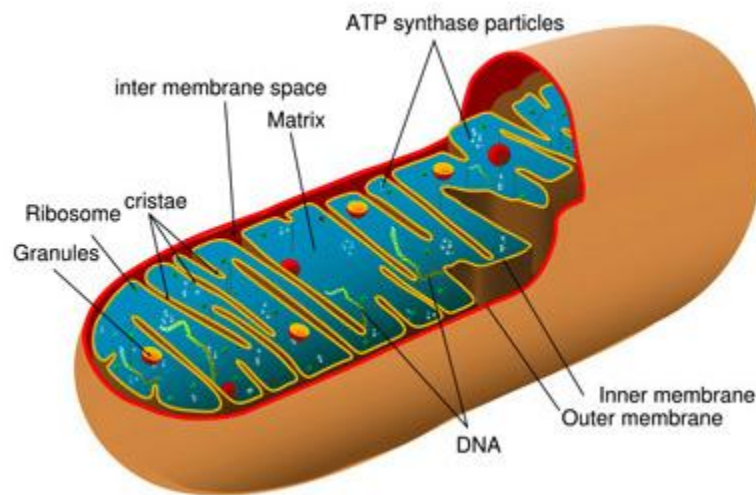


Anti-oxidants

Anti-oxidants protect your brain against reactive oxygen species (ROS), also known as free radicals.

Why does your brain produce reactive oxygen species?

The mitochondria in your cells combine oxygen with sugar to produce energy in the form of ATP. Sometimes, electrons get lost; they combine with other molecules to create reactive oxygen species. If you think of the mitochondria as a factory, then reactive oxygen species are toxic waste.



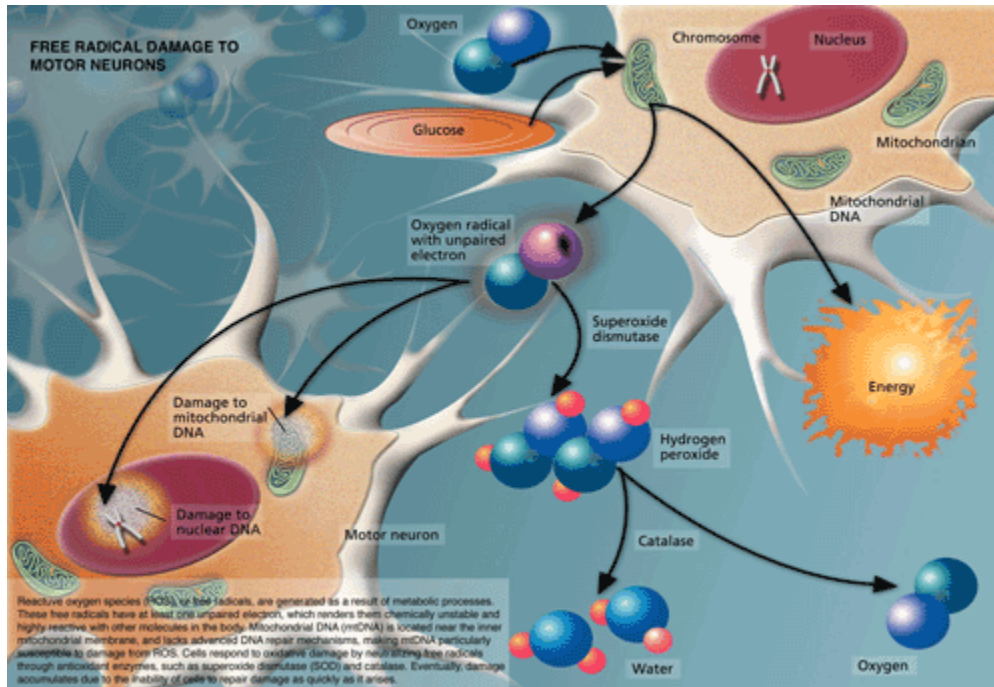
Reactive oxygen species have at least one unpaired electron. This unpaired electron makes them chemically unstable and highly reactive with other molecules.

