in plants to protect cells against oxidative stress. Polyphenol dietary intake could be as much as ten times higher than vitamin C and 100 times higher than vitamin E or carotenoids (Scalbert, Johnson & Saltmarsh, 2005). Wilson et al. (2006), studied the effects of blueberry polyphenols on lifespan and aging of Caenorhabditis elegans (C. elegans), a roundworm. This particular organism is easily studied in great detail, easy to maintain, has a short life span and has rapid generation time. Therefore, it is an ideal organism to be used for this type of experiment. Several different measures were recorded in this experiment. The most interesting finding is that the blueberry treatment group aged at a slower pace. Organisms that were given crude blueberry extract or a column fraction containing blueberry polyphenols had a mean increase in lifespan of 28% and the maximum life span was increased by 14%.

The speed of pharynx contractions can be a useful tool to indicate aging in the C. elegans. Pharynx contractions gradually decline with age. Young adult C. elegans will contract their pharynx approximately 250 to 300 times per minute. The C. elegans that were given blueberries, had more rapid contractions than the control group. In addition, cellular damage was also reduced in the blueberry treatment group. A 20% decrease level of lipofuscin showed this to be true. Lipofuscin is a substance that accumulates in aging cells (Wilson et al., 2006). These results support the hypothesis that blueberries can diminish age-related decline in organisms.

Blueberries and Aging

Anthocyanins

Anthocyanins, the natural pigments that cause certain foods to have red or blue color, belong to the secondary metabolite group of flavonoid polyphenols. Flavonoids such anthocyanin, catechins and flavones are best known for their strong antioxidant activity and their ability to eliminate harmful oxygen radicals. As previously mentioned, the ORAC scale has shown blueberries to have a high antioxidant capacity. In particular, blueberries contain a significant source of anthocyanins. The plant species, geographic location, harvesting procedures and cultivation conditions all play a role in the amount of anthocyanins found in berries (Nakajima, Tanaka, Seo, Yamazaki, & Saito, 2004; Sweeny, Kalt, Mackinnon, Ashby, & Gottschall-Pass, 2002; Bickford et al., 2000).

Lohachoompol, Srzednicki, and Craske (2004), conducted a study to determine the change in total anthocyanins in blueberries and their antioxidant effect after drying and freezing them. Blueberries are primarily sold either fresh or frozen. Freezing and drying are two possible alternative methods to preserve fruit. However, this process may also destroy anthocyanins or their antioxidant effect. For this experiment, frozen and fresh samples of blueberries were compared with two different dried samples of blueberries. The first dried sample was pretreated with 60% w/w sugar and 1% w/w NaCl solution for 4 hours and placed in a cabinet dryer for 90 minutes at 90°C. Next, the temperature was lowered to 70°C for 120 minutes. Subsequently, the temperature was lowered again to 50°C for another 120 minutes. The second dried sample of blueberries underwent the same drying process, without the sugar and salt treatment. All four of the

blueberry samples were separately blended in a food processor with a mixture of distilled water, acetic acid and methanol.

The total anthocyanin content of the fresh and frozen blueberries was much higher than that of the two dried samples. The loss of anthrocyanins in the pretreated sample was 49% and the loss in the untreated sample was 41%. It is believed the difference between the two dried samples is due to dewaxing, which is caused by stirring and soaking. Dewaxing is known to weaken the berry cuticle and cause the skin to rupture. This would explain the slight loss in anthocyanins in the pretreated sample.
The results show there is no significant difference between the fresh and frozen anthocyanin levels. However, anthocyanin levels of the fresh and frozen blueberry samples were significantly higher than either of the dried samples. Surprising, even though the anthocyanin levels were reduced, the antioxidant activity was not (Lohachoompol et al., 2004).

In a similar study, Nakajima et al. (2004), conducted an experiment to determine the radical scavenging activity of anthrocyanins between two types of blueberries and three types of other berries. Bilberry, a lowbush blueberry, and rabbiteye blueberry were compared to black currant, chokeberry and elderberry. Bilberry, black currant and rabbiteye blueberry were in the form of frozen fruit. Chokeberry and elderberry were in the form of fruit concentrates. Purified fractions from each berry containing anthocyanins were concentrated and freeze-dried to powder. Each powder was dissolved and diluted in ethanol at varying concentration levels. Anthocyanin content was estimated by measurements of ultraviolet absorbance at the appropriate wavelengths. All of the berries demonstrated radical scavenging activity on the ORAC assay. Bilberry

exhibited the highest, but black currant and chokeberry had almost identical results. However, black currant and chokeberry had only half of the anthocyanins that bilberry did. Since both anthocyanins and other phenolic compounds were extracted from the berries, it is difficult to conclude which caused the most scavenging activity. These findings support the idea that berries contain a variety of other phenolic compounds that act as antioxidants just as anthocyanins do.

Blueberries and Behavioral Correlates of Aging

As described earlier, research has shown that oxidative stress during aging causes damage to cells. Further, research has indicated that the effects of oxidative stress can be reduced by antioxidant compounds. Joseph et al. (1999), conducted a study to determine if antioxidant dietary supplements would be effective in reversing age-related deficits. The researchers evaluated neuronal and behavioral function in aged rats.

For this experiment, 40 male Fisher 344 rats were used. At the start of the experiment, the subjects were 19 months of age. Subjects were allowed to acclimate to the lab and put on a 5 day control diet. The rats were then randomly divided into four separate diet groups. The groups were maintained on a control diet, strawberry diet, spinach diet or blueberry diet. Rats were fed their corresponding diet for 8 weeks prior to any testing. The diets consisted of a standard base diet supplemented by 2% cornstarch or 2% freeze-dried aqueous extracts of strawberries, spinach or blueberries. Each of the four diets had equivalent amounts of calories and carbohydrates (Joseph et al., 1999).

