THE EFFECTS OF BERRY FRUITS ON COGNITION AND MOTOR FUNCTION IN AGING

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What Is Aging?

• Aging results from a complex set of processes that renders the body less able to activate protective mechanisms to counteract stressors that can damage the body leading to a progressive dysregulation with advancing age.

• Inflammation and oxidative stress increase as a function of age and the body becomes more sensitive to them. Aging is characterized by progressive losses in neuronal function accompanied by behavioral declines (decreases in motor and cognitive performance) in both humans and animals.
The Four Stages Of Life

1) You believe in Santa Claus.

2) You don't believe in Santa Claus.

3) You are Santa Claus.

4) You look like Santa Claus.
Aged rats demonstrate impaired motor performance on the rotarod

The rotarod evaluates balance and coordination

* = Mean is significantly different from 6 mo old rats ($p < 0.05$)

Typical age-related loss of spatial memory seen in old rats in Morris Water Maze paradigm

Reversal Test:
(1) represents original platform escape position on training days.
(2) represents reversed platform escape position on current test day.
Morris Water Maze

Rat Age Study

Latency to Platform (s)

Trial 1  Trial 2

Age (months)

6 12 15 18 22

0 17 34 51 68 85

*Statistically significant (P<.05).

Oxidative Stress And Inflammation In Brain Aging

• At rest the brain utilizes 20% of the body’s oxygen.
• Markers to both inflammation and oxidative stress increase as a function of age and the brain becomes more sensitive to them.
• When coupled with genetic changes the brain becomes more vulnerable to diseases such as Alzheimer disease.
Demographics & Brain Aging

By 2050, 30% of the US population will be over age 65

Many will exhibit impairments in motor and cognitive function.

This will be due to neurodegenerative diseases like AD & PD and normal brain aging.
• We must find strategies to improve behavior, possibly by changing the neuronal environment by altering oxidative stress and inflammatory components.

• Research in our lab and others has shown that the behavioral deficits seen in aging can be retarded or even reversed by the polyphenolics in berry fruits, possibly by increasing antioxidant and/or anti-inflammatory levels.
ORAC Units* (Micromole TE/grams)**

ORAC: Oxygen Radical Absorbance Capacity

*ORAC units include both fat- and water-soluble values.

**Micromole TE/gram means the number of micromoles of Trolox, a vitamin E equivalent, per 1 gram weight of fresh fruit.

Polyphenols

- Phenols

OH

Phenol

Neuroprotective Constituents Of Berries

- **Vitamins**
- **Polyphenolics**
  - Hydoxybenzoic acids
    - P-hydrobenzoic acid
    - Protocatechuic acid
    - Vanillic acid
    - Syringic acid
    - Gallic acid
  - Hydroxycinnamic acids
    - Coumaric acid
    - Caffeic acid
    - Ferrulic acid
    - Sinapic acid
    - Caffeoylquinic acid
- **Minerals**
- **Proanthocyanidins**
- **Flavonols**
- **Hydrolysable tannins**
  - Ellagitannins
  - Ellagic acid
- **Anthocyanins**
  - More than 45 identified water soluble anthocyanins
Berries and Health

**Neuroprotection**

- Anti-inflammatory
- Cardiovascular
- Anti-Cancer
- Structure / Absorption / Bioactivity
- Cultivar development

**Antioxidant**

- Anti-adhesion (Urinary tract health)
- Vision
- Glucose & energy metabolism
BEHAVIORAL STUDIES IN RODENT MODELS OF AGING
Procedures

• In all of our supplementation studies in aging, the rats were 19 months of age and supplemented for 8 weeks, typically at 2% of the diet.
• In the original study, an AIN 93 diet was used, and in all others an NIH-31 diet was used.
• In the purple grape juice and plum juice studies the animals drank the juice. For all others, the fruit, vegetable, or nut extract was added into the diet.
Summary Of Findings

• Nutritional interventions can forestall age-related deficits in learning and memory, but did not affect motor behavior.
• Nutritional interventions can reverse deficits in learning and memory and declines in motor behavioral performance.
• The beneficial effects of blueberries on behavioral performance were seen even when superimposed on an already well-fortified, healthy diet.
Rotarod