Mitochondrial DNA is near the inner mitochondrial membrane. Mitochondria have primitive DNA repair mechanisms, so it is particularly susceptible to free radical damage.

Cells produce the antioxidant enzymes superoxide dismutase (SOD) and catalase to clean up reactive oxygen species

Reactive Oxygen Species damage your brain in many ways:

- damage to proteins
- damage to nuclear DNA
- damage to membranes
- damage of mitochondrial DNA



Mitochondrial oxidative stress appears to play an important role Alzheimer's, Parkinson's and Huntington's disease (Schapira 1989, Mecocci 1993, Polidori 1999, Wang 2005).

Cells clean up the ROS in a series of steps. The free electrons in ROS get handed off from one anti-oxidant molecule (such as SOD or lipoic acid) to another in a series of transfers until they ultimately get transferred to make a stable oxygen molecule. It's like a game of passing the hot potato: "I don't want it, here, you take it."

ROS are also involved in cell signaling – this raises the concern that altering ROS may have some undesirable side effects. However, most of the signaling identified so far tells the cells to initiate clean up of ROS.

Antioxidants extend lifespan

Antioxidant treatments extend lifespan in animals from yeast, worms and fruit flies to mice, and probably in humans.

Your cells naturally produce anti-oxidant molecules and proteins that clean up the toxic waste produced by your mitochondria. You've already heard of some of these natural anti-oxidants, such as vitamins.

In 1956, in a now-famous and very influential paper, Denham Harman first proposed the free radical theory of aging (Harman 1956). This theory states that organisms age because of damage caused by free radicals. In 1972, Harman extended the theory to pinpoint

mitochondrial production as the critical source of free radicals that damage the cell (Harman 1972).

Anti-oxidants protect cells by cleaning up free radicals. Some of your cells' natural anti-oxidants are

- superoxide dismutase (SOD) protein
- catalase protein
- alpha-lipoic acid
- co-enzyme Q

William Orr and Rajindar Sohal at the Southern Methodist University in Dallas created transgenic fruit flies with three copies of the superoxide dismutase gene and the catalase gene (Orr 1994). Compared to the controls, the transgenic flies

- lived up to 30% longer than controls
- had less protein oxidative damage
- were more physically fit at older age
- had higher metabolic rate at older age

In humans, mutations in the SOD1 gene are associated with the loss of motor neurons in the brain of patients with the life-shortening disease Amyotrophic Lateral Sclerosis (ALS). ALS is also known as Lou Gehrig's Disease, and is the disease affecting the famous physicist Steven Hawking. Tony Parkes at the University of Guelph in Canada, with colleagues in Toronto, created transgenic fruit flies that over-expressed SOD1 specifically in their motor neurons (Parkes 1998). Compared to the controls, the transgenic flies

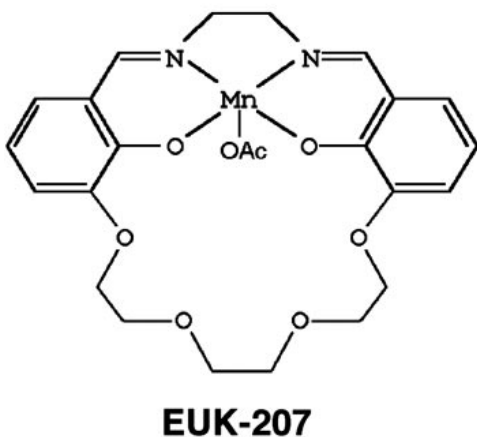
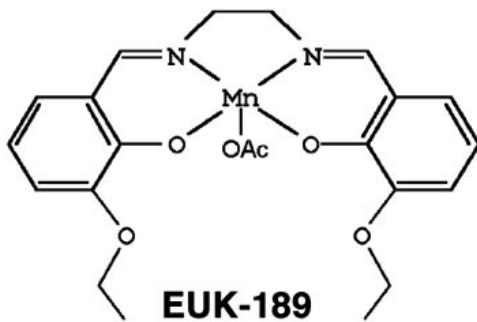
- lived up to 40% longer than controls
- had greater resistance to oxidative stress