After 2 months on their respective diets, the psychomotor behavior of the aged subjects was evaluated on five separate tests. Each test was administered once with at

least a 1 hour break in between each of the tasks involved. Rod walking was the first test. This consisted of having each subject balance on a stationary, horizontal rod. The second test was wire suspension. This test measures muscle strength and the prehensile reflex, which is an animal's ability to grasp a wire with its forepaw and remain suspended. To measure balance and coordination, the rats had to walk across varying sizes of horizontal planks. Muscle tone, strength, stamina and balance were measured by using an inclined screen. For this test, the rats were placed on a wire mesh screen that was inclined to 60°. The final test administered measured fine motor coordination, balance and resistance to fatigue. Rats were placed on a rotating rod that slowly accelerated. Subjects were measured on how long they were able to walk or remain standing on the rod (Joseph et al., 1999).

Cognitive functioning was assessed using the Morris water maze. For four consecutive days, in the morning and afternoon, the rats were tested on two separate trials. To test spatial learning and memory, a ten-minute intertrial interval between the two trial runs was implemented. There were four designated starting points where the rats were randomly submerged. The rats were allowed 2 minutes to locate the submerged platform. If the allotted time had passed, subjects were guided to the platform. Once at the platform, each subject stayed there for 15 seconds, and then was returned to its home cage. After the 10 minute interval, the subject was brought back to the water maze. For the second trial, each subject was placed at the same starting position as the first trial. Performance for both of the trial runs was videotaped and analyzed. Rats were measured on how long it took them to find the platform, the distance they swam while in the water and their swim speed at centimeters per second (Joseph et al., 1999).

The degree of oxidative stress was measured by the production of reactive oxygen species. To measure ROS, brain tissue was assessed using dichlofluorescin diacetate analysis. Striata were removed and synaptosomes, myelin and mitochondria were obtained for analysis. In addition to this, levels of the endogenous antioxidant, glutathione, were assessed. Vitamin E was also measured in the tissue by reverse-phase HPLC (Joseph et al., 1999).

The results for the psychomotor testing on rod walking and the accelerated rotarod were significant. The blueberry group had significantly longer latency to fall off than all three other groups on the rod-walking test. Also, the blueberry group had a significantly longer latency than the strawberry and spinach group on the rotarod task. Its latencies were also higher than the control group, but this did not reach statistical significance. There were no significant effects on the wire suspension task, inclined screen or plank walking between the diet groups. These results suggest that the blueberry diet improved some aspects of balance and coordination in aged rats (Joseph et al., 1999).

Morris water maze performance was better in the three antioxidant-supplemented groups than in the control group. In particular, the blueberry-supplemented group showed significantly better reference memory (improvement across days) than the controls. It also showed significantly better working memory (improvement based on learning a new platform position on a given day) compared with controls (Joseph et al., 1999).

There were no significant effects when the striatal glutathione levels were evaluated. However, the three dietary supplemented groups averaged higher than the control group. Finally, levels of vitamin E in the hippocampus were different between

the control group and the other three groups. All three diet groups did have higher levels of vitamin E, but not at a statistically significant level (Joseph et al., 1999).

The central nervous system is vulnerable to oxidative stress due to a lack of protection against free radicals. In addition, the aging body declines in its ability to protect against oxidative stress. Joseph et al. (1999), suggest that oxidative damage can be decreased when dietary supplements are used. Furthermore, these dietary supplements may also reverse some age-related neuronal and behavioral dysfunctions.

Blueberries and Object Recognition

A Recent UHCL study extended the Joseph et al. (1999), research to a different kind of memory task and a different neurochemical measure. Goyarzu et al. (2004), studied a four month blueberry-supplemented diet and its effects on object recognition memory and nuclear factor-kappa B (NF-κB) levels. Blueberries are one of the higher-ranking foods on the ORAC assay and Joseph et al. (1999) found that a diet supplemented with 2% blueberries produced significant benefits in aged rats. Therefore, Goyarzu et al. (2004) studied additional effects of a 2% blueberry-supplemented diet in aged rats, focusing specifically on object recognition memory.

The Object Recognition Memory Task

Recognition memory is the ability to identify that an item or stimulus has recently been encountered. It is often seen as having two distinct components. The first is recollection, which depends on the hippocampus. The second component, familiarity,

depends on the adjacent neocortex. There are two major advantages of assessing memory by using the object memory task. The task relies on animal's innate tendency to explore a novel, as opposed to familiar stimuli. Therefore, the subjects involved are never exposed to punishment. Secondly, subjects do not have to endure food or water deprivation in order to make them work. (Brown & Aggleton, 2001; Rugg & Yonelinas, 2003; Squire, Stark & Clark, 2004; Yonelinas, 1998).

The Goyarzu et al. (2004) study provided the basic model for the present research. In that study, the object recognition memory task was conducted as follows to measure each rat's memory ability. On the test day, each rat was allowed to rehabituate to the arena for 1 minute, returned to his home cage, and placed back in the arena. When the rat was placed back in the arena, also called the familiarization stage, there were two identical stimulus objects in symmetrical locations. The rat was positioned near the center of one wall, facing the objects on the opposite side of the arena. Subjects were allowed to explore either object for a total exploration time of 30 seconds or until 10 minutes had passed. Object exploration time was only recorded when the rats nose was within 1 centimeter of the object with the vibrissae (whiskers) moving. The delay stage was next. During this part of the experiment, the rats were removed from the arena for a predetermined amount of time. This delay can be varied to created different demands on memory. On the initial testing day, the delay was 1 hour. On a subsequent test 5 days later, the delay was for 30 seconds and different stimuli were used. (The short delay imposed hardly any demand on memory. Therefore, the task assessed whether poor performance might be attributed to sensory-motor instead of memory impairment.) The final portion of the object memory task was the retention test. The retention test was

given immediately following the delay stage. During this phase, two stimuli were placed in the arena. One of the stimuli was an identical copy of the object used during the familiarization process and the other was a novel object. The experimenters recorded how long the animal explored the familiar and the novel object. The object memory scores were calculated as the amount time spent exploring the novel object as a percentage of the rat's total exploration time (Goyarzu et al., 2004).

