Delaying the Degenerative Diseases of Aging

\[
\begin{align*}
O_2 & \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow \cdot OH \rightarrow H_2O \\
\text{e}^- & \text{e}^- & \text{e}^- & \text{e}^- & \text{e}^- \\
\end{align*}
\]

Bruce N. Ames
Children’s Hospital Oakland Research Institute
Professor, University of California, Berkeley

5 August ‘07
DDP Oakland
Estimated oxidative DNA adducts per rat liver cell

Young (4-mo)  24,000
Old (26-mo)   67,000
carbonyl content (nmol/mg protein)

Review

Oxidative damage and mitochondrial decay in aging
(bioenergetics / mitochondrial DNA / cardiolipin / acetyl-L-carnitine / neurodegeneration)

Mark K. Shigenaga, Tory M. Hagen, and Bruce N. Ames*
Division of Biochemistry and Molecular Biology,
401 Barker Hall, University of California, Berkeley, CA 94720

Contributed by Bruce N. Ames, July 27, 1994
Mitochondria from old rats compared to those from young rats:

1) Lower Cardiolipin

2) Lower Membrane Potential

3) Lower Oxygen Utilization

4) Increased Oxidant Leakage
Cardiolipin Levels in 3 and 24 Month Old Rat Hepatocytes

Cardiolipin (µg per 10^6 Cells)

Young

Old

* * **
R123 Fluorescence in old and young rat hepatocytes

![Graph showing R123 Fluorescence in old and young rat hepatocytes](image-url)
L-Carnitine/Acetyl-L-Carnitine (ALCAR)

- Transports long-chain fatty acids into mitochondria
- Removes short- and medium-chain fatty acids that accumulate
- Mediates the ratio of acetyl-CoA/CoA
- Decreases with age in plasma and in brain
- Improves cognitive function in rats
Effect of ALCAR Supplementation on Cardiolipin Levels

Cardiolipin (µg per 10 cells)

Young

Old

+ ALCAR

**
R123 Fluorescence in Young and Old Rat Hepatocytes
R-\(\alpha\)-Lipoic Acid (LA) in mitochondria

- LA reduced to dihydrolipoic acid, a potent antioxidant, & chelator of Fe & Cu
- Coenzyme of pyruvate and \(\alpha\)-ketoglutarate dehydrogenases
- Involved with carbohydrate utilization for ATP production
Lipoic Acid Lowers Mitochondrial Oxidants in Old Rats

Fl. Units/O₂ Consumed per Minute

Young

Old

+ LA

+ LA

**
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Young (MDA pmol/mg protein)</th>
<th>Old (MDA pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ LA</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>+ ALCAR</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>+ LA + ALCAR</td>
<td>40</td>
<td>90</td>
</tr>
</tbody>
</table>

*p < 0.001 vs. young rat group

*** p < 0.001 vs. young rat group
Ambulatory Activity before and After Supplementation with Lipoic Acid (LA) + Acetyl-L-Carnitine (ALCAR)

Distance Traveled (cm/hour/day)

* vs. young
# vs. old
Age-associated decrease in immune function and the effect of ALCAR (0.2%) + LA (0.1%) treatment for 2 months. Values are mean + SEM of 10-11 animals.

![Graph showing T cell stimulation index]

- Young
- Young Treated
- Old
- Old Treated

**P <0.001**

**P <0.01**
Spatial Memory relies on intact hippocampal function.

Treatments improved poor memory in old rats.
Spatial Memory Tested With Morris Water Maze

- Young
- Old
  - + ALCAR
  - + LA
  - + ALCAR + LA

Time in Seconds

P < 0.001

P < 0.05
Peak procedure: for measuring temporal memory. Associated with striatum, cerebellum, & hippocampus

**PEAK RATE:** measures learning and motivation.

**PEAK TIME:** measures internal clock, food is rewarded only when animals push lever 40s after sound or light signal.
Oxidative Damage to Nucleic Acid in Old Rats by mAb to oxo8G/oxo8dG: Immunohistochemical stain of neurons.
Staining of oxidized nucleic acid in neurons (mAb to oxo8dG in DNA/oxo8G in RNA)

RNA is Oxidized

(92% is removed by RNase)

*oxo8G: 8-hydroxyguanosine;  oxo8dG: 8-hydroxy-2’-deoxyguanosine
Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid

Jung H. Suh, Swapna V. Shenvi, Brian M. Dixon, Honglei Liu, Anil K. Jaiswal, Rui-Ming Liu, and Tory M. Hagen

Modulation of Gene Expression by Cancer Chemopreventive Dithiolethiones through the Keap1-Nrf2 Pathway

IDENTIFICATION OF NOVEL GENE CLUSTERS FOR CELL SURVIVAL

Mi-Kyoung Kwak, Nobunao Wakabayashi, Ken Itoh, Hozumi Motohashi, Masayuki Yamamoto, and Thomas W. Kensler

The Journal of Biological Chemistry

Proc. Natl. Acad. Sci. USA
Vol. 101, pp. 3381-3386, March 9, 2004
Modulation of Nrf-2-dependent gene expression by D3T in mouse liver.