LATENCY TO FALL (sec)

- CONTROL
- BLUEBERRY DIET
- STRAWBERRY

Legend:
- a
- b
- a, b
MORRIS WATER MAZE

TRIAL 1 vs TRIAL 2, DAYS 3-4

LATENCY TO PLATFORM (sec)

CONTROL
BLUEBERRY
DIET
STRAWBERRY

* = different than Trial 1
Reversals of Brain Aging Markers after Phytochemical Supplementation

**Dietary intervention improved behavior**
- Morris water maze performance
- Motor behavioral performance (rod walking, accelerod)
- Motor behavioral learning
- Exploration of a novel environment

**Dietary intervention improved neuronal function**
- Striatal calcium sequestration
- Cerebellar noradrenergic sensitivity
- Muscarinic receptor sensitivity

Localization of anthocyanins in the cortex & hippocampus was correlated with rat test performance.
Summary Of Findings

- Nutritional interventions can reverse deficits in learning and memory and declines in motor behavioral performance.

- The beneficial effects of berries on behavioral performance were seen even when superimposed on an already well-fortified, healthy diet.
It’s not just fruits and vegetables that are good for your brain

**WALNUTS**

- Polyphenols
  - twice as much as almonds, peanuts, hazlenuts
  - syringic acid, juglone, ellagic acid, proanthocyanidins
- PUFAs, Melatonin, folate, gamma tocopherol

3 USDA National Nutrient Database, 2005
Walnut Studies

TRIAL 1 vs TRIAL 2, DAYS 3-4

LATENCY TO PLATFORM (sec)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Memory</th>
<th>Motor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberries</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Black currant</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blueberries</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Concord grape juice</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cranberries</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Plum Juice</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Raspberries</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Spinach</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Strawberries</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Walnuts</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Coffee</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Animal Evidence

• Rodents experience behavioral declines with age

• Supplementing rodents’ diet with berry fruit can improve their mobility and cognition

• Different berry fruit can produce different beneficial effects on cognition
Different Effects

• Radiation-based model of accelerated aging

<table>
<thead>
<tr>
<th>2mo Sprague-Dawley</th>
<th>2 months</th>
<th>Cranial Irradiation</th>
<th>1 month</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n=20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% Blueberry, n=20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% Strawberry, n=20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.5Gy $^{56}$Fe radiation

• Morris Water Maze
  – Strawberry improved probe test performance
  – Blueberry improved reversal test performance

Do the results seen in animal studies translate to humans?
Effects of age on balance, gait, and cognition

Goals:
- Assess sensitivity of apparatus
- Replicate previous findings
- Assess protocol
- Identify target age range

Study Design

1 hour
## Sample

<table>
<thead>
<tr>
<th>Age Group</th>
<th>21-40y</th>
<th>41-50y</th>
<th>51-60y</th>
<th>61-65y</th>
<th>66-70y</th>
<th>71-75y</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>54%</td>
<td>50%</td>
<td>54%</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>28.8±5.1</td>
<td>45.5±3</td>
<td>56.2±3</td>
<td>62.8±1.53</td>
<td>68.3±1.5</td>
<td>73.3±1.29</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.74±0.12</td>
<td>1.72±0.07</td>
<td>1.69±0.08</td>
<td>1.76±0.11</td>
<td>1.67±0.09</td>
<td>1.75±0.12</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>76.4±16.7</td>
<td>67.5±14.3</td>
<td>70.7±13.2</td>
<td>81.8±14.6</td>
<td>74.4±15.5</td>
<td>76±14.2</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.3±6.03</td>
<td>22.7±4.61</td>
<td>24.8±3.91</td>
<td>26.4±2.98</td>
<td>26.6±5.1</td>
<td>24.9±4.72</td>
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<tr>
<td><strong>Education (y)</strong></td>
<td>16.1±3.45</td>
<td>17.1±3.9</td>
<td>16.1±5.66</td>
<td>18±3.54</td>
<td>15±3.28</td>
<td>14.5±2.25</td>
</tr>
<tr>
<td><strong>Falls (#)</strong></td>
<td>15%</td>
<td>25%</td>
<td>38%</td>
<td>17%</td>
<td>8%</td>
<td>25%</td>
</tr>
</tbody>
</table>
Sway Velocity