Nuclear Factor-Kappa B

Nuclear Factor-Kappa B (NF-κB) is a protein that induces altered gene expression. It is activated by a wide variety of conditions that induce oxidative stress (Li & Karin, 1999; Schreck et al., 1992). Therefore, brain levels of NF-κB can be used as an index of oxidative stress in the brain. Brain NF-κB levels are elevated in aged organisms (Paolisso et al., 1998; Toliver-Kinsky et al., 1997).

Effects of prolonged blueberry-supplemented diet in aged rats

In the study conducted by Goyarzu et al. (2004), the researchers determined how aged rats that were maintained on a blueberry-enriched diet would perform on the object recognition memory test, compared to aged rats on a control diet and young rats that were also on a control diet. In addition, they determined the effects on NF-κB in brain regions of rats who were give a blueberry-supplemented diet. They hypothesized that aged rats on the control diet would have impaired object memory in comparison with young rats on the same diet. They also hypothesized that rats maintained on the blueberry-supplemented diet would have better object memory than aged rats on the control diet. Also, the researches hypothesized that the aged rats on the control diet would have

increased levels of NF-κB levels in several area brain regions, as compared with the same regions in young rats. They further hypothesized that NF-κB levels would be less elevated in aged rats on the blueberry diet. Finally, they hypothesized that there would be a negative correlation between NF-κB levels and object memory performance, demonstrating the relevance of that biochemical measure to cognitive ability.

A total of 36 male Fisher-344 rats were used. There were 24 aged subjects who were randomly assigned to either the control group or the experimental group. The aged animals were 15 months old at the start of the diets, and were 19 months old at the time of testing. The remaining 12 subjects belonged to the young group. The young group was 4 months old at the start of the diet, and 8 months old at the time of testing.

Prior to testing, the 12 young rats and 12 of the aged rats were given a control diet. The base diet for the control group was highly nutritious NIH-31 rodent chow, supplemented with 2% dried corn. The other 12 aged rats were given the blueberry-enriched diet. This base diet was also NIH-31 rodent chow, but supplemented with 2% by weight freeze-dried blueberries instead of dried corn. The control and blueberry-enriched diets were isocaloric and equal in carbohydrates. All subjects were tested in an open top cube-shaped arena made of black Plexiglas. The stimulus objects used during testing were ceramic figurines that had been weighted to prevent the rats from moving the objects (Goyarzu et al., 2004).

Prior to testing, all of the rats were handled for 3 weeks and then allowed to habituate to the arena for 5 minutes a day for 5 consecutive days. On the test day, object recognition memory scores were determined with a 1 hour delay between object

familiarization and testing. Subsequently, object recognition was tested again, with only a 30 second delay to minimize the role of memory processes.

The outcome on the Goyarzu et al. (2004), study revealed some very interesting phenomena. With only a 30 second delay, there were no significant differences in object recognition between the groups, showing that the aged animals were not impaired as long as there was little demand on the memory. However, with a 1 hour delay, aged rats on the control diet had significantly lower object memory scores than young rats on the same diet. In fact, they performed no better than chance. The aged rats on the blueberry diet performed significantly better than the aged control group. They performed just as well as the young rats.

There were significant differences in NF-κB levels among the three groups. The aged rats on the control diet had significantly higher NF-κB levels than the young group in all areas of the brain except the striatum. These higher NF-κB levels were indicative of oxidative stress. Aged rats that were on the blueberry-enriched diet had significantly lower NF-κB levels than the aged control group in all areas of the brain region except the basal forebrain. As hypothesized, there were significant negative correlations between overall, cerebellar and hippocampal NF-κB levels and object memory, showing that biochemical oxidative stress status was relevant to cognitive ability.

Effects of Brief Blueberry Diets

A very recent study by Dr. Malin and his collaborates investigated whether decreasing the time that subjects were maintained on a blueberry-enriched diet would result in a reduced effect on memory impairment. This study employed 21 Fisher-344 rats that were initially 17 months old. The subjects were divided into three groups. The first group was fed a control diet, isocaloric with the blueberry diet. The second group was fed the control diet for 1 month and the 2% blueberry-enriched diet for the second month. The third group was fed a blueberry-enriched diet for two months. All of the rats were tested at the age of 19 months for object recognition memory, with a 1 hour delay between object familiarization and testing.

The rats that were on the 1 month and 2 month blueberry diets spent a significantly lower percentage of exploration time than the rats on control diet with the familiar object. The aged control diet rats showed no better than chance (50%) performance, as in the Goyarzu et al. (2004) study. However, both of the blueberry-enriched diet groups performed significantly better. The performance of the rats on the blueberry-enriched diets was almost identical to the previous study, where the rats were maintained on the diet for 4 months. Surprisingly, this experiment demonstrated that just 1 month of the blueberry diet was sufficient to prevent or reverse the object memory deficit in aged rats.

Purpose of the Present Study

The current study utilized the same rats in the study described above to determine the *aftereffects* of a blueberry-supplemented diet. Would the benefits of the antioxidant diet end as soon as the diet was terminated? Would they persist for 2 weeks? For 4 weeks? Would the effects of 1 month and 2 month antioxidant diets be equally persistent? To the author's knowledge, this is the first study regarding the persistence of benefits following an antioxidant diet.

In the present study, aged rats were fed a 2% blueberry-enriched diet for only 1 or 2 months, in hopes of defining a procedure that would result in approximately one half of the previous reversal of memory impairment. By using such a procedure, one would be able to detect either augmented or reduced benefits resulting from modifications of the antioxidant diet, such as supplementing it with a variety of antioxidant-containing fruits and vegetables, rather than blueberries alone. The specific goal of this thesis was to determine the aftereffects of the brief blueberry diets. This was done by testing for object recognition memory during the month following diet termination.

CHAPTER TWO: METHODS

Subjects and Treatment Groups

The subjects were 21 male Fisher 344 rats, 17 months of age at the beginning of the diets. Rats of this strain are commonly used in aging research because their size changes very little as they age. They had been handled for 5 minutes daily over the 2 previous weeks, to accustom them to human contact. For 2 months, Group A was fed a control diet supplemented with dried corn so as to be isocaloric with the 2% blueberry diet. Group B was fed the control diet for the first month and the blueberry-enriched diet for a second month. Group C was fed the blueberry-enriched diet for the 2 months. All rats were tested for object recognition memory at 19 months.