Bernard Malfroy, Susan Doctrow, and their colleagues at a the biotechnology company Eukarion in Bedford, Massachusetts, developed drug-like molecules to mimic the action of the body's natural anti-oxidants, superoxide dismutase and catalase (Melov 2000, Liu 2003). They tested the effects of the mimetics on the lifespan of worms (*C. elegans*) in collaboration with Simon Melov and researchers at the Buck Institute for Age Research, in Novato, California.



The Eukarion SOD mimetics

- increased the average lifespan of the worms by 44%
- restored the lifespan of prematurely aging worms to normal, an increase of 67%



We looked at the anti-oxidant cascade earlier. In this cascade, the free electrons that make ROS are handed off in a series of transfers until they ultimately get transferred to make a stable oxygen molecule. Remember passing the hot potato. Speeding up the flow in one

step of this series of transfers without increasing the capacity in a later stage may not be helpful – if we build a six-lane highway that suddenly ends on a two-lane city street, things will move fast down the highway, and come crashing to a stop at the city street. The same thing can happen with the electrons in free radicals.

In some experiments, increasing the levels of certain anti-oxidants has been harmful, rather than beneficial, for example, increased levels of Q10 shortened lifespan in some animal experiments. In one recent experiment in the roundworm *C. elegans*, lowering superoxide dismutase increased lifespan (Van Rammsdonk 2009).

A possible explanation of these results is that we are dumping all the traffic from a six-lane freeway onto a two-lane street: we don't have enough of the next components of the series to receive the free electrons. Getting the right amounts and right balance of anti-oxidants is probably important.

In drug clinical trials in humans, it is often challenging to find the right dose of drug that is beneficial while minimizing side effects. If the animal experiments with anti-oxidants used a dose that was too high, the adverse side effects could overwhelm any benefit. Again, choosing the right amounts and balance of anti-oxidants matters.

How do antioxidants affect your brain?

Antioxidants improve

- learning
- memory
- reaction time
- balance
- coordination

Antioxidants reverse age-related brain deficits

Earlier, we saw how small-molecule SOD mimetics (anti-oxidants) developed by Eukarion extended the lifespan of worms. Ruolan and Ingrid Liu at the University of Southern California collaborated with Eukarion to test if SOD mimetics could reverse age-related learning deficits in aging mice (Liu 2003).



Used two SOD mimetics from Eukarion: EUK-189 and EUK-207.

Female mice at 8 months old randomly assigned to 6 groups (16-18 per group)

- control
- untreated control
- low dose EUK-189
- high dose EUK-189
- low dose EUK-207
- high dose EUK-207

Implanted minipumps in anesthetized mice

- drug delivered for 28 days
- low rate ~9nmol/day
- high rate ~ 0.09 micromol/day

Results on tests of memory and learning

- control mice
 - significant loss of learning and memory abilities between 8 and 11 months.
- mice treated with the anti-oxidant Eukarion SOD mimetics
 - learning and memory deficits almost completely reversed

Results: Brain oxidative damage

- Learning and memory deficits were correlated with increased brain oxidative damage to proteins, lipids, and nucleic acids
- Compared to control mice, SOD mimetics
 - completely reversed the protein oxidation
 - reduced lipid peroxidation 50%
 - significantly reduced levels of oxidized DNA and RNA

Low doses were more effective than high doses.

Thus study showed that low molecular weight synthetic molecules that function like the enzymes superoxide dismutase and catalase improve cognitive performance and decrease oxidative stress in middle-aged wild type mice.