COP Path Length (mm)

Eyes Open
Eyes Closed

Age Group
21-40  41-50  51-60  61-65  66-70  71-75

[*, † different from young adults, p < 0.05]
Preferred Walking Speed

95% Confidence Ellipse Area (mm²)

Age Group

Preferred Max
Preferred
Preferred Min

[* different from young adults, \( p < 0.05 \)]
Virtual Water Maze

$R^2 = 0.49$

Total Latency (s) vs. Age (y)

Panoramic View

Acquisition (Block 3)

26 yo

72 yo
Virtual Water Maze

**Acquisition Block**

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-40y</td>
<td>max</td>
</tr>
<tr>
<td>41-50y</td>
<td></td>
</tr>
<tr>
<td>51-60y</td>
<td></td>
</tr>
<tr>
<td>61-65y</td>
<td></td>
</tr>
<tr>
<td>66-70y</td>
<td></td>
</tr>
<tr>
<td>71-75y</td>
<td></td>
</tr>
</tbody>
</table>

* Denote difference from 21-40y, error bars denote SEM.

**Platform Crossings**

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Target Crossings (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-40y</td>
<td></td>
</tr>
<tr>
<td>41-50y</td>
<td></td>
</tr>
<tr>
<td>51-60y</td>
<td></td>
</tr>
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<td>61-65y</td>
<td></td>
</tr>
<tr>
<td>66-70y</td>
<td></td>
</tr>
<tr>
<td>71-75y</td>
<td></td>
</tr>
</tbody>
</table>

* Denote difference from 21-40y, error bars denote SEM.
Conclusions

• Cognition and mobility decline with age. These results replicate previous findings.

• These methods are sufficient to detect age-related declines.

• Age-related declines are measurable younger than expected.

• These tests parallel the changes we see in rodent studies, where interventions with dietary supplements improve motor function and cognition.
Will dietary blueberry improve mobility and cognition among older adults?
Blueberry Intervention Study

- **Design**: Double-blind, placebo-controlled trial

- **Population**: Healthy older adults, age 60-75 years

- **Intervention**: freeze dried Tifblue blueberry or placebo, powder ≈1c/day fresh blueberries, 3 mo, abstinence from berry fruit consumption
Study Visits

• Screening visit – Preadmission screening consent, health history questionnaire, physical examination, blood draw, urine collection, Mini-Mental Status Exam, vital signs.

• Visit 1 – Consent, food frequency questionnaire, mobility and technology use questionnaires, practice versions of computer-based cognitive tests, practice walking on treadmill, begin berry fruit abstinence.

• Visits 2-4 – Fasting and postprandial blood and urine collection, anthropometrics, vital signs, and tests of: balance, gait, muscle activation, attention, short- and long-term memory, spatial navigation, executive function.
# Outcome Measures

## Mobility
- **Balance**
  - Postural Sway
  - Falls Efficacy Scale
- **Gait**
  - Gait Speed
  - Gait Variability

## Cognition
- **Executive Function**
  - Attention Network Test
  - Trail-Making Test
  - Task-Switching Test
- **Learning & Memory**
  - Digit Span
  - Virtual Morris Water Maze
  - California Verbal Learning Test
## Participant Demographics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Blueberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Women</td>
<td>63%</td>
<td>72%</td>
</tr>
<tr>
<td>Age (y)</td>
<td>67.3 ± 4.8</td>
<td>67.8 ± 4.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0 ± 2.5</td>
<td>24.1 ± 3.7</td>
</tr>
<tr>
<td>Mini Mental Status Exam</td>
<td>29.2</td>
<td>29.3</td>
</tr>
<tr>
<td>Bachelor’s Degree</td>
<td>68%</td>
<td>61%</td>
</tr>
<tr>
<td>Physical Activity Score</td>
<td>34.6 ± 1</td>
<td>37.6 ± 1*</td>
</tr>
<tr>
<td>Computer Use (h/w)</td>
<td>19.6 ± 14.4</td>
<td>10.7 ± 12*</td>
</tr>
<tr>
<td>Compliance</td>
<td>99.2%</td>
<td>99.2%</td>
</tr>
</tbody>
</table>