Diets

The base diet was NIH-31 rodent chow, as described in Youdim et al. (2000). This diet is specially formulated to be similar to a nutritionally balanced human diet, including ample amounts of vitamin E, a natural antioxidant. Thus, poor performance by the aged control group could not be attributed to malnutrition. To prepare the 2%

blueberry-supplemented diet, blueberries were homogenized in water, centrifuged, lyophilized and added to the NIH-31 rodent chow. The control diet was supplemented with 2% dried corn.

Memory Testing Procedure

Apparatus

The apparatus was a black Plexiglass arena (93 cm x 93 cm x 61 cm) with an open top. The stimulus objects the rats were allowed to explore were two identical copies of ceramic figurines one to two times the rats' size and weighted with pebbles so that the rats could not move them. On the test days, object recognition memory was evaluated by the following four-step procedure, modified from that of Clark, Zola and Squire (2000).

Handle and Habituation

All subjects were handled for 5 minutes and habituated to the arena for 5 minutes a day for 5 days.

Training and Familiarization Trial

Each rat was allowed to explore the empty arena for 1 minute, and then it was returned to its home cage. Immediately following the rehabituation phase, two identical stimulus objects were placed in symmetrical locations in the arena. The rat was allowed to explore until it reached a total of 30 seconds of object exploration or until 10 minutes had passed. Object recognition time was recorded only when the rat's nose was within 1

centimeter of the object, facing the object with the vibrissae (whiskers) moving. If a rat failed to spend at least 8 seconds in total exploration times, its data for that trial was excluded from analysis. Rats were removed from the arena for a preset amount of time (one hour) (Clark, Zola, & Squire, 2000).

Experimental Trial

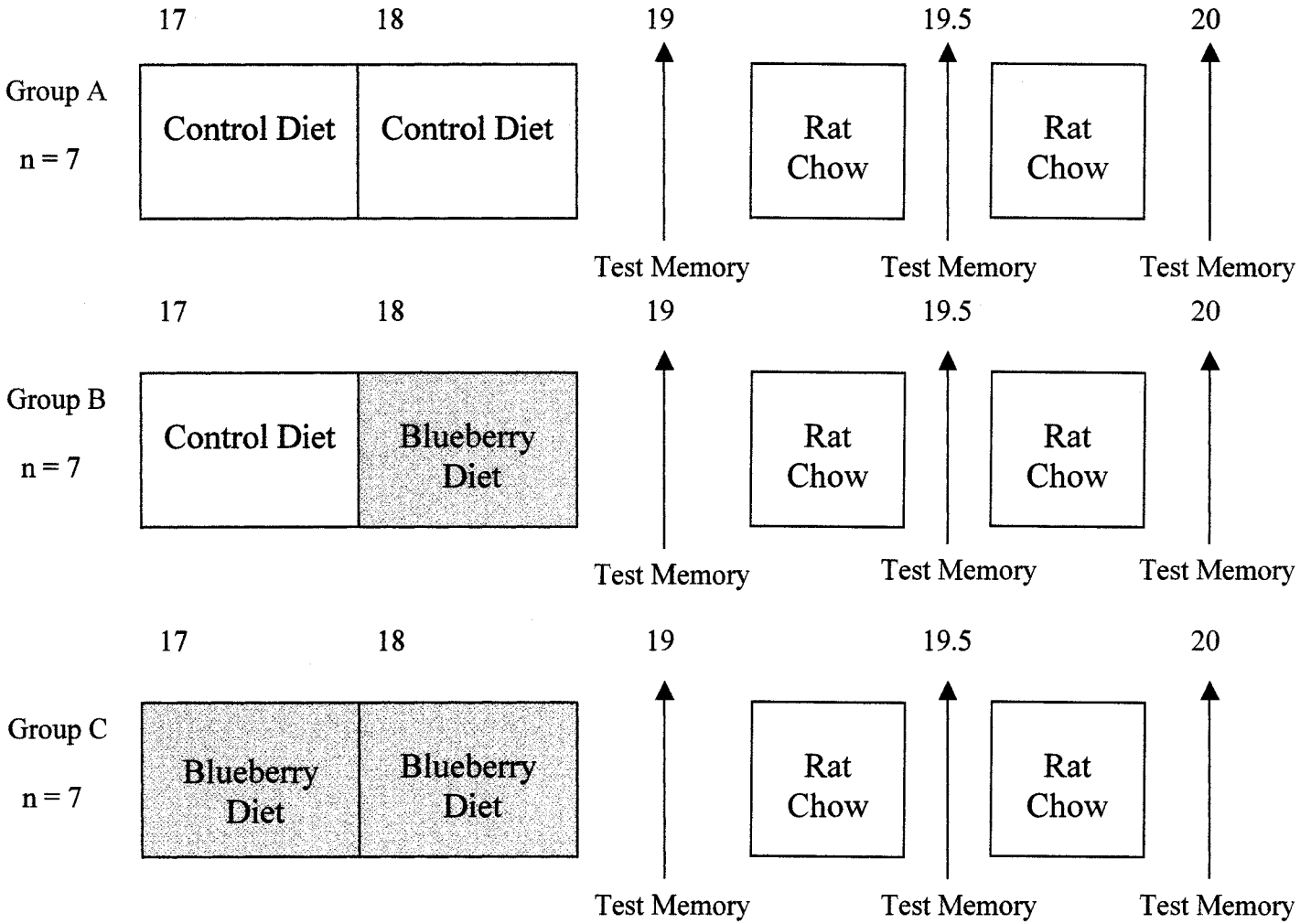
Immediately after the 1 hour delay, subjects were returned to the open field arena, which contained an identical copy of the previously explored object (the "familiar object") and a new object (the "novel object"). The rat was scored separately for time spent exploring each object. One dependent variable, designated as "object recognition memory score", was the time spent exploring the novel object as a percentage of the rat's total object exploration time (time spent with either object). A second dependent variable was the seconds spent exploring the novel object minus the seconds spent exploring the familiar object. This was designated as the "object difference score."