Aaron Clausen, Susan Doctrow and Michel Baudry (2008) in the Neuroscience Program at the University of Southern California extended the Liu work. They looked at SOD mimetic effects in older mice, at lower doses, and for longer periods of time.

17-month-old mice were randomly assigned to treatment groups:

- Vehicle control
- 4 EUK-treated groups (1.5 or 0.15mM EUK-189 or EUK-207)
- An 10 additional male mice were the untreated 16-month control group

Implanted minipumps in anesthetized mice

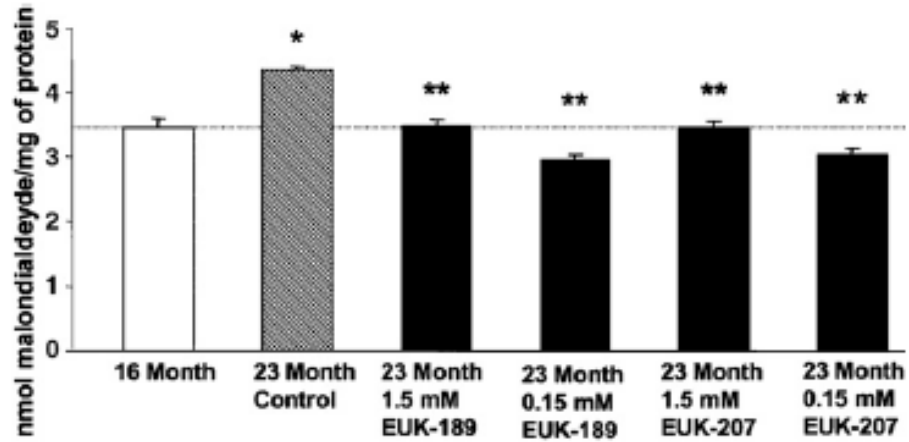
- drug delivered for 6 months
- low dose 0.15 mM
- high dose 1.5 mM

Results on tests of memory and learning

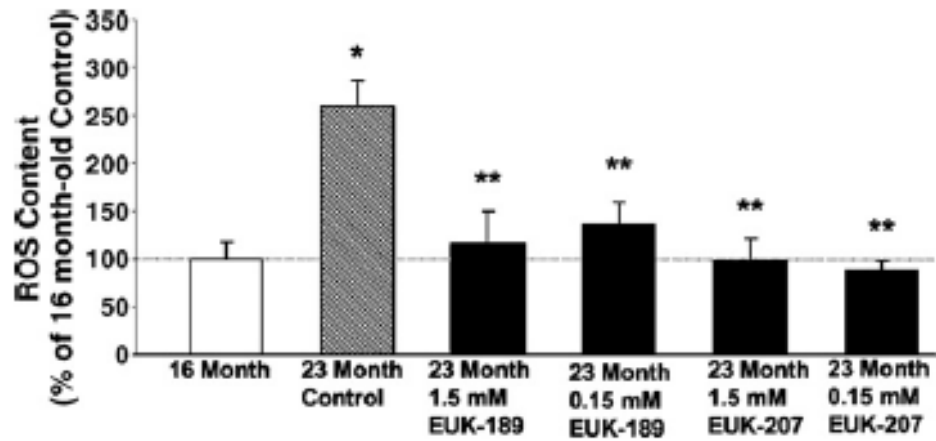
- control mice
 - significant loss of learning and memory abilities over 6-month trial period.
- mice treated with the anti-oxidant Eukarion SOD mimetics
 - learning and memory deficits much reduced