* Denote $p < 0.05$
Task Switching Test

Even vs Odd

Switch

+3

+2

+1

Switch

+3

+2

+1
Mental Flexibility

![Graph showing reaction time and errors over time for Control and Blueberry groups.](image-url)
CA Verbal Learning Test

- Acquisition
- Distractor
- Recall
- Cued Recall
- 20 min delay
- Recall
- Cued Recall
- Recognition
- 10 min delay
- Forced-Choice Recognition

{ Short Delay }

{ Long Delay }
CVLT: Free Recall

Control

Blueberry

Correct (#)

Day 0

Day 90

Distractor List

Short

Long

20

18

16

14

12

10

8

6

4

2

0

0

2

4

6

8

10

12

14

16

18

20
Response Monitoring/Inhibition

Interaction: $p = 0.032$
Blueberry Study Conclusions

- Blueberry improved measures of executive function.
- Both measures have been associated with prefrontal cortex function.
- Prefrontal cortex particularly vulnerable to age-related degeneration.
Will dietary strawberry improve mobility and cognition among older adults?
Strawberry Intervention Study

- **Population**: Healthy older adults, age 60-75 years

- **Intervention**: freeze dried strawberry or placebo, powder ≈2c/day fresh strawberries, 3 mo, abstinence from berry fruit consumption

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**Recruitment, letters**: 1,442
**Assent, telephone**: 326
**Pre-screen**: 96
**Screening, informed consent**: 46
**Baseline visit**: 39
**Day 0**: 38
**Day 45**: 37
**Day 90**: 37

**Analysis**
## Participant Demographics

<table>
<thead>
<tr>
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<th>Strawberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Women</td>
<td>42.1%</td>
<td>38.9%</td>
</tr>
<tr>
<td>Age (y)</td>
<td>68.5 ± 4.3</td>
<td>66.7 ± 4.4</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.9 ± 2.6</td>
<td>23.8 ± 2.4</td>
</tr>
<tr>
<td>Mini Mental Status Exam</td>
<td>29.1 ± 1.5</td>
<td>29.2 ± 1.6</td>
</tr>
<tr>
<td>Bachelor’s Degree</td>
<td>89.5%</td>
<td>77.8%</td>
</tr>
<tr>
<td>Physical Activity Score</td>
<td>35.6 ± 5.1</td>
<td>36.6 ± 4.3</td>
</tr>
<tr>
<td>Computer Use (h/w)</td>
<td>15.2 ± 11.2</td>
<td>17.7 ± 10.9</td>
</tr>
<tr>
<td>Compliance</td>
<td>97.4%</td>
<td>99.6%</td>
</tr>
</tbody>
</table>

* Denote $p < 0.05
Outcome Measures

**Mobility**
- Balance
  - Postural Sway
  - Falls Efficacy Scale
- Gait
  - Gait Speed
  - Gait Variability

**Cognition**
- Executive Function
  - Attention Network Test
  - Trail-Making Test
  - Task-Switching Test
- Learning & Memory
  - Digit Span
  - Virtual Morris Water Maze
  - California Verbal Learning Test
Spatial Memory

Virtual Morris Water Maze Latency

Virtual Morris Water Maze Quadrant Time

* Denotes p < 0.05, error bars denote SEM.

Panoramic Screen Capture
WORD RECOGNITION
California Verbal Learning Test Hits

Interaction: $p = 0.014$
Strawberry Study Conclusions

- Strawberry improved measures of spatial and recognition memory.

- Both measures have been associated with the function of the hippocampal and adjacent brain regions.

- The hippocampus is also particularly vulnerable to age-related degeneration.
Serum Biomarkers

### TNF-alpha

- Placebo
- Blueberry

### IL-6

- Placebo
- Blueberry
Cell Studies

- HAPI microglia cells
- Berry extracts can reduce pro-inflammatory signals (e.g., cytokines and nitric oxide) in cells after exposure to LPS (an inflammatory agent).

