Delaying (or Accelerating) the Degenerative Diseases of Aging

\[ \text{O}_2 \xrightarrow{e^-} \text{O}_2^- \xrightarrow{e^-} \text{H}_2\text{O}_2 \xrightarrow{e^-} \text{OH} \xrightarrow{e^-} \text{H}_2\text{O} \]
Base Excision Repair:
Specific DNA Glycosylase removes base.

Nucleotide Excision Repair:
Exonuclease removes stretch of DNA

Base is Damaged

DAMAGED DEOXYNUCLEOSIDE

TO URINE

DAMAGED BASE

Base Excision Repair:
Specific DNA Glycosylase removes base.
Estimated oxidative DNA adducts per rat liver cell

Old (26-mo) 67,000
Young (4-mo) 24,000
Oxidative damage and mitochondrial decay in aging

(bioenergetics / mitochondrial DNA / cardiolipin / acetyl-L-carnitine / neurodegeneration)

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401 Barker Hall, University of California, Berkeley, CA 94720

Contributed by Bruce N. Ames, July 27, 1994
Mitochondria in hippocampal neurons

Electron Microscopy Images
Cardiolipin Levels in 3 and 24 Month Old Rat Hepatocytes

Cardiolipin ($\mu$g per $10^6$ Cells)

- **Young**: High levels
- **Old**: Lower levels

Significance: ** at the 0.01 level
R123 Fluorescence in old and young rat hepatocytes

Fluorescence/cell

Normal cell number

0.03

0.02

0.01

0.00

10

100

1000

Fluorescence/cell

Old

Young
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
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 vs. Old

** + ALCAR
R123 Fluorescence in Young and Old Rat Hepatocytes
R-α-Lipoic Acid (LA) in mitochondria

- LA reduced to dihydrolipoic acid, a potent antioxidant, & chelator of Fe & Cu
- Coenzyme of pyruvate and α-ketoglutarate dehydrogenases
- Involved with carbohydrate utilization for ATP production
- Improves cognitive function in aged mice
Lipoic Acid Lowers Mitochondrial Oxidants in Old Rats

Fl. Units/O₂ Consumed per Minute

Young

Old

+ LA

**

- LA

**
MDA levels in young and old rats with LA, ALCAR, or both

** Young

- + LA
- + ALCAR
- + ALCAR + LA

** Old

- + LA
- + ALCAR
- + ALCAR + LA

*** p<0.001 vs. young rat group

P<0.01

P<0.05

20
Ambulatory Activity before and After Supplementation with Lipoic Acid (LA) + Acetyl-L-Carnitine (ALCAR)

Distance Traveled (cm/hour/day)

Young vs. young
Old vs. old

* 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.
Spatial Memory relies on intact hippocampal function.

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

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

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
Proc. Natl. Acad. Sci. USA
Vol. 101, pp. 3381-3386, March 9, 2004

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

The Journal of Biological Chemistry

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
Induction of Phase 2 Enzymes

Modulation of Nrf-2-dependent gene expression by D3T in mouse liver.

**Figure A**

- Graph showing enzyme activity vs. [ALCAR, mM] for different age groups (Young, Old, Old + ALCAR, Old + LA, Old + ALCAR + LA).
- Dual Y-axes for 1/v vs. 1/[ALCAR, mM].

**Figure B**

- Graph showing enzyme activity vs. [CoA, mM] for different age groups.
- Dual Y-axes for 1/v vs. 1/[CoA, mM].

**Bar Graph**

- Comparison of Vmax between young and old groups.
- Comparison of Km for ALCAR and CoA between young and old groups.

**Legend**

- * = vs. young
- # = vs. old

**Units**

- nmol/min/g
- μM
Age-related loss of undamaged mitochondria in hippocampal neurons

Electron Microscopy Images
“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.