Results: Brain oxidative damage

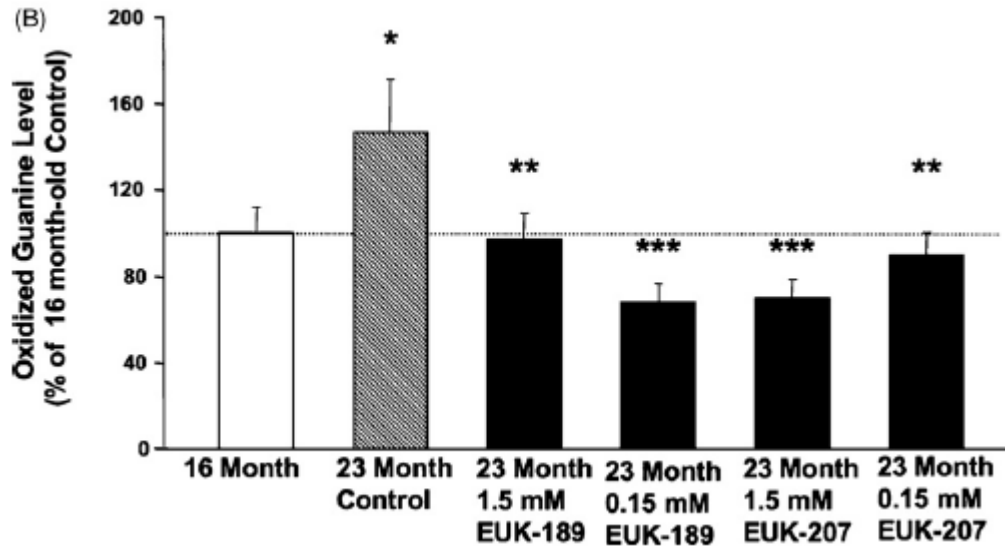
- Learning and memory deficits were correlated with increased brain oxidative damage to proteins, lipids, and nucleic acids in controls, not in treated mice
- Compared to control mice, SOD mimetics
 - significantly reduced levels of Reactive Oxygen Species concentration
 - significantly reduced lipid peroxidation
 - significantly reduced levels of oxidized DNA and RNA



Effects of chronic treatment with EUK-189 or EUK-207 on lipid peroxidation



Effects of chronic treatment with EUK-189 or EUK-207 on Reactive Oxygen Species concentration



Effects of chronic treatment with EUK-189 or EUK-207 on oxidized nucleic acids

Clausen summarized the results:

"Chronic treatment with EUK-189 or EUK-207 was initiated at a relatively late stage in the lives of these mice, but our 6 month-long treatments were still able to provide protection against cognitive declines that occurred between 16 and 23 months of age. Thus, our results suggest that these compounds might prove to be beneficial in preventing further cognitive impairment in relatively old individuals that already exhibit mild cognitive impairment. In addition, while this study was not intended to address chronic toxicity, it is well worth noting that long term, sustained treatment with these compounds was beneficial to the mice without showing any indications of toxicity. Similar observations have been made in other long-term treatment studies, for example, chronic administration of EUK-189 in a mouse Alzheimer's disease model."

Which antioxidants to choose?

Thousands of studies have examined anti-oxidant effects in-vitro and in-vivo, in animals and in humans. I won't attempt to summarize them here.

Many foods such as berries are rich in anti-oxidants. Lists are readily available. However, the amount of anti-oxidant you can get from food is a tiny fraction of what you get by taking supplements.



Photograph by The Wandering Angel

In addition to taking a regular vitamin pill (which includes anti-oxidant vitamins), I suggest you consider alpha lipoic acid.

Alpha lipoic acid

Here are reasons why I think that alpha lipoic acid is a particularly good anti-oxidant

- proven neuroprotective benefit in multiple animal studies and multiple double-blind, randomized, controlled clinical trials in humans
- very good safety profile in human clinical trials; side effect rates similar to placebo
- soluble in both fat and water, so it reaches all parts of the neuron and mitochondria
- re-activates other anti-oxidants (Vitamins A and C, co-enzyme Q)
- reverses memory impairment and oxidative damage in aged mice

Alpha lipoic acid has a relatively short half-life, meaning that it is cleared out of your body fairly quickly. However, that doesn't mean it can't work. Your garbage collectors only visit your house for a few minutes a week, but you are still glad they come.