Methods

- Can serum from humans supplemented with blueberries also reduce these pro-inflammatory signals?
HAPI Cells

**Nitrite**

- CONTROL
- BB

**iNOS**

- Control
- Blueberry

**TNF-α**

- Control
- Blueberry

**COX-2**

- Control
- Blueberry
Conclusions

• Functional declines in mobility and cognition are key features of aging in humans and rodents.

• Berry fruit can improve some aspects of cognition in healthy older adults.

• Effects on some measures of mobility and cognition may not have been detected due to the healthiness of the sample.

• Different berry fruit can improve different aspects of cognition.

• Dietary blueberry did not alter levels of circulating inflammatory biomarkers. However, serum from older adults that consumed blueberry reduced LPS-induced inflammatory-stress-mediated signals in stressed HAPI microglia \textit{in vitro}.
MECHANISMS
Possible Mechanisms in the Beneficial Effects of the Polyphenolics

- Functional antioxidant/anti-inflammatory effects
  - Decreased sensitivity to oxidative stress
  - Decreased sensitivity to neurotoxins and inflammatory agents
- Direct effects on the brain
  - Increased calcium clearance
  - Membrane effects
  - Alterations in signaling
    - Decreased oxidative stress/inflammatory signaling
    - Increased protective signaling
    - Signaling in learning and memory
  - Increased neurogenesis
  - Increased arborization
  - Increased autophagy (natural house-keeping)
Models of Aging that Produce Oxidative Stress and Inflammation

• Exposure to radiation particles of high energy and charge.

• High-fat diets.
Michelangelo's famous statue, David
Michelangelo's famous statue, David, returns to Italy this week after a successful 12 week, 20 city, tour of North America.
HIGH FAT DIET EFFECTS

• Consuming a high fat (HF) diet may result in behavioral deficits and brain alterations that are similar to those observed in aging animals.

• Diets supplemented with blueberries (BB) can allay behavioral deficits and reduce neurological correlates associated with aging.

• Supplementation of a HF diet with BB may offer protection against the high fat diet-related behavioral declines and brain alterations, possibly by decreasing inflammation associated with consuming a high-fat diet.

9 month old C57Bl/6 mice were fed one of four diets for five months (n=15/diet)

- low fat (10% calories from fat)
- low fat + 4% BB (freeze-dried, TIF-blue)
- high fat (lard-based, 60% calories from fat)
- high fat + 4% BB
Results

Blueberry Did Not Prevent Weight Gain over the 5 month period

Percent Increase in weight after 5 months on diet. (*p<0.05 from both low fat diets, Tukey’s HSD)
Novel Object Recognition Task

Phase 1
2 Die
10 min

Phase 2

Phase 3
Object B replaced with marble
10 min

% Recognition Index = \left( \frac{\text{Time attending to object B}}{\text{Time attending to object A} + B} \right) \times 100
Phase 3 performance after 2, 3, and 4 months on the diets

- * $p < 0.05$ different from 50%
- # $p < 0.05$ different from training phase
CONCLUSIONS

• BB supplementation does not affect weight.

• Mice fed HF diet showed impaired novel object recognition performance.

• Mice fed HF + BB diet initially showed impaired performance, but their performance improved over subsequent test sessions.

• Over time BB may be able to reverse some of the impact that eating a HF diet may have on certain types of learning & memory.
Making small changes to a poor diet, like incorporating more nutrient dense foods, may reduce cognitive dysfunction associated with consumption of a high fat diet.
Possible Mechanisms in the Beneficial Effects of the Polyphenolics

• Functional antioxidant/anti-inflammatory effects
  – Decreased sensitivity to oxidative stress
  – Decreased sensitivity to neurotoxins and inflammatory agents

• Direct effects on the brain
  – Increased calcium clearance
  – Membrane effects
  – Alterations in signaling
    • Decreased oxidative stress/inflammatory signaling
    • Increased protective signaling
    • Signaling in learning and memory
      – Increased neurogenesis
      – Increased arborization
      – Increased autophagy (natural house-keeping)
It is possible that the polyphenolics can have direct effects on the brain, i.e., not mediated through oxidative stress or inflammatory pathways, by directly increasing signaling and neurogenesis. Some support for this contention comes from studies demonstrating that flavonoids may protect neurons by activating protein kinase signaling cascades.
Microglial cells are the brain’s immune cells and become activated in response to inflammatory challenges. These cells are in the hippocampus of aged rats.
Activated Microglia in the CA3 Hippocampal Region

