The Effects of a High Antioxidant Diet on Two Behavioral Measures in Aging Rats

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Abstract

Neurodegeneration refers to the progressive loss of function or structure or neurons, including the neuronal death. This pattern of cell loss is central to the processes of many neurological diseases, including Alzheier's, Parkinson's, and Huntington's. The greatest risk factor in neurodegeneration is aging. Over time, free radicals in an organism's central nervous system will begin to cause widespread damage to neurons, particularly in the hippocampus which plays an important role in memory consolidation and spatial recognition. It has been previously demonstrated that treatment with diets enriched by specific antioxidants may reduce neurodegenerative changes in aging animals. In this study, we examined the effects of a diet enriched with high antioxidant foods in aging female rats, using two behavior tests - Morris Water Maze, a test of spatial memory, and Novelty Suppressed Feeding, a measure of anxiety. Groups were tested at14 mos. (n=20) and 18 mos. (n=22) with half of each group eating standard lab feed and the other half eating lab feed supplemented with strawberries and/or spinach.

Background & Rationale

Oxidative stress:
- Free radicals, which may cause oxidative stress and cell death, have been shown to play a role in the neurodegeneration involved with aging
- Caused by the disparity between the production of reactive oxygen and the body's ability to detoxify the reactive intermediates and easily repair the resulting damage.
- Occurs as a result of aging
- Has been implicated in memory loss, anxiety, and depression [2]
- Neuroprotective measures may reverse these behavioral defects

Antioxidants:
- It has been previously demonstrated that treatment with diets enriched by antioxidants may reduce neurodegenerative changes in aging animals [1]
- A high antioxidant diet has been shown to reverse age-related declines in cognitive function [3]
- Memory loss, induced by chronic stress, has been shown to decrease as a result of a high antioxidant diet [4]

Methods

•Twenty female Long-Evans Hooded rats
•Maintained on 12 hour light/dark cycle
•Partially socially housed: two rats per cage, separated by a Plexiglas divider during feeding period (avg. feeding period = 12 hrs/day)
•Two groups: antioxidant diet and control diet
•Diet group given a normal diet supplemented with freeze-dried spinach (4.93 g/kg body weight) and strawberries (3.03 g/kg body weight)
•Rats handled for 4 days prior to testing

Behavioral tests:
- Morris Water Maze – measure of spatial hippocampal-dependent memory
  - •Started on the day diet was suspended
  - •Four days of training, 4 sessions each
  - •5 minutes minimum between sessions
  - •60 seconds max latency per session
  - •Probe trail on the 4th day, immediately following final trial
- Novelty Suppressed Feeding – measure of anxiety
  - •12 days after diet suspended
  - •Food deprived 24 hours
  - •15 minutes maximum per session
  - •Testing occurred between 1 and 6 pm
- Sucrose Preference Test – test of anhedonia ; omitted from results due to inconsistency in methodology

Data Analysis
- Water Maze data analyzed using SMART Video Tracking System by Harvard Apparatus
- Statistical analysis completed using IBM SPSS statistical software and Minitab 16 statistical software

References

[1] Bickford, P. C., Gould, T., Briederick, L., Chadman, K., Pollock, A., Young, D Joseph, J. (2000). Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. *Brain Research, 866*(1-2), 211-217
[2] Bouayed, J, Hassan Rammal, and Rachid Soulimani, "Oxidative Stress and Anxiety: Relationship and Cellular Pathways," Oxidative Medicine and Cellular Longevity, vol. 2, no. 2, pp. 63-67, 2009. doi:10.4161/oxim.2.2.7944
[3]James A. Joseph, Barbara Shukitt-Hale, Natalia A. Denisova, Donna Bielinski,Antonio Martin, John J. McEwen, and Paula C. Bickford
Reversals of Age-Related Declines in Neuronal Signal Transduction, Cognitive, and Motor Behavioral Deficits with Blueberry, Spinach, or Strawberry Dietary Supplementation*The Journal of Neuroscience, 15 September 1999, 19(18):8114-8121*
[4] Barbara Tagliari, Emilene B. Scherer, Fernanda R. Machado, Andre´a G. K. Ferreira, Carla Dalmaz, Angela T. S. Wyse. (2011). Antioxidants Prevent Memory Deficits Provoked by Chronic Variable Stress in Rats. Neurochem Res. 36:2373–2380

Results: Novelty Suppressed Feeding (NSF)

Latency:
- Question: How long did the animal hesitate prior to picking up the food and eating it?

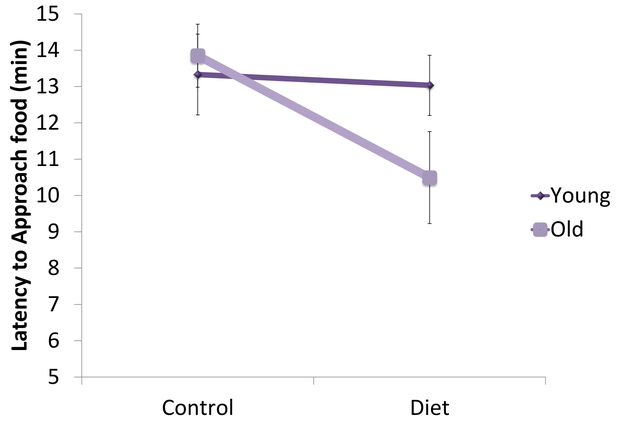

Figure 3. Latency to approach food pellet within the 15-minute NSF test for young (n=10) and old (n=11) rats in each group. Error bars = SEM. Significant correlations between age group and diet were determined by between-subjects ANOVA. No difference was seen between young and old control animals (p=.833); however, old diet animals have an average latency that is 3.36 min shorter than their control counterparts.