Can antioxidants prevent, slow, or treat Alzheimer's disease?

In 1906, Alois Alzheimer autopsied the brain of a patient with severe memory loss and found her entire brain filled with what looked like wads of old chewing gum (plaques) inside the neurons, and matted hair (tangles) between the neurons.

Two proteins dominate the brains of Alzheimer's patients:

- amyloid beta forms the plaques
- tau forms the tangles

Both contribute to the disease, but it's not clear if one or both are the initial causes.

In a 2004 review in the New England Journal of Medicine, Jeffrey Cummings summarized current knowledge of amyloid beta in Alzheimer's (Cummings 2004):

- Mutations in the amyloid precursor protein lead to early-onset Alzheimer's disease
- All currently known mutations in other genes associated with Alzheimer's disease increase the production of amyloid beta
- Transgenic mice that produce human amyloid precursor protein have learning and memory deficits and their brains have plaques similar to Alzheimer's
- The apolipoprotein E4 allele, a major risk factor for Alzheimer's disease, accelerates deposition of amyloid

Amyloid beta generates free radicals; the Met³⁵ amino acid of amyloid beta is responsible for free radical production.

Amyloid beta works in the nerve synapse. Excess production of one form of amyloid beta, called amyloid beta 42, is closely linked to Alzheimer's.

There are many studies of oxidative stress in Alzheimer's disease and more specifically the activity of amyloid beta in producing reactive oxygen species (Markesbery 1997, Rinaldi 2003, Grundman 2002).

Increased oxidative damage in Alzheimer's:

- increased lipid peroxidation
- increased protein and DNA oxidation
- advanced glycation end products (AGE)
- decreased polyunsaturated fatty acids

Oxidative damage appears to be one of the earliest pathophysiological events in Alzheimer's preceding the formation of amyloid plaques and neurofibrillary tangles.

Further evidence of oxidative stress in Alzheimer's:

- Transgenic mice expressing amyloid beta reproduce the clinical symptoms and pathological progression of AD including oxidative stress.
- In amyloid beta transgenic mice, memory impairment is correlated with increased levels of amyloid beta
- Active and passive beta-amyloid-directed immunization removes beta-amyloid plaques and restores memory.
- In transgenic mice expressing both amyloid beta and presenilin 1 genes required for memory are down-regulated. In studies of cortical tissue from Alzheimer's patients, the same memory-associated genes are down-regulated.

Plasma levels of most antioxidants are lower in patients with Alzheimer's and Mild Cognitive Impairment (MCI) compared to healthy age-matched controls.

Free radicals cause neuron degeneration and death in vitro and in vivo

Anti-oxidants have been shown to protect against neurodegeneration

Anti-oxidants, particularly Vitamin E, have shown efficacy in clinical trials for treating Alzheimer's disease and in epidemiologic studies anti-oxidants are linked to reduced risk of Alzheimer's disease (Cummings 2004).

In a 1997 New England Journal of Medicine article, Sano and colleagues described a double-blinded, placebo-controlled, randomized, multi-center trial of two years of Vitamin E in 341 patients with moderate Alzheimer's (Sano 1997).

- Primary outcome was time to the occurrence of any of the following:
 - death
 - institutionalization
 - loss of the ability to perform basic activities of daily living
 - severe dementia
- After adjustment for baseline Mini-Mental State Examination (MMSE) scores, there were significant delays in the time to the primary outcome for the patients treated with Vitamin E (670 days) compared with the placebo group (440 days).
- No significant differences in cognitive function between the two groups at two years.