Immunohistochemistry for Ox-6 (brown) demonstrates the number of activated microglia in the CA3 region hippocampus of aged animals.
Walnuts Decrease the Number of Activated Microglia in the Aged Hippocampus

Quantification of Ox-6 positive microglia reveals that dietary walnut supplementation is associated with significantly fewer activated microglia in the dentate, CA3, and the hippocampus as a whole. (mean ± SEM; n=5 control, 7 for 6% walnut, and 6 for 9% walnut; *p<.05, **p<.01)
Kainic Acid Diet Study
Morris Water Maze-Day 3 Probe

The Effects of Kainic Acid on OX-6 Activation

a=KA different than matched diet Ringer’s group; b=different than control Ringer’s group; c=different than control KA group.
Inflammatory and Protective Signals in Hippocampus in Control, BB or PXM-Treated Animals Given KA or Vehicle

Means not sharing a common letter are significantly different from each other (p < 0.05; Fisher's LSD)
Means not sharing a common letter are significantly different from each other (p < 0.05; Fisher's LSD).

Therefore, it appears that the significant effects of polyphenolics on motor and cognitive behavior are due to a multiplicity of direct and indirect actions, the former involving effects on neuronal communication and the latter involving antioxidant and anti-inflammatory activity.
INDIVIDUAL DIFFERENCES
17-mos old male Fischer 344 rats (n=60)

Cognitive assessment: Radial arm maze

- Poor cognitive (n=20)
  - Blood draw: Tail
    - Control (n=10)
    - Blueberry (n=10)
    - 4 wks
      - Blood draw: Tail

- Average cognitive (n=20)
  - Blood draw: Tail
    - Control (n=10)
    - Blueberry (n=10)
    - 4 wks
      - Blood draw: Tail

- Good cognitive (n=20)
  - Blood draw: Tail
    - Control (n=10)
    - Blueberry (n=10)
    - 4 wks
      - Blood draw: Tail

4 wks

Cognitive assessment: Radial arm maze

Decapitation and sample collection
Radial Arm Water Maze
- 5 trials/day for 5 days
- Trials 1-4, consecutive acquisition trials
- Trial 5, 30-min delayed retention trial

ERRORS
- reference (long-term) memory (entering an arm that does not contain the platform)

- working (short-term) memory errors (reentries into an arm).
Latency in the RAWM
Trial 5 Average

LATENCY TO PLATFORM (SEC)

POOR  AVERAGE  GOOD  POOR  AVERAGE  GOOD

CONTROL  BLUEBERRY

# * *
Nitric Oxide (NO) production

- **CONTROL PRE-DIET**
- **CONTROL POST-DIET**
- **BLUEBERRY PRE-DIET**
- **BLUEBERRY POST-DIET**

**NITRITE CONCENTRATION (µM)**

- **POOR**
- **AVERAGE**
- **GOOD**

**CONTROL**

**BLUEBERRY**
TNF-alpha expression
$r = 0.765, p < 0.001$
Latency to platform (sec) against nitrite concentration (µM). The data points are color-coded for control (green) and blueberry (blue).

- CONTROL: $r = -0.229, p = 0.585$
- BLUEBERRY: $r = 0.886, p = 0.001$
CONCLUSIONS

- Daily consumption of blueberry improves learning and memory of poor cognitive performers, and preserves cognitive functioning of good performers.

- Circulating anti-inflammatory factors may predict cognitive function. Daily consumption of blueberry may have beneficial effects on these factors, depending on the baseline cognition.
Conclusions

• Eating berries, grapes and walnuts may help prevent or reverse age related declines in cognition and brain functioning.

• This may be due to the activity of polyphenols and other components such as omega fatty acids.

• These components may act to inhibit or reduce inflammation and oxidative stress and/or enhance protective mechanisms.

• Whole foods may be more effective in combating oxidative stress and inflammation.