WALNUTS AND BERRIES FOR THE BRAIN

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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.

• In the neuroscience world, 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**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Latency to Platform (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>34 ± 17</td>
</tr>
<tr>
<td>12</td>
<td>17 ± 34</td>
</tr>
<tr>
<td>15</td>
<td>68 ± 34</td>
</tr>
<tr>
<td>18</td>
<td>51 ± 34</td>
</tr>
<tr>
<td>22</td>
<td>68 ± 34</td>
</tr>
</tbody>
</table>

*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 and walnuts, possibly by increasing antioxidant and/or anti-inflammatory levels.
Neuroprotective Constituents Of Berries

**Vitamins**
- Polyphenolics
  - Flavonols
  - Proanthocyanidins
- Minerals
- Hydrolysable tannins
  - Ellagitannins
  - Ellagic acid
- Anthocyanins
  - More than 45 identified water soluble anthocyanins

**Polyphenolics**
- Hydroxybenzoic acids
  - P-hydrobenzoic acid
  - Protocatechuic acid
  - Vanillic acid
  - Syringic acid
  - Gallic acid
- Hydroxycinnamic acids
  - Coumaric acid
  - Caffeic acid
  - Ferrulic acid
  - Sinapic acid
  - Caffeoylquinic acid
100g of walnuts contain apx. 38g of LA and 9g of ALA (USDA National Nutrient Database)

Neuroprotective constituents

- Folate
- polyphenollics
- Melatonin
- Vitamin E
- linoleic acid (18:2n-6)
- α-linolenic acid (18:3n-3)

Polyphenols: twice as much as almonds, peanuts, hazlenuts, to include syringic acid, juglone, ellagic acid, and proanthocyanidins
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 berry fruit on behavioral performance were seen even when superimposed on an already well-fortified, healthy diet.
Rotarod

<table>
<thead>
<tr>
<th>DIET</th>
<th>LATENCY TO FALL (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
</tr>
<tr>
<td>BLUEBERRY</td>
<td></td>
</tr>
<tr>
<td>STRAWBERRY</td>
<td></td>
</tr>
</tbody>
</table>
**MORRIS WATER MAZE**

TRIAL 1 vs TRIAL 2, DAYS 3-4

![Bar graph showing latency to platform (sec) for different diets and trials.](image)

- **Control**
- **Blueberry Diet**
- **Strawberry Diet**

* = different than Trial 1
Walnut Studies

TRIAL 1 vs TRIAL 2, DAYS 3-4

<table>
<thead>
<tr>
<th>Neurological Benefit</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>
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)
Serum Walnut Metabolites – Possible Rescuing Effects on Neurons

A high-fat diet containing whole walnuts (*Juglans regia*) reduces tumour size and growth along with plasma insulin-like growth factor 1 in the transgenic adenocarcinoma of the mouse prostate model.

Paul A. Davis¹, Vihas T. Vasu², Kishorchandra Gohil², Hyunsook Kim¹, Imran H. Khan³, Carroll E. Cross² and Wallace Yokoyama⁴

<table>
<thead>
<tr>
<th>Table 5. Liver metabolomic results</th>
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<tr>
<td></td>
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<tr>
<td><strong>Biochemical</strong></td>
</tr>
<tr>
<td>Campesterol</td>
</tr>
<tr>
<td>Sphingosine</td>
</tr>
<tr>
<td>Sphinganine</td>
</tr>
<tr>
<td>Myristate (14:0)</td>
</tr>
<tr>
<td>1-Myristoylglycerophosphocholine</td>
</tr>
<tr>
<td>1-Oleoylglycerophosphocholine</td>
</tr>
<tr>
<td>1-Palmitoylglycerophosphocholine</td>
</tr>
<tr>
<td>1-Palmitoylglycerophosphoethanolamine</td>
</tr>
<tr>
<td>1-Palmitoylglycerophosphoinositol</td>
</tr>
<tr>
<td>2-Oleoylglycerophosphocholine</td>
</tr>
<tr>
<td>2-Palmitoylglycerophosphocholine</td>
</tr>
<tr>
<td>2-Palmitoylglycerophosphoethanolamine</td>
</tr>
<tr>
<td>2-Hydroxystearate</td>
</tr>
<tr>
<td>α- or γ-Linolenate (18:3n-3 or 6)</td>
</tr>
<tr>
<td><strong>n-6 Fatty acid-related</strong></td>
</tr>
<tr>
<td>Arachidonate (20:4n-6)</td>
</tr>
<tr>
<td>2-Arachidonoylglycerophosphocholine</td>
</tr>
<tr>
<td>Linoleate (18:2n-6)</td>
</tr>
<tr>
<td>1-Linoleoylglycerophosphoethanolamine</td>
</tr>
<tr>
<td>2-Linoleoylglycerophosphoethanolamine</td>
</tr>
<tr>
<td>13-HODE + 9-HODE</td>
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<tr>
<td><strong>n-3 Fatty acid-related</strong></td>
</tr>
<tr>
<td>Docosapentaenoate (n-3 DPA; 22:5n-3)</td>
</tr>
<tr>
<td>2-Docosahexaenoylglycerophosphocholine</td>
</tr>
<tr>
<td>2-Docosahexaenoylglycerophosphoethanolamine</td>
</tr>
<tr>
<td>EPA (20:5n-3)</td>
</tr>
</tbody>
</table>

HODE, hydroxy-octadecadienoic acid.

*Statistically significant lipid related metabolite changes (*P* < 0.05) are presented as the ratio of the whole walnut diet or the high fat diet abundance at 9 and 18 weeks.