Testing for Aftereffects of the Diets

After the above experiment, all rats were placed on a normal balanced rodent diet (Harlan Teklad Rodent Chow). In order to determine the loss or retention of any benefits from the previous diets, rats were retested for object recognition memory 2 and 4 weeks later following the same method described above. Subjects were handled and re-habituated to the apparatus for 2 days prior to each retest. Each retest involved new sets

of familiar and novel objects, so that there would not be any carryover from previous trials. The overall research design is summarized in Figure 1.

Fig. 1 Experimental Design Age in Months



CHAPTER THREE: RESULTS

Figure 2 shows the percentage of total exploration time spent with the novel as opposed to the familiar object during the retention trial, 1 hour after the familiarization trial. At 2 and 4 weeks after diet termination, the control diet group increased its performance to slightly above 50%. The performance of the group that had been on the blueberry-enriched diet for 1 month declined progressively. Four weeks after diet termination, its performance was indistinguishable from the control group. In contrast, the performance of the 2 month blueberry diet group remained steady throughout. According to one-sample t-tests, the control group failed to perform significantly better than chance (50%) at the end of the diet $t(6) = -0.96$, NS, at 2 weeks later, $t(6) = 0.79$, NS, or at 4 weeks later, $t(6) = 1.18$, NS. The 1 month blueberry diet group performed significantly better than chance at the end of the diet, $t(5) = 2.65$, $p < 0.05$. However, its performance was not significantly better than chance 2 weeks later, $t(4) = 1.40$, NS, or 4 weeks later, $t(6) = 0.61$, NS. At the end of its diet, the 2 month blueberry diet group approached significantly better than chance performance, $t(5) = 1.76$, $0.05 < p < 0.10$. Its performance was significantly better than chance 2 weeks later, $t(6) = 2.33$, $p < 0.05$, as well as 4 weeks later, $t(4) = 2.60$, $p < 0.05$. It should be noted that the degrees of freedom may vary between trials, since animals that fail to explore the objects sufficiently during the familiarization trial (at least 8 seconds) have their retention trial scores disqualified for that trial.

One-sample t-tests were used to evaluate each treatment group's change in object memory scores (percentage time with novel object) from the end of the diet to 4 weeks later. The analyses excluded data from any subject that failed to explore adequately during the familiarization trials at either the end of the diet or 4 weeks later. The 1 month diet group had a significant reduction in its object memory scores over the 4 weeks, $t(5) = -3.05$, $p < 0.02$. There was no significant change in either the control diet group, $t(6) = 1.30$, NS, or the 2 month diet group, $t(4) = -0.46$, NS.

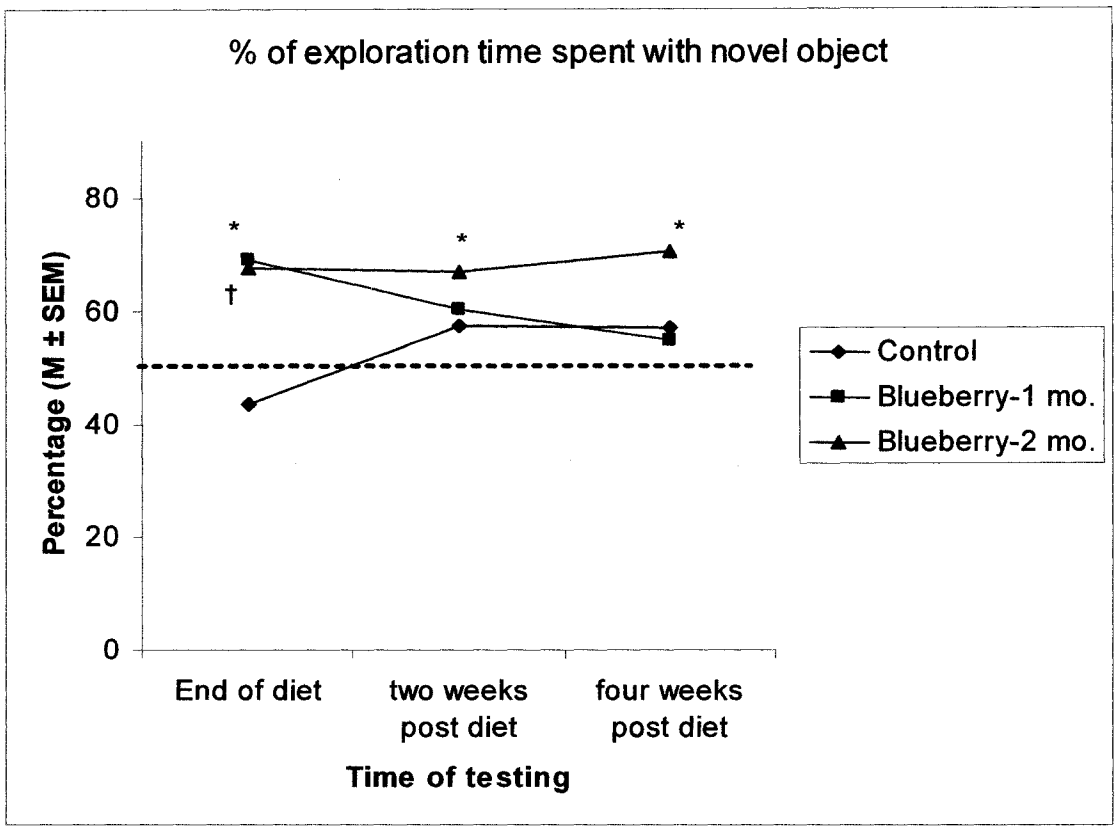


Figure 2. Mean object recognition memory scores (percentage of exploration time spent with the novel object) of rats at 2 and 4 weeks following termination of a 1 month blueberry diet (squares), a 2 month blueberry diet (triangle) or the control diet. Dashed line indicates chance performance.