“More quarters! For God’s sake, more quarters!”
“You’re fifty-seven years old. I’d like to get that down a bit.”
Meta-analysis of acetyl-L-carnitine versus placebo for mild cognitive impairment and mild Alzheimer’s disease

Treatment with alpha-lipoic acid significantly improves both neuropathic symptoms and deficits in diabetic patients with symptomatic diabetic neuropathy.


ITT analysis of 4 phase II-III RCTs plus meta-analysis: 600 mg i.v. per day for 3 weeks

Total Symptom Score (TSS): *relative improvement at 3 weeks vs baseline*

**Percent**

<table>
<thead>
<tr>
<th></th>
<th>ALADIN I</th>
<th>ALADIN III</th>
<th>SYDNEY</th>
<th>NATHAN II</th>
<th>Meta-Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>77</td>
<td>338</td>
<td>60</td>
<td>241</td>
<td>716</td>
</tr>
<tr>
<td>α-lipoic acid</td>
<td>79</td>
<td>60</td>
<td>72</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>placebo</td>
<td>66</td>
<td>60</td>
<td>25</td>
<td>48</td>
<td>55</td>
</tr>
</tbody>
</table>

* p<0.05 vs Placebo
Life Expectancy of Men and Women at Birth

SOURCE: National Institute on Aging
Accelerating the Degenerative Diseases of Aging
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Population Group</th>
<th>% Ingesting &lt; EAR * From Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minerals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>Women 14 - 50 years</td>
<td>16 %</td>
</tr>
<tr>
<td>Magnesium</td>
<td>All</td>
<td>56 %</td>
</tr>
<tr>
<td>Zinc</td>
<td>All</td>
<td>12 %</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>Women &gt; 70 years</td>
<td>49 %</td>
</tr>
<tr>
<td>Folate</td>
<td>Adult Women</td>
<td>16 %</td>
</tr>
<tr>
<td>E</td>
<td>All</td>
<td>93 %</td>
</tr>
<tr>
<td>C</td>
<td>All</td>
<td>31 %</td>
</tr>
</tbody>
</table>

* USDA What we Eat in America (NHANES 2001-2002) Sept. 2005
Serine

SHMT

MTHFR (polymorphism)

CH$_2$=THF

TS

dUMP

dTMP

CH$_3$-THF

Methionine

MS

Homocysteine

B$_6$

B$_{12}$
Base excision repair processing of opposed lesions

Gap three or more nucleotides away from base lesion

DNA double strand break formed by processing the second lesion
Micronuclei in: RNA positive erythrocytes
RNA negative erythrocytes

TIME (DAYS)
1 year preRx

Micronuclei per 1000 cells

Folinic Acid
Folic Acid

Normal range
In a series of studies, we have been able to confirm that the micronucleus index in cytokinesis-blocked lymphocytes is significantly negatively correlated with plasma vitamin B12 (B12) concentration and significantly positively correlated with plasma homocysteine (HC). Furthermore we have shown in a randomized double-blind placebo-controlled dietary intervention study that intake of 3.5 times the RDI of folic acid and B12 significantly reduces the micronucleus index only in those with above average levels of micronucleus frequency. Micronucleus frequency is minimized when plasma HC is below 7.5 µmol/l and plasma B12 is above 300 pmol/l. Therefore, it is important to take account of the effect of B12 and HC when using the micronucleus assay for human biomonitoring studies.
Each of the six dependent variables (that were analyzed by nonlinear regression in former figures) were transformed to Z scores and modeled as a quadratic function of the ln-liver nonheme iron as the independent variable. The equation for the RCR ratio's Z score was obtained from inverted RCR values (1/RCR) so that normal rats had the lower instead of the higher values. For presentation purposes each model line was obtained from 9 values of liver iron. All statistics were performed as in materials and methods.
ADJUSTED ODDS RATIOS FOR INADEQUATE PREGNANCY OUTCOME AMONG ANEMIC PREGNANT WOMEN.
(Source: Scholl et al., AJCN 1992)

* Significant

- LBW
- PRETERM
- SGA
- POOR WT GAIN

Odds Ratio:
- ANEMIC
- Fe DEF
- OTHER ANEM

* Significant
An overview of evidence for a causal relationship between iron deficiency during development and cognitive or behavioral function in children

Joyce C McCann and Bruce N Ames (2007) AJCN in press
Is docosahexaenoic acid, an n3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals

Joyce C McCann and Bruce N Ames
An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring

Joyce C McCann, Mark Hudes, and Bruce N Ames
Zinc Deficiency Induces Increased Oxidative Stress in C6 Glioma Cells
Zinc Deficiency Induces Fapy Glycosylase (Fpg)-sensitive Single Strand Breaks in Human Lung Fibroblasts
Synthesis of Heme