Collected the blood serum-Walnut-fed rats

Treated on primary hippocampal neurons for the possible rescuing effects

Analyzed for the antioxidant enzyme

No enough samples for metabolomic analysis

Possible future analysis
WALNUT SERUM STUDIES

NITRITE

![Bar graph showing uM Nitrite levels for different conditions.](image-url)

- Control
- LPS

- uM Nitrite

- 0%
- 6%
- 9%

- a
- ab
WALNUT SERUM STUDIES

iNOS

Relative Band Intensity (Thousands)

Control  |  LPS
---|---
Control  |  ab
0%       |  ab
6%       |  ab
9%       |  ab
WALNUT SERUM STUDIES

COX-2

Relative Band Intensity (Thousands)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0%</th>
<th>6%</th>
<th>9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WALNUT</td>
<td>Serum</td>
<td>b</td>
<td>ab</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td>Studies</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
WALNUT SERUM STUDIES

TNF

Control

LPS

pg/mg

0% 6% 9%

0 500 1000 1500 2000 2500

Control 0% 6% 9%

ab ab a

WALNUT SERUM STUDIES
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.
Walnut Effects of Microglia in the Aged Rats
Microglial Cells

Microglial cells are the brain’s immune cells and become activated in response inflammatory challenges. These cells are in the hippocampus of aged rats.

Slightly activated

Very activated
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

Latency to Cross the Platform

Control
Blueberry
Piroxicam

Ringer’s
Kainic acid

Control
Blueberry
Piroxicam

Latency to Cross the Platform

Control
Blueberry
Piroxicam

Ringer’s
Kainic acid

Diet

a=KA different than matched diet Ringer’s group; b=different than control Ringer’s group;
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

Therefore, polyphenolics can have direct effects on the brain, i.e., not mediated through oxidative stress or inflammatory pathways, by directly increasing signaling and neuronal communication. Additionally, berry fruits and walnuts may protect neurons by activating protein kinase signaling cascades.
In addition to neutralizing free radicals, berries may prevent the deleterious effects of inflammation and oxidative stress by blocking the stress signals whose generation results in downstream production of cytokines.
Will these effects 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>n</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>54%</td>
<td>50%</td>
<td>54%</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Age (y)</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>Height (m)</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>Weight (kg)</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>BMI (kg/m²)</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>
</tr>
<tr>
<td>Education (y)</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>Falls (#)</td>
<td>15%</td>
<td>25%</td>
<td>38%</td>
<td>17%</td>
<td>8%</td>
<td>25%</td>
</tr>
</tbody>
</table>
Balance

• Postural sway
  – constant displacement and correction of the center of gravity within the base of support

• Postural sway increases with age.

• Postural sway predicts falls among older adults.
  – Pajala, et al. (2008)
  – Maki, Holliday & Topper (1994)

Sway Velocity

Overstall, et al. (1977)
Sway Velocity

[*, Ü different from young adults, p < 0.05]
Gait

• Gait speed decreases with age.
  – Prince, Corriveau, Hébert & Winter (1997)

• Gait variability increases with age.

• Gait speed and variability predict falls among older adults.
  – Brach, et al. (2005)
  – Verghese, et al. (2009)

• Gait speed positively associated with life-expectancy.
  – Studenski, et al. (2011)
Preferred Walking Speed

95% Confidence Ellipse Area (mm²)

Age Group

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

[* different from young adults, \( p < 0.05 \) ]
Spatial Navigation

• Spatial orientation declines with age.

Panoramic View
Acquisition
(vMWM)

\[ y = 9.1974x + 93.784 \]

Age (y)

Total Latency (s)

26 yo
72 yo

20
25
30
35
40
45
50
55
60
65
70
75

0
200
400
600
800
1000
1200

Acquisition
(Block 3)
vMWM Latency

Acquisition Trials

* different from those 40 and younger, $p < 0.05$
Ü different from those 50 and younger, $p < 0.05$
á different from those 65 and younger, $p < 0.05$
Quadrant Preference

![Quadrant Preference Graph](image)

**Probe Trial**

- Time in Target Quadrant (%)

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

- **Probe Test**: 26 yo, 72 yo

[* different from young adults, p < 0.05]
Target Crossings

Probe Trial

Target Crossings (#)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>21-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-65</th>
<th>66-70</th>
<th>71-75</th>
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<tbody>
<tr>
<td></td>
<td><img src="image" alt="Bar chart" /></td>
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<td></td>
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</tr>
</tbody>
</table>

[* different from young adults, $p < 0.05$]
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.
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.
Neuroscience Lab

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A. Martin, MD, Ph.D.
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I. Cantuti-Castelvetri, Ph.D.
R. Galli Ph.D.
A. Neuman, K. Luskin
L. Simon, H. Stellwagen
A. Szprengiel, V. Cheng
Walnut diet reduces accumulation of polyubiquitinated proteins and inflammation in the brain of aged rats

Poulouse et.al 2012
Walnut effects on Autophagy: Normal Aging – 22 M old Rats

P62-hippocampus

<table>
<thead>
<tr>
<th>Walnut diet (%)</th>
<th>Control</th>
<th>6% WN</th>
<th>9% WN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Density (10000’s)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: $p<0.05$ vs C
b: $p<0.05$ vs R
MAP1B-LC3; Formation and Turnover of Autophagosomes in Hippocampus
Reduced OS/Inflammation-Reduced accumulation of Damaged Proteins? Phosphorylation of MAPK and NfKB

**Mean Density (1000’s)**

- **p-P38-MAPK**
  - 0: 420, 6% WN: 320, 9% WN: 220

- **p-NfKB**
  - 0: 400, 6% WN: 300, 9% WN: 200

*Significance:
- *a; p<0.05 vs Control*
- *b; p<0.05 vs 6%*