* $p > 0.05$, †$0.05 < 0.10$ vs. chance performance.

Figure 3. shows memory retention in terms of time spent with the novel object minus time spent with the familiar object on each retention trial. Once again, the control diet group did not perform significantly better than chance at the end of the diet, $t(6) = -0.96$, NS, 2 weeks later, $t(6) = 0.70$, NS, or 4 weeks later, $t(6) = 0.96$, NS. The 1 month blueberry diet group performed significantly above chance at the end of its diet, $t(5) = 2.46$, $p < 0.05$. However, it failed to perform significantly better than chance 2 weeks later, $t(5) = 1.17$, NS, or 4 weeks later, $t(6) = 0.067$, NS. The 2 month blueberry diet group displayed a different temporal pattern. At the end of the diet, it failed to significantly outperform chance, $t(5) = 1.32$, NS. However, those rats performed significantly better than chance 2 weeks later, $t(5) = 2.16$ $p < 0.05$, and 4 weeks later, $t(4) = 2.54$, $p < 0.05$.

One-sample t-tests were also used to evaluate each treatment group's change in object difference scores (exploration time with the novel object minus time with the familiar object) from the end of the diet to 4 weeks later. The analyses excluded data from any subject that failed to explore adequately during the familiarization trials at either the end of the diet or 4 weeks later. The 1 month diet group had a significant reduction in its object difference scores over the 4 weeks, $t(5) = -2.78$, $p < 0.05$. There was no significant change in either the control diet group, $t(6) = 1.16$, NS, or the 2 month diet group, $t(4) = -0.06$, NS.

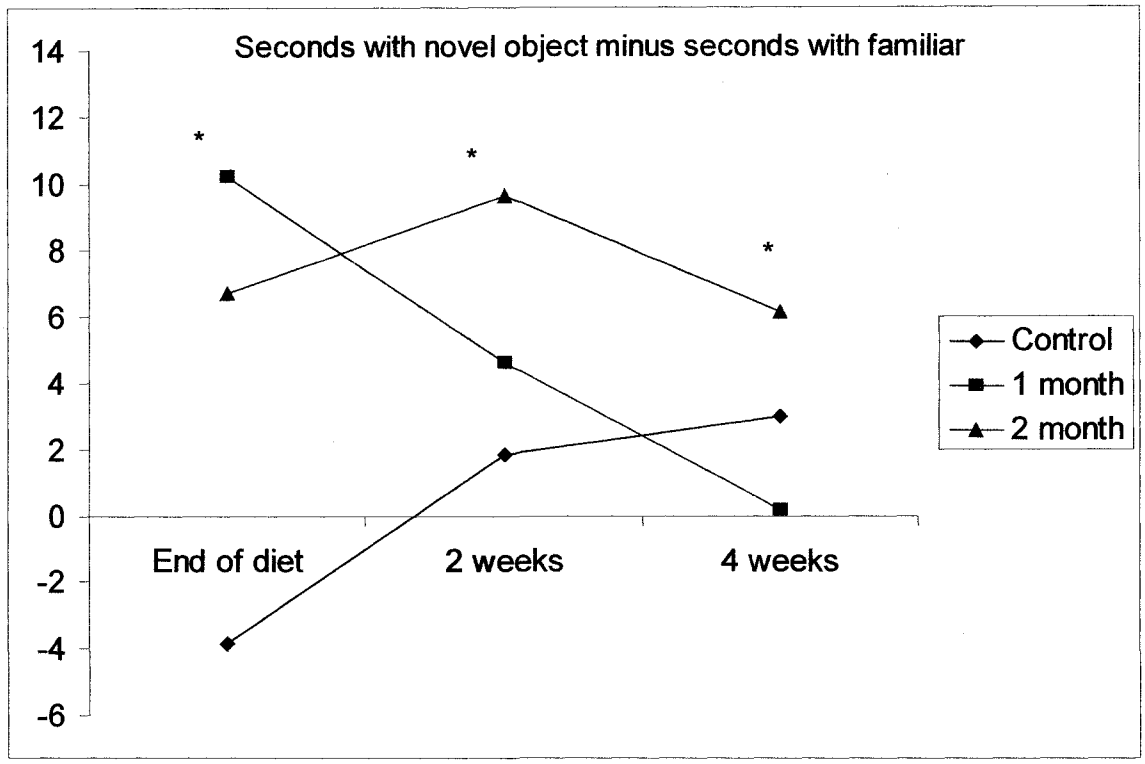


Fig. 3 Object memory performance as indicated by seconds spent with the novel object minus seconds spent with the familiar object during retention trials at the end of the three diets and 2 and 4 weeks later. The control group received the control diet for 2 months. The 1 month group received the control diet for 1 month, followed by the 2% blueberry diet for 1 month. The 2 month group received the 2% blueberry diet for 2 months. The animals were 19 months old at the end of the diet.

* $p < 0.05$ vs. chance performance.

CHAPTER FOUR: DISCUSSION

Evidence indicates that both 1 and 2 months of a blueberry-supplemented diet prevents memory impairment in rats at 19 months of age. The performance of the aged rats on the control diet in the current study is almost identical with that of the aged control rats in the earlier Goyarzu et al. (2004), study. In both cases, performance was no better than chance. Also, the performance of rats that had been maintained on 1 and 2 month blueberry diets was almost identical to that of rats maintained for 4 months on the blueberry diet in the earlier study. That performance was as high as that of young rats. Therefore, by the measure of object recognition memory, 1 month, 2 month and 4 month diets all totally reversed the memory impairment found in 19 month old rats. This illustrates the power of an antioxidant dietary intervention. In one way, though, this is a disappointment, since it would have been useful to identify a treatment that only partially reduced the difference between young and old rats. That would have allowed a comparison with other types of diets, without the problem of a "ceiling effect."