Source: Professor Daniel Ziegler of the Diabetes Research Institute, Düsseldorf, Germany:
Meta-Analysis Provides Highest Level of Evidence, Diabetes Monitor (2002, p6)

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

- **α-lipoic acid**
- **placebo**

* p<0.05 vs Placebo

Source: Professor Daniel Ziegler of the Diabetes Research Institute, Düsseldorf, Germany:
Meta-Analysis Provides Highest Level of Evidence, Diabetes Monitor (2002, p6)
Micronutrient Undernutrition in Americans

<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 → CH₂=THF → MTHFR (polymorphism) → CH₃-THF → B₁₂ → MS → Homocysteine → Methionine

- Serine
- B₆
- SHMT
- CH₂=THF
- MTHFR (polymorphism)
- CH₃-THF
- B₁₂
- MS
- Homocysteine
- Methionine
- dUMP
- TS
- dTMP
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

Folinic Acid

Folic Acid

Micronuclei per 1000 cells

20 50 100 150 200 250 300 350

0 10 30 80 130

Normal range

1 year preRx 50 100 150 200 250 300 350
Folate, Vitamin B12, Homocysteine Status and Chromosome Damage Rate in Lymphocytes of Older Men

Folate, Vitamin B12, Homocysteine Status and DNA Damage in Young Australian Adults

Micronucleus Frequency in Human Lymphocytes is Related to Plasma Vitamin B12 and Homocysteine

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)
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
(2006) Submitted
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

DCF Fluorescence Intensity (RFU)

Control  ZnAD  ZnDF
Zinc Deficiency Induces Fapy Glycosylase (Fpg)-sensitive Single Strand Breaks in Human Lung Fibroblasts

![Comet Score Graph]

- **Control (+Fpg)**
- **ZnAD (+Fpg)**
- **ZnDF (+Fpg)**

* *
Synthesis of Heme

Cytosol

Porphyrrins

PBG

2ALA

Heme-a

ALA

Heme-a

PPIX

PPGIX

Porphyrins

Succ-CoA + Gly

ALA

PLP

Mitochondria

FeII

FC

Heme
Maturation of heme-$b$ to heme-$a$ is rate limiting for the assembly of complex IV

(1) Farnesylation

Heme-$b$

(2) Oxidation

Heme-$a$

(Only in complex IV)
Biotin deficiency accelerates cell senescence
<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, [+ ] Literature
Magnesium Deficiency Shortens Fibroblast Lifespan
Magnesium Deficiency Induces Senescence Marker
Magnesium Deficiency Induces DNA-Protein Crosslinks
Calcium Deficiency
  Fenech: chromosome breaks
  Lipkin: colon cancer mice

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

Vitamin D Deficiency
  Garland: epi colorectal cancer humans

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

Zinc Deficiency
  Fong: esophageal cancer humans/rodents

Vitamin B12
  Fenech: Chromosome breaks

Selenium
  Rao: DNA damage
  Combs/Trumbo: Cancer humans

Omega-3 FA
  Denkins: Cancer

Niacin
  Kirkland/Depeint: DNA damage

Choline
  da Costa: DNA damage in humans
Many micronutrient deficiencies are found to cause DNA damage in mice or human cells in culture and, where assayed, earlier senescence.

_Thomson hypothesis that:_ 1) episodic shortage of each micronutrient throughout evolution caused natural selection to favor short-term survival of the organism at the expense of long-term health; 2) this was achieved by allocating scarce micronutrients by enzyme triage through an adjustment of the binding affinity of each enzyme for its required micronutrient.

The consequences of the triage are evident at all levels. For example, in metabolic reactions, ATP synthesis would be favored over DNA-protecting enzymes; in cells, erythrocytes over leukocytes; and in organs, the heart over the liver.

If this hypothesis is validated, ensuring micronutrient adequacy in humans throughout life is essential for maximizing longevity and minimizing the degenerative diseases of aging.
## Energy Sources - 1999-2000

<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, 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
END