Approach Behavior:
- Question: How many animals from each condition approached food?

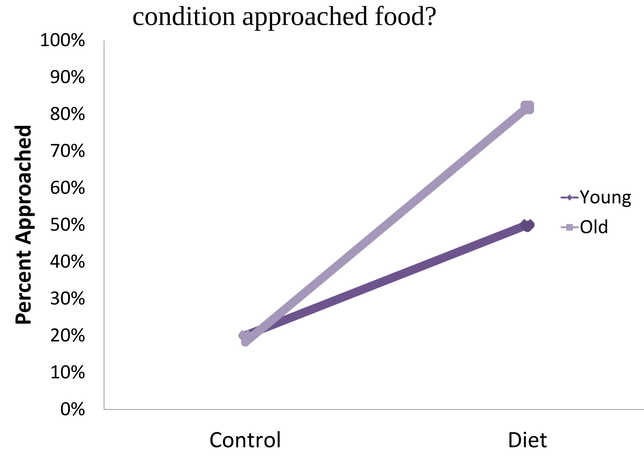

Figure 4. Percentage of old and young rats in each group that approached the pellet within the 15 minute test. Significant correlations between experimental variables were determined by Two-Way chi-square tests of independence. No difference in approach behavior was seen in the young animals between conditions (χ^2 = 3.600; df=3; p=.30); however, approach behavior in old rats was higher in the diet group (χ^2 = 8.909; df=3; p=0.03).

Results: Morris Water Maze

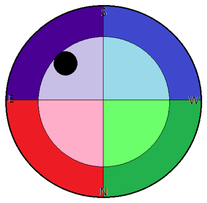

Figure 1. Schematic drawing of Morris Water Maze. Each color represents one quadrant – North West, South West, North East, and South East. Platform was placed in SE quadrant.

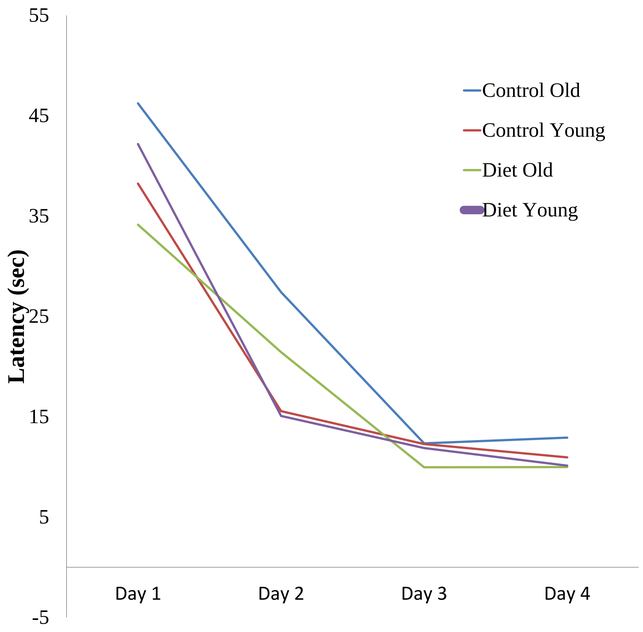

Figure 2. 4x4 ANOVA. ME of day, Trial, and Day*Trial were significant across all groups (p < .01); ME of condition by day was significant (p=.017). ME of Day*Trial*Condition and Trial*Condition , however, were not significant (p>.05). Specifically, old rats on the high antioxidant diet performed better than their control counterparts (p=.045)

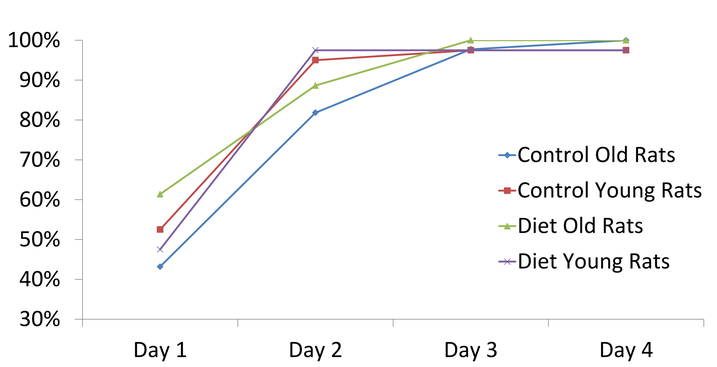

Figure 3. Average percentage of rats to reach platform in each group by day. At 18 mos. of age, a larger portion of high antioxidant diet rats recalled the water maze from day 1 to day 2 than their control counterparts.

Discussion

Novelty Suppressed Feeding
- In 18 moth old animals, a high antioxidant diet resulted in a decrease in anxiety as measured by latency to approach food in a novel situation
- A greater proportion of old rats on the high antioxidant diet approached food compared to their young counterparts
- Major implication: Antioxidants have a protective effect on anxiety only in old rats. This is most likely the case because oxidative stress does not begin before 15 mos. in rats.

Morris Water Maze
- All rats, regardless of condition or age, learned the Morris Water Maze in 4 days.
- After an 8 week regimen of high antioxidant diet, the 18 month old rats outperformed rats fed normal lab feed.
- These differences were not present in the young animals, implying that the high antioxidant diet did in fact protect the brain from hippocampal cell death and memory loss.

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