Rutten and colleagues reviewed antioxidant clinical trials in Alzheimer's disease (Rutten 2002):

“Recent prospective studies have indicated that dietary intake of several exogenous antioxidants is associated with a lower risk for Alzheimer's disease. This suggests that people at risk for developing Alzheimer's disease or being in the early phases of this disease may benefit from intervention with exogenous antioxidants. The clinical studies carried out so far, however, do not provide the final answer to whether antioxidants are truly protective against Alzheimer's disease...

There is compelling evidence that oxidative stress is involved in Alzheimer's disease pathogenesis, and several lines of evidence indicate that administration of antioxidants may be useful in prevention and treatment of Alzheimer's disease.

Further clinical studies, based on larger cohorts studied over a longer period of time, are needed, however, to test this hypothesis. Furthermore, for the future one might expect balanced upregulation of both exogenous and endogenous antioxidants as one of the best treatment strategies for preventing or at least slowing down the progression of Alzheimer's disease.”

Small, initial studies of alpha lipoic acid in mild dementia or Alzheimer's disease show promising results, but larger studies are required (Hager 2007, Maczurek 2008)

Are anti-oxidants and supplements worth the cost?



It's easy to spend many dollars per day on anti-oxidants and supplements. Are they worth it, if we don't know for sure that they work?

We could ask, compared to what?

- Compared to \$100 billion spent each year in the US to treat and care for Alzheimer's patients?
- Compared to how much you would pay to reverse Alzheimer's after you were diagnosed?
- Compared to the cost of a hamburger, fries and shake?

Better yet, if you really want to reduce your risk of Alzheimer's,

- skip the hamburger and fries
- walk to your nearest farmers market
- get some fresh vegetables and fruit to go along with your salmon for dinner

Supplements

Caveat: Pills containing supplements often don't contain the claimed amount
Herbal supplements particularly problematic – amount of active ingredient in a plant product depends on many factors:

- the soil it grows in
- how much water
- how much sun
- how much fertilizer
- insects and parasites
- when it was harvested
- how it was processed
- how it was stored and shipped
- how long it has been on the shelf

Therefore hard to evaluate in clinical trials

See PowerPoint files:

Ginkgo Biloba

Q10

Coffee effects brain

Folic acid

Malouf 2008 (Cochrane)

"The small number of studies which have been done provide no consistent evidence either way that folic acid, with or without vitamin B12, has a beneficial effect on cognitive function of unselected healthy or cognitively impaired older people. In a

preliminary study, folic acid was associated with improvement in the response of people with Alzheimer's disease to cholinesterase inhibitors. In another, long-term use appeared to improve the cognitive function of healthy older people with high homocysteine levels. More studies are needed on this important issue."

Caffeine for pain, migraine, and tension headaches

Caffeine has long been known to relieve pain, either by itself or as an adjuvant to over-the-counter pain-relief medications such as aspirin, ibuprofen and acetaminophen. It also increases the pain-relieving effect of narcotics such as morphine. In the U.S., caffeine is not included in standard over-the-counter formulations because some people may experience the undesirable effects of sleeplessness or anxiety if they take the medicine and are not aware that it contains caffeine. But if you have pain or a headache and want a more effective pain-killer, here's what you might consider.

In a study reported in 2000, Seymour Diamond and his colleagues looked at the effects of ibuprofen with or without caffeine for treating tension headaches. When they had a tension headache, the patients took one of four treatments: placebo, 400 mg ibuprofen (two standard tablets), 200 mg caffeine (about a cup and a half of coffee), or the combination of 400 mg ibuprofen plus 200 mg caffeine. Neither the patients nor the doctors knew which drug the patient was getting. The results were that six hours after taking the medications, 71% of patients taking caffeine plus ibuprofen had complete relief, compared to 58% for those taking ibuprofen alone, 58% for those taking caffeine alone, and 48% for those taking placebo. The median time to what the patients considered a meaningful improvement was 108 minutes for patients taking caffeine plus ibuprofen, 161 minutes for those taking ibuprofen alone, 132 minutes for those taking caffeine alone, and 279 minutes for those taking placebo. The patients who got caffeine had higher rates of nervousness, dizziness, or nausea. Other studies have shown that higher doses of caffeine worsen these side effects without improving pain relief. So, you are more likely to get relief, and to get it about an hour sooner, if you have a cup of coffee along with your ibuprofen. Of course, you are likely to get some of the coffee side effects. Similar studies of caffeine in combination for migraine headaches and post-surgical pain show that it is effective for these types of pain, too.