Unless the rats' memory impairment developed totally between 18 and 19 months of age, an improbably sudden decline, the 1 month blueberry diet must have reversed some already existing decline in object memory. This conclusion can be directly tested. Rats could be evaluated for memory function at 19 months of age, placed on blueberry enriched or control diets, and re-evaluated a month later. This experiment could then be continued with each group kept on its respective diet and tested for object memory every successive month. Would the object memory impairment at 19 months of age be at least

partially reversed? The answer to that question would shed further light on the benefits of a blueberry-enriched diet.

An interesting result in the current study is that enhanced object memory performance persisted virtually unchanged at 2 and 4 weeks after termination of the 2 month blueberry-enriched diet. In contrast, performance dropped steadily at 2 and 4 weeks after termination of the 1 month blueberry diet.

This raises the question, why the large difference in persistence after 1 or 2 month diets? One possible explanation is a "threshold hypothesis". Assume there is a threshold concentration of antioxidants needed to prevent memory impairment. Assume also that, after discontinuing an antioxidant diet, brain antioxidant concentration steadily decreases. Assume further that the 2 month diet produced a large surplus of antioxidant nutrients over the threshold, while the 1 month diet produced only a bare surplus above the threshold. Then the 1 month diet would soon lose its ability to prevent memory impairment, while this loss of effectiveness would take much longer for the 2 month diet.

Another explanation could be a "secondary antioxidant hypothesis": blueberry-supplemented diets can increase brain levels of the powerful endogenous (naturally occurring in the body) antioxidant glutathione (Joseph et al., 2000). It is thus reasonable to suppose that antioxidant anthocyanins in the blueberries produce a secondary effect by "re-charging" the brains endogenous antioxidants, notably glutathione. This would account for an "after-effect" of anthocyanins, even after they have been metabolized and eliminated from the body. Perhaps the longer diet was more effective at activating the longer-acting endogenous antioxidant compounds. This would be possible to test by

measuring the brain glutathione at the end of 1 month and 2 month blueberry enriched diets and at weekly time points thereafter.

Virtually all of the published work on the benefits of blueberries and aging has used rodent as subjects. The current study is encouraging in terms of potential human application. First of all, a relatively brief blueberry diet may be helpful. It might possibly even reverse some impairment that has already developed. Secondly, the benefits of several months of the diet may not be immediately lost if the diet is interrupted. The time may have arrived to begin clinical studies regarding the effects of blueberry-enriched diets on the memory decline in aged human beings. After all, it is a low-risk intervention. There is very little risk of serious side effects in consuming blueberries. Furthermore, it is something that could be evaluated right now, without waiting for extensive safety tests and Food and Drug Administration (FDA) approval. Also, there is very little concern that blueberries would interact with various prescription medications that an aged person might be taking. If in fact blueberries were found to be useful, it is something that older people can do for themselves right now, at a relatively modest cost compared to prescription medication, while waiting for some new wonder drug to prevent those "senior moments".

REFERENCES

Albert, M.S. (1994). Age-related changes in cognitive functions. In: M.L. Albert & J.E. Knoefel (Eds.). Clinical neuorology of aging. New York. Oxford University Press. 2nd Edition.

Andres, P., Van der Linden, M., & Parmentier, F. B. R. (2004). Directed forgetting in working memory: Age-related differences. *Memory, 12(2),* 248-256.

Bickford, P.C., Gould, T., Briederick, L., Chadman, K., Pollock, A., Young, D., Shukitt-Hale, B., & Joseph, J. (2000). Antioxidant rich diets improve cerebellar physiology and motor learning in aged rats. *Brain Research, 866,* 211-217.

Birren, J. E., & Schaie, K. W. (Eds). (2006). *Handbook of the psychology of aging* (6th ed.). San Francisco, CA: Elsevier.

Bjork, E. L., & Bjork, R. A. (Eds). (1996). *Memory* (2nd ed.). San Diego, CA: Academic Press.

Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus. *National Review Neurosci., 2,* 51-61.

Carmeli, E., Lavian, G., & Reznick, A. Z. (2000). The role of antioxidant nutrition in exercise and aging. In Z. Radak (Ed.), Free radicals in exercise and aging (pp. 73-115). Budapest, Hungary: Human Kinetics.

Clark, R. E., Zola, S.M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *Journal of Neuroscience, 20,* 8853-8860.

Cong, Y. S., Wright, W. E., & Shay, J. W. (2002). Human telomerase and its regulation. *Microbiology and Molecular Biology Reviews, 66,* 407-425.

Dellu, F., Mayo, W., Cherkaoui, J., Le Moal, M., & Simon H. (1992). A two-trial memory task with automated recording: Study in young and aged rats. *Brain Research, 588,* 132-139.

Digiovanna, A. G. (1994). *Human aging biological perspectives.* New York, NY: McGraw-Hill, Inc.

Ferraro, K. F. (1997). *Gerontology perspectives and issues* (2nd ed.). New York, NY: Springer

Green, R. L. (1992). *Human memory paradigms and paradoxes.* Hillsdale, NJ: Lawrence Erlbaum Associates.

Goyarzu, P., Malin, D. H., Lau, F. C., Taglialatela, G., Moon, W. D., Jennings, R., et al. (2004). Blueberry supplemented diet: Effects on object recognition memory and nuclear factor-kappa B levels in aged rats. *Nutritional Neuroscience, 7(2),* 75-83.

Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. *Journal of Gerontology, 11,* 68-94.

Ji, L. L., & Hollander, J. (2000). Antioxidant defense: Effects of aging and exercise. In Z. Radak (Ed.), Free radicals in exercise and aging (pp. 35-72). Budapest, Hungary: Human Kinetics.

Joseph, J.A., Denisova N.A., Bielinski, D., Fisher, D.R., & Shukitt-Hale, B. (2000). Oxidative stress protection and vulnerability in aging: Putative nutritional implications for intervention. *Mech. Ageing Dev. 116,* 141-153.

Joseph, J.A., Shukitt-Hale, B., Denisova, N.A., Bielinski, D., Martin, A., McEwen, J.J., & Bickford, P.C. (1999). Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with blueberry, spinach or strawberry dietary supplementation. *Journal of Neuroscience, 19,* 8114-8121.