Cytosol

Porphyrrins

PBG

2ALA

Heme-a

ALA

Heme

Succ-CoA + Gly

PLP

ALA

PPGIX

PPIX

Fe^{II}

Mitochondria

FC
Biotin deficiency accelerates cell senescence
Micronutrient deficiency and heme synthesis in human cell culture

<table>
<thead>
<tr>
<th>Micronutrient Deficiency</th>
<th>Heme Deficit</th>
<th>Complex IV Deficit</th>
<th>Oxidative Stress</th>
<th>DNA Damage</th>
<th>Early Senescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxine</td>
<td>[+]</td>
<td></td>
<td></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td>+</td>
<td>#</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td>+</td>
<td>[+</td>
<td>[+]</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td>[+</td>
<td>[+</td>
<td>[+]</td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipoic Acid</td>
<td></td>
<td></td>
<td>[+]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenate</td>
<td></td>
<td>[+]</td>
<td>[+]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = Atamna/Ames, ++Askree /Ames, #Ho/Ames [+] Literature
Magnesium Deficiency Shortens Fibroblast Lifespan
Magnesium Deficiency Induces DNA-Protein Crosslinks

![Graph showing the relationship between culture conditions (Mg content) and DNA-protein crosslinks as a percentage of total DNA.](image)
Calcium Deficiency
   Fenech: chromosome breaks
   Lipkin: colon cancer mice

Folate Deficiency
   MacGregor/Ames/Fenech: chromosome breaks mice/humans
   humans
   Willett: epi colon cancer humans

Vitamin D Deficiency
   Garland: epi colorectal cancer humans

Magnesium Deficiency
   Bell: chromosome breaks humans damage
   Larsson: epi colorectal cancer humans

Zinc Deficiency
   Fong: esophageal cancer humans/rodents humans

Vitamin B12
   Fenech: Chromosome breaks

Selenium
   Rao: DNA damage
   Combs/Trumbo: Cancer

Omega-3 FA
   Denkins: Cancer

Niacin
   Kirkland/Depeint: DNA

Choline
   da Costa: DNA damage in
Inadequate dietary intakes of vitamins and minerals are widespread, most likely due to excessive consumption of energy-rich, micronutrient-poor, refined food. Inadequate intakes may result in chronic metabolic disruption, including mitochondrial decay. Deficiencies in many micronutrients cause DNA damage, such as chromosome breaks, in cultured human cells or in vivo. Some of these deficiencies also cause mitochondrial decay with oxidant leakage and cellular aging, and are associated with late onset diseases such as cancer. I propose DNA damage and late onset disease are consequences of a triage allocation response to micronutrient scarcity. Episodic shortages of micronutrients were common during evolution. Natural selection favors short-term survival at the expense of long-term health. I hypothesize that short-term survival was achieved by allocating scarce micronutrients by triage, in part through an adjustment of the binding affinity of each protein for its required micronutrient. If this hypothesis is correct, micronutrient deficiencies that trigger the triage response would accelerate cancer, aging, and neural decay but would leave critical metabolic functions, such as ATP production, intact. Evidence that micronutrient malnutrition increases late onset diseases, such as cancer, is discussed. A multivitamin-mineral supplement is one low-cost way to ensure intake of the Recommended Dietary Allowance of micronutrients throughout life.
<table>
<thead>
<tr>
<th>Food</th>
<th>Cumulative Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Regular soft drinks</td>
<td>7.1</td>
</tr>
<tr>
<td>2. Cake, sweet rolls, doughnuts, pastries</td>
<td>10.6</td>
</tr>
<tr>
<td>3. Hamburgers, cheeseburgers, meatloaf</td>
<td>13.8</td>
</tr>
<tr>
<td>4. Pizza</td>
<td>16.8</td>
</tr>
<tr>
<td>5. Potato chips, corn chips, popcorn</td>
<td>19.7</td>
</tr>
<tr>
<td>6. Rice</td>
<td>22.4</td>
</tr>
<tr>
<td>7. Rolls, buns, English muffins, bagels</td>
<td>25.0</td>
</tr>
<tr>
<td>8. Cheese or cheese spread</td>
<td>27.6</td>
</tr>
<tr>
<td>9. Beer</td>
<td>30.2</td>
</tr>
<tr>
<td>10. French fries, fried potatoes</td>
<td>32.4</td>
</tr>
</tbody>
</table>

Gladys Block from National Health and Nutrition Examination Survey (NHANES) 2000.
CAUTION: HAZARDOUS WAIST

Visceral fat increases your risk of heart disease, cancer, diabetes, etc.

Start a waist disposal program today.
“The main distinguishing characteristic between man and the lower animals is the desire to take pills”

Mark Twain
If you want fiber, Madame, I suggest you eat the menu.
Life Expectancy of Men and Women at Birth

SOURCE: National Institute on Aging
END