References

- Clausen A, Doctrow S, Baudry M.
Neurobiol Aging. 2008 Jun 18. [Epub ahead of print]
Prevention of cognitive deficits and brain oxidative stress with superoxide dismutase/catalase mimetics in aged mice.
- Contestabile, Antonio
Benefits of Caloric Restriction on Brain Aging and Related Pathological States: Understanding Mechanisms to Devise Novel Therapies
Current Medicinal Chemistry, 2009, 16, 350-361
- Cummings, Jeffrey L.
Alzheimer's Disease
N Engl J Med 2004;351:56-67.
- Hager K, Kenklies M, McAfoose J, Engel J, Münch G.
J Neural Transm Suppl. 2007;(72):189-93.
Alpha-lipoic acid as a new treatment option for Alzheimer's disease--a 48 months follow-up analysis.
- Harman, D (1956). "Aging: a theory based on free radical and radiation chemistry".
Journal of Gerontology 11 (3): 298–300. PMID 13332224.
- Harman, D (1972). "A biologic clock: the mitochondria?". Journal of the American Geriatrics Society 20 (4): 145–147. PMID 5016631.
- Maczurek A, Hager K, Kenklies M, Sharman M, Martins R, Engel J, Carlson DA, Münch G. Adv Drug Deliv Rev. 2008 Oct-Nov;60(13-14):1463-70.
Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease.
- Malouf R, Grimley Evans J.
Folic acid with or without vitamin B12 for the prevention and treatment of healthy elderly and demented people.
Cochrane Database Syst Rev. 2008 Oct 8;(4):CD004514.
- Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ.
Extension of life-span with superoxide dismutase/catalase mimetics.
Science. 2000 Sep 1;289(5484):1567-9.
Buck Institute for Age Research, Novato, CA 94949, USA.
- Mecocci, P.; MacGarvey, U.; Kaufman, A.E.; Koontz, D.; Shoffner, J.M.; Wallace, D.C.; Beal, M.F. Oxidative damage to mitochondrial

DNA shows marked age-dependent increases in human brain. *Ann. Neurol.*, 1993, 34, 609-16.

Orr, W.C. and Sohal, R.S. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263: 1128-1130, 1994.

Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons. *Nat Genet.* 1998 Jun;19(2):171-4.

Polidori, M.C.; Mecocci, P.; Browne, P.; Senin, U.; Beal, M.F. Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex. *Neurosci. Lett.*, 1999, 272, 53-6.

Van Rammsdonk, Jeremy M., and Hekimi, Siegfried (2009). "Deletion of the Mitochondrial Superoxide Dismutase sod-2 Extends Lifespan in *Caenorhabditis elegans*". *PLoS Genetics* 5 (2): e1000361.

Rendeiro C, Spencer JP, Vauzour D, Butler LT, Ellis JA, Williams CM. The impact of flavonoids on spatial memory in rodents: from behaviour to underlying hippocampal mechanisms. *Genes Nutr.* 2009 Sep 2. [Epub ahead of print]
School of Psychology and Clinical Language Sciences, University of Reading, Reading, RG6 6AP, UK.

Schapira, A.H.; Cooper, J.M.; Dexter, D.; Jenner, P.; Clark, J.B.; Marsden, C.D. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet*, 1989, 333, 1269.

Wang, J.; Xiong, S.; Xie, C.; Markesbery, W.R.; Lovell, M.A. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.*, 2005, 93, 953-62.