Kregel, K. C., & Zhang, H. J. (2006). An integrated view of oxidative stress in aging: Basic mechanisms, functional effects, and pathological considerations. *Am J Physiol Regul Integr Comp , 292,* 18-36.

Lohachoompol, V., Srzednicki, G., & Craske, J. (2004). The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. *Journal of Biomedicine and Biotechnology, 5,* 248-252.

Li, N., & Karin, M. (1999). Is NFkB the sensor of oxidative stress? *The FASEB Journal, 13,* 137-143.

Liu, J., Head, E., Gharib, A. M., Yuan, W., Ingersoll, R. T., Hagen, T. M., et al. (2002). Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid. *PNAS , 99,* 2356-2361.

Matsuo, M., & Kaneko, T. (2000). The chemistry of reactive oxygen species and related free radicals. In Z. Radak (Ed.), Free radicals in exercise and aging (pp. 1-30). Budapest, Hungary: Human Kinetics.

Morrison, J. P., Coleman, M. C., Aunan, E. S., Walsh, S. A., Spitz, D. R., & Kregel, K. C. (2005). Aging reduces responsiveness to BSO and heat stress-induced perturbations of glutathione and antioxidant enzymes. *Am J Physiol Regul Integr Comp , 289,* 1035-1041.

Nakajima, J., Tanaka, I., Seo, S., Yamazaki, M., & Saito, K. (2004). LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. *Journal of Biomedicine and Biotechnology, 5,* 241-247.

Navarro, A., Gomez, C., Sanchez-Pino, M. J., Gonzalez, H., Bandez, M. J., Boveris, A. D., et al. (2005). Vitamin E at high doses improves survival, neurological performance, and brain mitochondrial function in aging male mice. *Am J Physiol Regul Integr Comp Physiol , 289,* 1392-1399.

Naveh-Benjamin, M., Guez, J., Craik, F. I. M., & Kreuger, S. (2005). Divided attention in younger and older adults: Effects of strategy and relatedness on memory performance and secondary task costs. *Journal of Experimental Psychology: Learning, Memory and Cognition, 31,* 520-537.

Paolisso, G., Tagliamonte, M. R., Rizzo, M. P., Manzella, D., Gambardella, A., & Varricchio, M. (1998). Oxidative stress and advancing age: Results in healthy centenarians. *Journal of American Geriatric Society, 46,* 833-838.

Rugg, M. D., & Yonelinas, A. P. (2003). Human recognition memory: A cognitive neuroscience perspective. *Trends Cogn. Sci., 7,* 313-319.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: Antioxidants and beyond. *The American Journal of Clinical Nutrition ,81,* 215-217.

Schneider, E. L., & Rowe, J. W. (Eds). (1996). *Handbook of the biology of aging* (4^{th} ed.). San Diego, CA: Academic Press.

Schreck, R., Albermann, K. J., & Bauerle, P. A. (1992). Nuclear factor kB: An oxidative stress response transcription factor of eukaryotic cells. *Free Radical Research Communications, 17,* 221-237.

Sprenger, M. (1999). *Learning and memory: The brain in action.* Alexander, VA: Association for Supervision and Curriculum Development.

Squire, L.R., Bloom, F. E., McConnell, S.K., Roberts, J.L., Spitzer, N.C., & Zigmond, M.L., (2003). Fundamental Neuroscience, (2nd ed.). New York: Academic Press

Squire, L. R., Stark, C. E., & Clark, R. E. (2004). The medial temporal lobe. *Annual Review Neurosci., 27,* 279-306.

Strehler, B. L. (1977). *Time, cells and aging* (3rd ed.). New York, NY: Academic Press.

Sweeny, M.I., Kalt, W., Mackinnon, S.L., Ashby, J. & Gottschall-Pass, K.T. (2002). Feeding rats diets enhanced in lowbush blueberries for six weeks decreases ischemia induced brain damage. *Nutritional Neurscience, 5,* 427-431.

Tileston, D. W. (2004). *What every teacher should know about learning, memory, and the brain.* Thousand Oaks, CA: Cornwin Press.

Toliver-Kinsky, T., Papaconstantinou, J., & Perez-Polo, J. R. (1997). Age-associated alterations in hippocampal and basal forebrain nuclear factor kappa B activity. *Journal of Neuroscience Research, 48,* 580-587.

Weinstein, B. S., Ciszek, D. (2002). The reserve-capacity hypothesis: Evolutionary origins and modern implications of the trade-off between tumor-suppression and tissue-repair. *Experimental Gerontology, 37,* 615-627.

Wilding, J., & Valetine, E. (1997). *Superior memory.* East Sussex, United Kingdom: Psychology Press.

Wilson, M. A., Shukitt-Hale, B., Kalt, W., Ingram, D. K., Joseph, J. A., & Wolkow, C. A. (2006). Blueberry polyphenols increase lifespan and thermotolerance in caenorhabditis elegans. *Aging Cell, 5,* 59-68.

Wolfe, P. (2001). *Brain matters: Translating research into classroom practice.* Alexandria, VA: Association for Supervision and Curriculum Development.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the united states. *Journal of Agricultural and Food Chemistry, 52,* 4026-4037.

Yonelinas, A. P., Kroll, N. E., Dobbins, I., Lazzara, M., & Knight, R. T. (1998). Recollection and familiarity deficits in amnesia: Convergence of remember-know, process dissociation, and receiver operating characteristic data. *Neuropsychology, 12,* 323-339.

Youdim, K.A., Shukit-Hale, B., Martin, A., Wang, H., Denisova, N., Bickford, P.C., & Joseph, J.A., (2000). Short-term dietary supplementation of blueberry polyphenolics: Beneficial effects of aging brain performance and peripheral tissue function. *National Neuroscience, 3,* 383-397.

Zacks, R. T., Hasher, L., & Radvansky, G. (1996). Studies of directed forgetting in older adults. *Journal of Experimental Psychology: Learning, Memory and Cognition, 22,* 143-156.